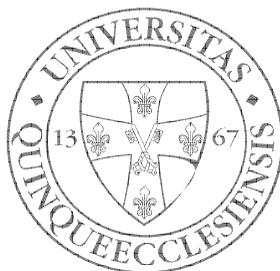


# DEVELOPMENT OF SELECTIVE CHEMICAL SENSORS AND METHODS

Thesis for PhD Dissertation

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## 1. **Introduction**

Amperometry is one of the voltammetric methods of electro analytical chemistry. Up till our present days high number of voltammetric methods with more or less different measuring program has been worked out. Amperometry is the most simple of them.

Generally saying in case of voltammetric measurements an electrode potential value determined by an appropriate time program is imposed on the working electrode being in the measurement cell, and the current value as a response is recorded in appropriate time instances determined by the data collecting program of the method. The analytical information is obtained on the basis of current – concentration dependence

In case of conventional amperometry the electrode potential is kept constant and the current observed at that potential is continuously recorded. The concentration of electroactive species in the cell is reflected by the difference of the instantaneous current and the residual current. In the cell intensive convection is applied by electrode rotation, vibration, solution streaming or stirring.

Using amperometry advantageously very small lower limit of detection can be achieved, however the selectivity provided is poor. Further more difficulties can arise when the product of the electrolysis stays on the electrode surface passivating it.

In early times of instrumental analysis, therefore amperometry was mostly used for indication of redox titration. In the twenties of the last century Folk and Badwen reported on a “new type electrometric” method that could be used well for indicating iodometric titration.

Stock published paper about the well known “dead stop” technique that uses two identical indicating electrodes. He calls the method “amperometric titration”. John T. Stock (1911-2005) during his long active life highly contributed to development of amperometry. All the way to our days the electrochemical community recognizes his comprehensive monograph as a popular, basic, useful work.

A major step in developing of voltammetric methods have been made by the school of Heyrowsky by using the dropping mercury electrode that is inventing the polarography. The renewing dropping mercury electrode eliminates the uncertainty that electrode passivation could bring in. Using the dropping mercury electrode highly accurate direct analysis in quite low concentration range could be carried out voltammetrically. Therefore solid electrode indicated amperometric titration lost a bit of popularity in electro analytical laboratories. Soon polarography became the fifth most often applied method of instrumental analysis. In polarography electrode potential – current dependences are recorded and the concentration is determined on the basis of these, comparing them with

calibration curves (i-c plots) obtained recording polarograms in standard solutions of known concentration. In early times of polarography metal analysis was its major field of application.

Just limited number of organic molecules showed electroactivity in the potential range available with mercury electrode, therefore spreading voltammetric methods of potential scanning in organic analysis was hindered. Further more the observation of electrode fouling when application of solid electrodes were tried, discouraged researchers seeking applicability.

Regardless that by the seventies of the last century more and more effective polarographic methods were worked out, or instruments, the popularity of polarography very much decreased. With the newly developed atom spectroscopic methods the polarography could not compete in respect of speed and simplicity.

The interest of electroanalytical schools dealing with developing voltammetric methods was turned to organic analysis. Considerable part of organic analytes can be oxidized electrochemically. Therefore, electrodes capable for providing the potential windows for oxidation were needed. The importance of solid electrodes increased. Ralph N. Adams (1924-2005) authored the first book (1969) about electroanalytical application of solid electrodes. His book dealing with the properties of electrodes, with the mechanisms of electrode processes, with the voltammetric methods, with the methods

applicable for studying electrode reaction still is a “classic base work” today.

Solid electrodes used in voltammetry often serve simple as electron source or electron drain. However, a structural change e.g. a layer formation can happen or can be facilitated on the working electrode surface that beneficially influences the measuring property of it. Taking advantage on this resulted the development of the chemically modified electrodes. In our days the research dealing with chemically modified electrodes represent one of the most dynamically developing field of electroanalytical chemistry.

An appropriate modifying layer – in certain cases – can avoid electrode passivation, and can provide selectivity. In this way by using chemically modified electrodes the disadvantageous property of amperometry can be corrected. This contributed to the recent increase in popularity of amperometry. For example, the amperometric biosensors prepared by coating the electrode surface with enzyme layer that facilitates selective detection of different species, are applied broad scale in every day analytical practice today.

The development and vast use of methods of separation sciences are the other source of recently increased popularity of amperometry. For detection of “peaks” of electroactive analytes separated by HPLC columns or capillar electrophoretic devices high number of different

amperometric detectors have been worked out and have been used in broad scale.

I got involved in research work aiming to develop voltammetric analytical methods about ten years ago. My experiments have been carried out partly at the Institute for Chemistry of the Faculty of Sciences at the University of Pécs, partly at the University of Memphis, Faculty of Biomedical Engineering, in its Electroanalytical Laboratory.

The dissertation summarizes the result of my research achieved in three more or less separated fields:

- The first, longest part deals with results achieved studying in basic media the voltammetric behaviour of the copper electrode coated with electrocatalytic oxide film. In this part of the work the nature of electrochemical oxidation of different carbohydrates were investigated. Copper working electrode containing amperometric detector cells were also prepared, their properties were investigated and they were used in solving analytical problems connected to an IC analytical system.
- The second part of the dissertation is about development, investigation and application of amperometric biosensors based on oxidoreductase enzymes.

- In the third part of the work a new, amperometric measuring technique is introduced. The Periodically Interrupted Amperometry (PIA), developed in our laboratory proved well applicable for enhancing the measuring capability of membrane coated electrodes, like amperometric enzyme sensors.

## The aims of the work

The objective of my experimental work has been the development of high performance electroanalytical methods and selective sensors, advantageously applicable ones for purposes of clinical diagnosis, and for food and environmental analysis. In the initial times of the studies it could be concluded that application and improvement of certain chemically modified electrodes is needed for stepping forward in this field. It was obvious to select amperometry from the full scale of voltammetric methods. Planning the development of special amperometric cells and electrode assemblies seemed very promising in that time.

Literature data and our preliminary observations suggested that copper working electrodes in basic media can well be used for determination of carbohydrates and certain organic acids. Some important details of electrode processes allowing the determinations were not known.

My goal was investigating the nature and properties of this electrochemical oxidation process. Further more I wanted to develop copper electrode containing amperometric detector cells for application in HPLC analysis or in FIA determinations. It was obvious to prove the applicability of the different detector cells by solving real analytical problems with their use.

In HPLC analysis of carbohydrates the used different detector cells are far not optimal. Some of them lack the sensitivity needed, some require pre or post column derivatization, some need pulsed amperometric electrode cleaning. Accordingly the application of

copper electrode in sugar analysis appeared very promising.

Therefore my goal was:

**Investigation of electrochemical oxidation reactions of different mono and disaccharides at electrocatalytic copper electrodes in basic media and application of the electrode:**

-Investigation of electrochemical oxidation in basic media

-Working out amperometric copper electrode containing detector cells for HPLC and FIA applications

-Application of the detector cell in IC analysis of sugars.

The most often applied of biosensors are based on amperometric detection and catalytic action of oxidoreductase enzymes. Regardless that their first practically useful types appeared more than forty five years ago, their development is still in the focus of interest today. Their improvement in several respects of measuring properties like stability, sensitivity, selectivity, *in vivo* applicability is necessary to broaden their application field.

**My intention was contributing to the work carried out in the field of oxidoreductase enzyme based amperometric biosensor development**

- My goal was to extend the lower limit of detection of amperometric putrescine enzyme sensor to bring it into the range needed for application in clinical diagnosis.

- My other goal was working out an amperometric biosensor containing electrode assembly for in vivo animal experiments, and investigating its applicability.

During my experiments it appeared as a new idea that the sensitivity of membrane coated amperometric electrodes, like enzyme sensors can be enhanced by applying periodically interrupted polarization program replacing the conventional continuous electrode polarization one. My objective was investigation of the possibilities and limitations of this sensitivity enhancement initiative. .

**Development of new amperometric measuring technique for improvement of performance improvement of membrane coated electrodes**

- Periodically interrupted amperometry (PIA)
- Applications of PIA

### 3. Methods

The instruments and tools available at the Institute for Chemistry of University of Pécs, Faculty of Sciences and at the Electroanalytical Laboratory of Faculty of Biomedical Engineering, University of Memphis (TN) were used in the experiments.

In voltammetric measurements Princeton Applied Research type 273A computerized potentiostat/galvanostat (EG&G, PAR, USA), Autolab12 potentiostat/galvanostat electrochemistry workstation (EchoChemie B.V. Hollandia) és CHI 760C electrochemistry workstation ( Shanghai CH Instruments, China) were

applied. The Autolab apparatus was controlled by GEPES 4.9.005 measurement and evaluation program, while the CHI 760C by the version 6.20 CHI program. Both workstation has bipotentiostat and microelectrode units.

Radelkis type OH 404 coulometer made of potentiostat, controller and coulometer units served for macroelectrolysis. Its magnetic stirrer and electrolysis cell was used.

Perkin-Elmer type 372 (Germany) flame atomization atomic absorption spectrophotometer was used for determination of copper content of solution obtained after macroelectrolysis. It was connected to computerized data collecting unit for achieving higher reproducibility and sensitivity.

Orion 720 A and Thermo Orion 420A (Orion Research Inc. Beverly, MA, USA ) pH meters were used.

Fiber optics ( Ocean Optics Inc. Dunedin, FL, USA) spectrophotometer and conventional Specord UV-VIS (Carl Zeiss Jena, Germany) spectrophotometers were used for enzyme activity measurements following standard procedures. For putrescine oxidase Toyobo method 510 nm, for glucose oxidase o.dianisidine method 500 nm)

For checking the mechanic steps of electrode making, optical (Carl Zeiss Jena, Germany) microscope and Olympos CKX 31 ( Olimpos LTD, UK) inversios microscope were used )

Radiometer Copenhagen type EDI 101 rotating disc electrode with CVT101 controller (Radiometer

Analytical S.A. France ) was used in rotating disc experiments.

Type RK 52H sonicator bath (Bandelin Sanorex, Germany ) was used for preparation of solution and degassing the eluents.

Home made FIA apparatus was put together for testing the detector cell performance while units of Waters HPLC apparatus (Waters, Milford, MA, USA ) together with Dionex type AMMS-III suppressor , Dionex type CD25 conductivity detector, Dionex CarboPac PA' and IonPac AS5A analytical and safety columns (Sunnyvale, CA, USA) were combined for IC and HPLC studies.

Determination of number of electrons taken part in oxidation:

#### I. Method involving macro electrolysis

Small volumed basic solution of the sugar was oxidized at large area copper electrode using constant electrode potential. The electric charge passed through the cell was measured ( $\Delta Q$ ) as well as the amount of sugar disappeared ( $\Delta m$ ). Based on the material balance the number of electrons exchanged ( $n$ ) was calculated

$$n = \frac{M\Delta Q}{F\Delta m} \quad M \text{ is the molecular weight, } \textit{charge}$$

$$\Delta Q = Q_{\textit{sample}} - Q_{\textit{background}}$$

#### II Koutecky –Levich method:

Current values were measured with rotating disc electrodes at different rotation rate in solution of different concentrations. From these values the well

known Levich plots were made. From the slope of the  $1/i_{\omega} - 1/\omega^{1/2}$  plot, using literature data for viscosity and diffusion coefficients, the  $n$  value was calculated. From the intersection values of the plots with the vertical axis the reaction rate coefficient could be also determined.

Results summarised as thesis points

1. Different copper electrodes were prepared - domestically in the first time -for voltammetric experiments. Papers were published in international scientific journals about results obtained investigating the voltammetric behavior of copper electrodes.
2. Investigating the redox processes taking place at the surface film of copper electrodes in basic media the thickness of the oxide film was estimated. The measuring conditions appropriate for voltammetric carbohydrate analysis was determined.
3. The number of electrons exchanged during electro oxidation of different carbohydrates at copper electrode surface in basic media was measured using two entirely different methods. In this way it was proven that the number of electrons taking part in electro oxidation of one carbohydrate molecule is unusually high in conditions studied. ( $n=2xC_N$ )
4. IC and AA analysis were performed of the product of solutions obtained by electrochemical macroelectrolysis of carbohydrates at copper electrode in basic media. As a result of this an earlier supposed hypothetical mechanism

of the electrochemical oxidation could be experimentally proven. Accordingly the electro oxidation is electrocatalytic by nature and the main product is formate.

5. Three different kinds of flow through detector cells, containing copper working electrodes were constructed. Their performance was investigated in FIA and in IC conditions. Methods were worked out for carbohydrate analysis and they were tested solving problems of real sample analysis.

6. A new enzyme immobilization method was used for binding putrescine oxidase enzyme with high specific activity on electrode surface

7. Optimising preparation technology of putrescine electrode and by fine tuning of the measurement method, the dynamic range of putrescine electrode -that originally was for detection of bacterial infection- was lowered substantially. The new measurement range overlaps with the range needed for clinical diagnosis.

8. Glucose enzyme electrode containing amperometric measurement cell assembly was developed for *in vivo* animal experiments. Its applicability was proved making glucose tolerance tests with anaesthetized rats inserting the assembly under their skin.

9. A new amperometric measurement method was worked out. The method called periodically interrupted amperometry (PIA) improves considerably the

performance of membrane coated electrodes, like biosensors. Providing advantageously high signal/noise ratio it extends the lower limit of detection towards the smaller concentrations.

10. The applicability and advantage of the PIA method was proved carrying out experiments with different electrodes, with amperometric enzyme sensors.