

Novel reagentless chronopotentiometric method for assessing antioxidant activity using chemically modified working electrode

Doctoral thesis
PhD

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Introduction

I started my research work at the University of Pécs as a Stipendium Hungaricum supported foreign student in the end of August 2019. My thesis summarizes the results of my almost four year-long efforts aiming to work out novel electroanalytical method applicable solving current practical task. I hope the results obtained will find application in every day practice of quantitative analytical chemistry and possibly get listed in the broad techniques of electroanalysis.

Looking at the history of instrumental analysis, it can be concluded that in the beginning, electrochemical methods played fundamental roles. Their invention and application started as early as in the second part of the nineteen century. From the early times the development of electroanalytical methods still goes on at a high pace in different laboratories. A high number of reports appear about new and improved electroanalytical methods, instruments, electrodes and about advantages of their applications. Regardless of these, the real every day applications of electroanalytical methods is getting more and more overpassed by other more recent methods.

There are however, several areas where the electroanalytical methods still compete well with the mentioned ones. As example, we can mention the measurements with ultra-micro electrodes in assessing local concentration data in living biological organisms or in gathering local chemical information for scanning electrochemical microscopy studies. Similarly when very low concentration of certain species has to be measured, then inverse voltammetric methods can give help. The application of cost effective, on line or at in-situ methods using selective electrochemical sensors gives electroanalytical methods a considerable comparative advantage. Developing appropriate chemically modified electrodes can enhance the chance of gaining application in every day practice.

In my studies working with carbon electrodes chemically modified with a reversible redox mediator layer, it was learned that the confined mediator layer could easily be electrochemically brought into its oxidized or reduced redox state. This guided us into investigating the applicability of the mediator layer as an analytical redox reagent. Furthermore, potentiometry could be used to detect the progress of heterogeneous redox reactions. These encouraged us to experiment with working out practically applicable “reagentless” analytic methods. Based on literature information and on earlier or on my recent

experimental findings we concentrated our efforts in dealing with determination of the action of reductive species (of antioxidant character) in liquid samples.

In living organisms the oxidizing materials, like reactive oxidizing species (ROS) or free radicals have important beneficial roles in different biochemical reactions, in signaling or in self-defenses. On the other hand however, their elevated level can be extremely harmful. It can mediate generation of oxidative stress a cell damaging condition. Presence of certain bioactive species, called antioxidants can counteract these unwanted seriously aging and multiple pathogen condition by adding electron or hydrogen to the oxidizing molecule or to the radical, respectively. Therefore determination of antioxidants in physiological samples or in nutrients has high practical importance. Depending on their source, solubility, action mechanism and chemical structure the physiologically important antioxidants are usually sorted in different groups. Real samples taken for chemical analysis usually contain a mixture of antioxidant species. We can say that the antioxidant character is a collective property. Therefore the results of the analysis are often given in relation to the action of a species used for calibration as antioxidant activity or capacity. The measured values obtained for food, beverage, plant tissue or physiologic samples with different methods usually are not in good correlation.

The collective antioxidant activity (AOA) is related to reaction rate. Higher activity shows higher reaction rate in radical quenching or electron exchange. (The antioxidant capacity reflects how many oxidant molecule is reduced by certain amount of the sample).

In my work a reagentless method was worked out. It uses for gaining analytical signal the rate of a heterogeneous redox reaction by detecting the initial change of open circuit redox potential in the first few milliseconds. The reagent reacting with the analyte is a reversible redox mediator confined on the surface of a carbon working electrode. A controlled potential step is used for setting the redox state of the reagent. It needs to be mentioned here that most of the advanced electrochemical workstations are equipped with potentiometric measuring program that is capable of executing the measuring protocol. It was hoped that the obtained signal can be used well for estimating the antioxidant activity of certain samples.

Aim

The main aim of my work was to develop a simple, affordable and accurate method for the measurement of antioxidant activity in samples. Chemically modified electrodes were planned to prepare, study and use. Controlled potential treatment and open circuit potentiometric detection techniques seemed proper for the measuring protocol

The specific objectives were,

- To develop a reagentless chronopotentiometric measuring method using chemically modified graphite electrodes among conventional-size Glassy carbon-, miniaturized “pencil lead graphite”- and graphene oxide layer modified graphite electrodes.
- To prepare nano-size graphene oxide as a docking layer for adsorbed redox mediator and to evaluate the increase of sensitivity and stability provided by the docking film .
- To investigate the response in case of different antioxidant species and in case of their mixtures in different samples.
- To postulate the shapes of E-t and $\Delta E/\Delta t$ -c curves by model calculation.
- To investigate the applicability of miniaturized chemically modified pencil graphite electrodes in antioxidant measurement
- To evaluate the collective antioxidant activity in mixed solutions of reducing species.
- To compare analytical characters of the reagentless electroanalytical measurement with “classical” differential pulse voltammetric antioxidant measuring method.
- To study the redox activity of mediator confined graphite surfaces using scanning electrochemical microscopy.

Methods

In this study chemically modified carbon electrodes were used in making antioxidant activity measurements using the new chronopotentiometric method. Controlled potential step for bringing the mediator in oxidized state was used while open cell potentiometric detection served for following the rate of heterogeneous redox reaction. In addition, cyclic voltammetry was used in characterizing the electrode and in determining the electron transfer characteristics of the working electrode. Cyclic voltammetry was applied in electrochemical pretreatment of the electrode, electrodeposition of graphene oxide and polymerization of MB on the carbon electrode surfaces. Differential pulse voltammetry was used as a parallel measuring technique for antioxidant measurement. Scanning electrochemical microscopy was used in characterizing the redox activity on the surface of a modified carbon electrode.

Results and discussion

A theoretical model was used by postulating the expected Nernstian response from the redox activity of the immobilized mediator. The shapes of E vs. t and $\Delta E/\Delta t$ vs. concentration curves were predicted by model calculation. The amount of mediator on the surface was expected to influence the sensitivity of the modified electrode.

Reagentless chronopotentiometric measurements were made using conventional-size Glassy carbon electrode modified with an immobilised Meldola Blue mediator layer. An initial investigation was made to determine the electrochemical responses of electropolymerised and adsorbed mediator layers.

To evaluate the stability of Meldola Blue layer on, several cyclic voltammograms (CV-s) were made at 100 mV/s. The charge in regard to each subsequent CV was monitored to reveal the amount of Meldola Blue that was retained between the first and last CV-s. It was found that the electropolymerized layer was thicker and more stable than the adsorbed one. However; in sensitivity the adsorbed one seemed superior.

The repeatability of chronopotentiometric measurement results in case of the two differently Meldola Blue modified Glassy carbon electrode was determined by making more than twenty serial repeats of a particular concentration of antioxidant. The decrease in the signal between the final and the initial indicated the percentage repeatability of results and the stability of the Meldola Blue layer during the measurement of antioxidants.

In order to enhance stability of the Meldola Blue layer on the smooth Glassy carbon surface, an assessment of nano-size graphene oxide as a docking layer for adsorbing mediator was tested. It was found that graphene oxide conferred better stability to the mediator.

The influence of the amount of mediator confined on sensitivity was investigated. As it was expected higher amount resulted in smaller sensitivity. The sensitivity of the method could be tuned to the concentration range needed.

The method was then used to investigate the differences in antioxidant activity for selected pure species. It was found that different antioxidants have different reaction rate constants therefore each antioxidant gave a unique signal. However, the method is not specific for a particular antioxidant. The activity is reported relative to the equivalent gallic acid concentration.

The adsorption of redox mediator layer on the pencil graphite surface was found satisfactory.

To challenge the method further, an investigation was done in regard to the applicability of miniaturized (OD=0.2mm) chemically modified pencil graphite electrode in antioxidant measurement. Miniaturization alters the diffusion characteristics to the electrode surface. In case of miniaturized electrodes the convection was not found essential for obtaining reliable results. The electrode could be applied directly on a real sample in chronopotentiometric measurements.

The conventional size electrode was used to investigate the response of the modified electrodes to collective antioxidant activity in mixed solutions of reducing species. The results showed an initial slope that was higher than the gallic acid of equivalent. Interestingly, the mixed sample was of a lower concentration. This implied that the collective activity was determined, not the concentration but by the activity of the species.

The new method was compared in parallel measurements to the classical reagentless differential pulse voltammetric one. It was noted that at the lower concentrations of antioxidant, differential pulse voltammetry was more sensitive. The lower limit of quantitation in differential pulse voltammetry was lower than in the new method. However, the dynamic range of the new method was better than differential pulse voltammetric one..

The redox activity of the modified graphite electrodes was tested using the scanning electrochemical microscopy operating in approach curve mode. A

positive feedback was obtained implying that the redox mediator layer rather acts as catalyst, it has no insulating function.

Conclusions

Antioxidant activity of physiologic, clinical, food, beverage etc. samples is a very important property that is more often measured.

In my work a novel measuring method has been worked for assessing collective antioxidant activity value of complex samples. The method employs carbon working electrode chemically modified with confined reversible redox mediator layer. Based on literature studies and preliminary experiments the Meldola Blue was selected for preparation of the mediator layer.

The measuring procedure worked out is made of two steps: in one short controlled potential step the confined mediator brought up to its oxidized state. In the second step the potential control is terminated and the open cell potential between the working electrode and a reference is followed in time. The initial slope of the open cell potential – time trace is used as analytical signal. The applicability of the method has been proved experimentally. The dependence of the signal on different parameters was checked. Using a rough model the theoretically expected dependence of the analytical signal on experimental parameters was studied.

Thesis points:

1. Working principle of a novel method for antioxidant activity measurements were introduced and it was experimentally realized .
2. Using different methods Meldola Blue mediator containing stable redox mediator layers were immobilized on surfaces of different carbon electrodes.
3. The properties like thickness, activity, number of confined MB molecules, stability of the mediator layer has been studied with different methods
4. Model calculation were employed for explanation of the expected dependence of analytical signal on different measuring parameter
5. Different carbon electrodes, Glassy carbon, Pencil graphite, Glassy carbon with Graphene oxid docking layer were chemically modified and tested in the studies.
6. Analytical signal provided the method in case of different antioxidant species were investigated. Using gallic acid equivalence for expressing collective antioxidant activity value has been proposed.
7. Preliminary SECM measurements were made for studying redox activity of MB containing layer modified carbon electrodes.
8. Applicability of the method for assessing antioxidant activity of real fruit juice samples was tested.
9. It was proved that the confined redox reagent gets renewed in controlled potential step of the method. Therefore no addition of reagent was needed for the sample –reagent interaction. The novel method can be considered as “reagentless” one

List of publications

Publications related to the PhD dissertation

1. Kinyua Muthuri, L., Nagy, L. & Nagy, G.
Chronopotentiometric method for assessing antioxidant activity: A reagentless measuring technique, **Electrochem. Commun.** 122, 106907 (2021).
2. Kinyua Muthuri, L., Nagy, L. & Nagy, G.
Evaluating the antioxidant activity of different species using a novel reagentless chronopotentiometric method, **Electrochem. Commun.** 139, 107311 (2022).
3. Muthuri, L. K., Nagy, L. & Nagy, G.
Chemically modified pencil electrodes for application in reagentless chronopotentiometric antioxidant activity measurement. **Electroanalysis** 2023, e202200505.

Other publication

none

Conference presentations related to the PhD thesis

1. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Chemically Modified Electrode and Method for Chemical Analysis of Reducing Species, International Conference on Nanotechnology and Biosensors, May 03-04, 2021 in Rome, Italy
The Best presentation IRC award was given to our group
2. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Application of chemically modified carbon electrodes in developing novel reagentless chronopotentiometric antioxidant activity measuring method, Electrochemistry V4 Workshop 3-5th November 2021, Pécs Hungary (PPT)
3. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Chronopotentiometric antioxidant measuring method with improved measuring properties, Spring electrochemical meeting 2022, 17-18 March 2022, Kosice, Slovakia (PPT)

4. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Graphene Oxide Film Improves Stability of Meldola Blue Mediator Layer on Glassy Carbon Electrode, International Conference on Chemical Sensors June 12 – 17, 2022, Mátrafüred 2022, Visegrád, Hungary (Poster)
5. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Reagentless chronopotentiometric measurement of antioxidant activity at physiological pH, XXII. Working meeting of physical chemists and electrochemists, 29 June 2022, Brno, Czech Republic (PPT)
6. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Comparison of measured and simulated calibration curves of a novel chronopotentiometric antioxidant measurement method, 20th international symposium and Summer school on bioanalysis, June 24-30, 2022, Pécs, Hungary (PPT)
7. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Recent results with a novel electrochemical antioxidant measuring method, 1st Forum of Young Researchers on Heterogeneous Catalysis, YOURHETCAT 2022, 11 – 13th of July, 2022, Szeged, Hungary (poster)
8. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Chronopotentiometric Method for Assessing Antioxidant Activity: a Reagentless Measuring technique, Szentágothai Conference, Szervező: University of Pécs, Szentágothai Szakkollégium, 26th March 2021 (PPT)
9. Lawrence K. Muthuri*, Lívía Nagy and Géza Nagy.
Assessing antioxidant activity using a reagentless chronopotentiometric method, XIV. Szent-Györgyi Albert Konferencia, Szervező: BME Szent-Györgyi Albert Szakkollégium 16-17th April 2021. (PPT)

Conference presentations not related to the PhD thesis

Lawrence K. Muthuri, Attila Csicsor, Etelka Tombácz, Lívia Nagy, Géza Nagy
Electrochemical Method for Detection and Measurement of Humic Substances Using Chemically Modified Electrodes, Electrochemistry V4 Workshop 3-5th November 2021, Pécs Hungary (poster)

Proposals and competition

Lawrence K. Muthuri
Reagentless Chronopotentiometric Measuring Technique for Antioxidants in Food, Environmental and Physiological Samples

Participated in the PTE innovations awards (PhD category) competition May 2022
Awarded 3rd position.

Recognition

Elsevier recognition for a record of 182 views of the article in July 2022, Chronopotentiometric method for assessing antioxidant activity: A reagentless measuring technique. *Electrochem. Commun.* 122, 106907 (2021) within a month.

Project participation

OTKA, NKFIH Grant number K 125244

