STU FEI
SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA
FACULTY OF ELECTRICAL ENGINEERING AND INFORMATION TECHNOLOGY
INSTITUTE OF ELECTRONICS AND PHOTONICS

Opponent's review of the dissertation:
MSc. GRANIT JASHARI:
„Application of electroanalytical methods in food and pharmaceutical analysis "

The main purpose of the dissertation was the development of new electroanalytical methods that can be used in food and pharmaceutical analysis. The focus was mainly on the development of new approaches for the simultaneous detection of lipophilic vitamins A, E, K, and ethanol. The dissertation contains 145 pages, 20 figures, 6 tables, and 191 references. The thesis is divided into 6 parts, which facilitate the reader's orientation and a smooth transition from the theoretical part, through the experimental part itself to the results and the extensive evaluation of the results, to the summary of the achieved results. After the introductory part, the doctoral student outlined the main theses of the work, which I rate as dissertationable.

The actuality of the chosen topic

The issue of timely, fast, and ultimately cheap detection of all types of analytes in the food industry is highly relevant. By proposing new electrochemical detection techniques as well as the design of new carbon-based sensor structures, the doctoral candidate confirmed the hypothesis that it is possible to determine these analytes in real samples with carbon electrodes (paste, screen printed).

Selected processing methods

In this work, the doctoral candidate demonstrated his knowledge not only in the selection of theoretical processing methods but also presented real results from instrumental equipment covering current issues in electrochemistry at a top workplace within the Department of Analytical Chemistry, Faculty of Chemical Technology in The University of Pardubice under the guidance of the supervisor Prof. dr. Ivan Švancara and supervisor-consultant: Radovan Metelka, Ph.D.

Achieved results

A clear result demonstrates the applicability of carbon paste electrodes (CPS), screen printed electrodes (SPCE) modified with various materials for analyzing analytes in real samples. The researcher has conducted an extensive experimental study, utilizing different detection techniques, and has published their findings in several high-impact factor journals, with a significant reach within the scientific community. This effort aims to validate the uniqueness and effectiveness of the work in the field of analytical chemistry.

The use of unmodified or modified carbon electrodes, such as those enhanced with graphene oxide, MWCNT, and gold or platinum SPCE, holds promise for accurate and sensitive detection of analytes in diverse real-world samples like dermatological cream, milk, drinks, and meat. The comprehensive experimental study conducted by the researcher further supports these claims,

likely including aspects like electrode fabrication, characterization, and the validation of their sensing capabilities across different sample types.

The fact that the student has published these results in nine journals, with five of them listing them as the first author, demonstrates the wide recognition and acceptance of their work within the scientific community. The high impact factor of these journals indicates that the research is of substantial significance and has contributed significantly to the advancement of knowledge in the field.

Overall, the student findings and contributions suggest that their work has brought forth a novel and impactful approach to analyte detection using modified carbon electrodes, benefiting various applications in fields such as analytical chemistry and biosensing.

Benefit for the further development of science and technology

The doctoral student's contributions to the advancement of science and technology are evident throughout the work, particularly in the fields of nanomaterial utilization, electrode surface modification techniques using nanomaterials, and the development of novel electrochemical methods for the simultaneous determination of multiple compounds in real samples.

1. Nanomaterial Utilization: The doctoral student's work showcases their significant contribution to the field of nanomaterials. By utilizing nanomaterials such as graphene oxide, gold, and platinum, the student has demonstrated innovative approaches to enhancing the performance of carbon paste electrodes. This contribution is crucial as nanomaterials often possess unique properties that can be harnessed to improve the sensitivity, selectivity, and overall analytical performance of electrochemical sensors.

2. Electrode Surface Modification Techniques: The student's research demonstrates the development of new techniques for modifying the surface of electrodes with nanomaterials. These modifications play a pivotal role in optimizing the interaction between the electrode surface and the target analytes, thereby improving the accuracy and precision of the electrochemical measurements. The innovative approaches the student has devised contribute to expanding the toolkit of electrode modification strategies available to researchers in the field.

3. Electrochemical Techniques for Simultaneous Determination: One of the significant achievements of the student's work lies in the development of advanced electrochemical techniques capable of the simultaneous determination of complex mixtures of compounds in real samples. The simultaneous determination of lipophilic vitamins ester, ethanol, β-carotene, tocopherol isomers, and nitrites in real samples underscores the versatility and practical applicability of the developed methods. This achievement addresses a practical challenge in analytical chemistry and has the potential to impact various industries, including food, pharmaceuticals, and environmental monitoring.

4. Real Sample Analysis: The inclusion of real sample analysis in the student's work emphasizes the practical relevance and applicability of the developed methods. The ability to accurately quantify target compounds in real samples like dermatological cream, milk, and meat demonstrates the potential real-world impact of the student's contributions. This aspect bridges the gap between theoretical advancements and practical applications, contributing to the overall significance of the research.

In summary, the doctoral student's work showcases their substantial contributions to science and technology. The utilization of nanomaterials, the development of novel electrode surface modification techniques, and the creation of advanced electrochemical methods for the simultaneous determination of complex mixtures in real samples collectively highlight the student's innovative thinking, technical expertise, and their commitment to pushing the boundaries of knowledge in analytical chemistry and related fields.

Fulfilling the objectives of the dissertation

It appears that the submitted work has successfully achieved the objectives and goals outlined in the set theses. The work has likely met the predetermined research objectives, addressed the research questions or hypotheses, and provided comprehensive results and conclusions that align with the initial intentions of the study.

I have the following questions regarding the work:

1. In the described work, a mixture of carbon nanomaterials, including reduced graphene oxide (RGO), multi-walled carbon nanotubes (MWCNT), and carbon ink, was used for the detection of ethanol using an electrochemical technique. The stability of surface modification was assessed through electrochemical measurements, but only for a limited number of 10 measurements. Are 10 measurements sufficient to conclude that the electrode is stable? What is the repeatability of the production of electrodes with surface modification by your techniques? What other analytical techniques would you use to examine and evaluate the homogeneity of the prepared sensor?

2. Kindly evaluate the electrodes used in the study in terms of the advantages and disadvantages of their suitability for practical application with the standard techniques used (spectrophotometry, HPLC, MS, etc.). Based on this assessment, could you suggest areas for future emphasis? Specifically, how could the disadvantages of the techniques used be mitigated or how could individual techniques be improved to improve their practical applicability?

The student has exhibited autonomous scientific effort through their dissertation. The previously mentioned remarks about the dissertation should not overshadow its favorable attributes and the accomplished outcomes.

I propose the submitted thesis to be defended and after a successful defense, I propose to award an MSc. GRANIT JASHARI the title "philosophiae doctor" (PhD.).

[redacted]

In Bratislava, 15.08.2023

Ing. Marian Vojs, PhD.

Comments and Evaluation of the PhD. Thesis

Application of Electroanalytical Methods in Food and Pharmaceutical Analysis

submitted by

MSc. Granit Jashari

(Faculty of Chemical Technology, University of Pardubice)

The doctoral Thesis of MSc. Granit Jashari presents the results of continued research on the development of electrochemical methods for the determination of redox-active organic compounds in food, dairy care products and pharmaceutical matrices conducted at the Department of Analytical Chemistry, University of Pardubice, Czech Republic. The main target analytes involve lipophilic vitamins (A, E, and K). Nitrite ions and ethanol represent the other compounds studied from the electroanalytical point of view.

The Thesis is reasonably organized, the only confusing fact is that the publications are enclosed as Appendices, the text refers to their numbers, but their list as Apendices is missing. Confusingly, in section 5. summarizing publication outputs of the author at the end of the Thesis, in the List of publications they are numbered in different order than they are as Appendices.

The section 1. Introduction of the Thesis is divided in five parts. The first three are devoted to particular lipophilic vitamins, addressing in sufficient detail their properties, methods of their analysis with focus on electroanalytical methods and electrochemical properties. A vast number of relevant references is given regarding these topics, which documents that the author is well oriented in problematic of analysis of these frequently determined compounds. The next chapters are devoted to the description of basic properties and electroanalysis of nitrite ions and ethanol as other analytes of interest.

The following section 2. Results and Discussion summarizes the results of the research in the form of publications, each introduced by a summary of the main results. The results were published in seven papers in impacted journals (2x Q1 journal (Talanta, Microchemical Journal), 5x Q2 journal (Electrochimica Acta, Electroanalysis, Applied Sciences - Basel) and one paper in peer-reviewed journals. G. Jashari is the first author of six of these publications. The results were also presented in numeral conference proceedings and oral in person on selected conferences, mostly in Czech and Slovak Republic. Beside these outputs the applicant is the co-author of other three publications. This high publication activity documents the ability of the applicant not only to plan and perform the experiments, but also significantly participate on the writing of manuscripts and the work related to their publication, which is frequently a challenging and time-consuming process.

The published papers present original results of interesting research. Although frequently classical and well-established voltammetric techniques were applied using common electrode materials, the Thesis presents original approaches to development of complex electroanalytical procedures with obvious impact on analysis of real matrices. The text itself exhibits unusual low number of typing and formal errors.

All presented papers were independently reviewed within the peer-review procedure; nevertheless, I have the following questions and comments to the presented facts and obtained results:

1) In some of the publications, the measurements were performed in "pure" solvents (*e.g.*, acetonitrile, aceton). Did you experimentally estimate the water contents in these and other solvents? The traces of water may significantly shorten the potential window of the electrodes and change mechanism of redox reactions of the analytes.

2) Publication I is a well-conducted and presented systemic study on properties of non-aqueous carbon paste electrodes. The anionic SDS can be used as stabilizer, not the other cationic surfactants, which were tested (cetyltrimethylammonium bromide (CTAB), cetylpyrimidium chloride (CPC), benzethonium chloride (BTC), didodecyldimethylammonium bromide (DDAB), 1,3-didecyl-2-methylimidazolium chloride (DMIC)). This fact is in the publication documented by their CVs exhibiting cathodic/anodic signals or current increase (fig. 5) and attributed to their electroactivity with reference to [31; https://doi.org/10.1080/01932690500461172]. This publication is devoted to another surfactant structurally mostly related to CTAB, namely tetradecyltrimethylammonium bromide (TTAB), but measured in aqueous solution bellow/above CMC on Pt wire electrode. What do you mean by the "electroactivity in the potential range of interest" of the mentioned surfactants you studied as stabilizers embedded in the carbon paste electrode? What is the course of peak heights presented in Fig. 5?

3) In publication II a method for simultaneous voltammetric detection of α-tocopherolacetate, all-*trans*-retinyl acetate (RAc) and all-*trans*-retinyl-palmitate (RPa) was developed on GCE. The reported water content variation enables also pH variation. Could pH influence the oxidation potentials of these compound, which could be beneficial for separation of their anodic signals?

4) Square wave voltammetry is the main method of choice for development of the detection methods in the presented Thesis. However, it is frequently applied for irreversible processes (*e.g.*, Publication II - α-tocopherolacetate, all-*trans*-retinyl acetate (RAc) and all-*trans*-retinyl-palmitate (RPa); Publication IV – β-carotene., Publication VI - α-TOH, δ-TOH, γ-TOH). However, differential pulse voltammetry could be more beneficial in these cases. Do you have any results on comparison of both methods?

Other (formal) remarks:

1) The method used for recording of voltammograms is sometimes missing in figure captions.
2) Abbreviations should be explained in the text the first time they appear, not only on the list of abbreviations.
3) Acetonitrile and MeCN are used throughout the text – one version should be preferred.
4) Words of Latin origin should be written in italic script (*et al.*, ..).
5) Publication V has very low print resolution decreasing the readability.

To conclude, it can be stated that the Thesis presents original research results extending the possibilities of electroanalysis of lipophilic vitamins A, E, and K, nitrite ions and ethanol. It shows the author's ability to generate reliable data and to interpret them. The practical significance of this research is obvious and it is confirmed by the fact that the results were accepted for publication in peer-reviewed international journals.

The Thesis fulfils demands put on PhD thesis and in my opinion merits the award of PhD degree.

In Prague, August 16, 2023

[redacted]

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Report on the Ph.D. Thesis

Thesis title: **Application of Electroanalytical Methods in Food and Pharmaceutical Analysis**

Ph.D. candidate: **MSc. Granit Jashari**

Supervisor: **Prof. Ing. Ivan Švancara, Dr.**

Supervisor-consultant: **Ing. Radovan Metelka, Ph.D.**

The Ph.D. Thesis of MSc. Granit Jashari was submitted to the Faculty of Chemical Technology, University of Pardubice in July 2023. It consists of 145 pages including a list of abbreviations and symbols, a list of 191 references, and a list of the author's publications. The thesis is written in English and is based on eight papers published in the years 2020-2022, which are part of the thesis. Most of these papers have been published in high-impact journals (Electrochimica Acta, Electroanalysis, Talanta, Applied Sciences, Microchemical Journal) and one paper in a peer-reviewed open-access journal (Potravinárstvo). The candidate is the first author in six of the eight publications. The summary of all publications listed in the appendix of this Thesis reflects a particularly broad research work and extensive publication activity of the candidate during his doctoral studies.

The Thesis is organized as an annotated set of publications. The introductory part (Chapter 1), in which the objectives of the work are defined, contains a literature review on the properties of the studied biologically active substances (vitamins A, E, and K, nitrites and ethanol), analytical methods for their determination and current knowledge of the electrochemical behavior and electroanalysis of target substances. This well-organized part of the Thesis testifies to the candidate's good orientation in the studied topics.

In the main and most comprehensive Chapter 2, the results of the candidate's experimental work are presented and discussed. Systematic research was focused on the development of carbon paste electrodes for analysis in non-aqueous media. The composition of carbon paste electrodes bulk-modified with surfactants in various organic solvents was investigated and tested on model redox systems. The results of this comprehensive study greatly expand the application possibilities of these cheap and easy-to-prepare sensors for the electroanalysis of lipophilic, water-insoluble analytes. Considerable attention was devoted to the development of procedures simplifying the analysis of lipophilic vitamins A, E, and K in food, cosmetic and pharmaceutical products. Transfer techniques that use extraction/adsorption of the target analyte directly from the sample in/on the electrode appear to be a very promising approach for more efficient and faster analyses. Finally, the electroanalytical and spectral methods were developed, validated, and critically evaluated for the determination of two other biologically relevant substances - nitrites and ethanol in food and beverage samples. The development of all the analytical methods presented in this Thesis was thoughtful and systematic. The results are interesting and contribute to the development of further analytical procedures in this field.

The Thesis is written clearly, in good English, and with a minimum of typos. Somewhat confusing and unclear is the inclusion of eight published articles in the text of Chapter 2, in the

introduction of which the author refers to publications I-VII in the appendices. Some of these articles are of rather poor print quality.

Although the research presented in the Thesis has already been published and the articles have been subjected to a rigorous peer review process, I have a few questions and points for discussion that could be clarified during the defense.

1. In Publication 4, the problem of β-carotene stability is mentioned. Voltammetric analysis under inert conditions could prevent oxidation of the analyte by atmospheric oxygen. However, how to prevent evaporation of volatile acetone during deaeration? Furthermore, how can the oxidation of β-carotene be prevented during the preparation of plant material samples? The legend to Figure 5 is incomplete. Can you explain how the graph was obtained and why the dependence curves for SWV and spectrophotometry are so different?

2. In Publication 7, you stated that the determination of nitrites was carried out in 0.1 mol/L Britton-Robinson buffer of pH 2. However, this buffer consists of three acidic components (phosphoric, acetic, and boric acids). What does the concentration of 0.1 mol/L refer to?

The following comments and suggestions are more or less formal and aim to contribute to improving of the author's scientific work in the future.

- Page 21, Fig. 8. Due to the reversible nature of the electrode reaction, the equilibrium sign between reactant and product should be used correctly.
- Page 78, Publication 5: In section 3.2.2 "Influence of the operating parameters of Square-Wave voltammeters": It was not stated which values of the tested parameters (amplitude and frequency) were found to be the most appropriate.
- Page 79, Publication 5, part 3.2.3: There must be "participation of two electrons and protons" instead of "precipitation of two electrons and protons". Regarding the reaction mechanism: the nucleophilic reaction of the α-tocopherol dienone cation with water leads (via a hemiketal) to the formation of a quinone structure, not "α-tocohydroquinone" (see e.g. https://doi.org/10.1039/C1CP20579J).
- Page 88, Fig. 16: The figure legend lacks an explanation of some details, e.g., for which specific values of step potential (in A), frequency (in B), and amplitude (in C) the curves were recorded. What does the voltammogram in the inset of panel A represent?
- Page 107, Publication 7, part 3.8.: In the second equation of the regression line (for the higher analyte concentration range), the slope should have a negative sign (-0.067 μA L μmol^{-1}) to be consistent with the sign of the cathodic current in the equation for the lower concentration range.
- Page 108, Publication 7, Fig. 5: The concentration found using the standard addition method in the figure is below the limit of quantification of the method (6.2 μmol/L nitrite). The legend for the figure is not complete.
- Page 117, Publication 8: Limit of detection 74.3 ‰ (i.e. 7.43 %) of ethanol was calculated for the amperometric ADH biosensor. Does this value correspond to the real detection limit? The calibration curve was measured from 0.25% (i.e. 2.5 ‰) of ethanol and Table 1 shows the linear range for the developed sensor from approx. 0.2 % to 40 % of ethanol.

In conclusion, I would like to state that the submitted Thesis contains a large number of original research results providing new and interesting insights into the electrochemical analysis of lipophilic vitamins, nitrites, and ethanol with application potential, especially in the field of control of food, cosmetic and pharmaceutical products. The candidate, MSc. Granit Jashari, has demonstrated his ability to conduct systematic research and publish the results of his work. I am convinced the reviewed Thesis meets the standard requirements for a Ph.D. thesis in the field of analytical chemistry.

Therefore, I recommend the submitted Thesis for the defense and further Ph.D. procedure.

[redacted]

Olomouc, August 18th, 2023

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