Czech Academy of Sciences
J. Heyrovský Institute of Physical Chemistry, v. v. i.
Dolejškova 2155/3, 182 23 Prague 8, Czech Republic
VAT Nr. CZ61388955
phone: +420 26605 2011, e-mail: director@jh-inst.cas.cz

J.Heyrovský Institute of Physical Chemistry

REPORT ON THE THESIS

University	University of Pardubice
Faculty	Faculty of Chemical Technology
Department	Department of Analytical Chemistry
Specialization	Analytical Chemistry
Student	MSc. Amir Shaaban Farag Mohamed Nawar
Title	Electroanalytical methods in determination of selected biologically active compounds
Supervisor	Prof. Ing. Karel Vytřas, DrSc. Prof. Ing. Ivan Švancara, Dr.
Referee	Prof. Ing. Tomáš Navrátil, Ph.D.
	J. Heyrovsky Institute of Physical Chemistry of the Czech Academy of Sciences

The submitted Thesis was realized at Department of Analytical Chemistry, the University of Pardubice, under the supervision of prof. Ivan Švancara. It is written in English and consists of 118 pages (87 pages of the text and 4 appendixes - papers published in impact journals). The name "Farag AS" and the address "Pardubice" has been indexed in Web of Science (Claritive) 8 times (6 papers and 2 conference proceedings – all of them in the years 2018 - 2019). The papers were published in the following journals: Sensors (4th author, 8 times cited, IF 3.576 - Q2), Journal of Pharmaceutical and Biomedical Analysis (1st author, 4 times cited, IF 3.209 - Q2), Electroanalysis (1st author, 4 times cited, IF 2.544 - Q2/Q3), Talanta (1st author, 3 times cited, IF 5.329 - Q1), and Monatshefte für Chemie 2 times (2nd and 1st author, 4 times and 3 times cited, respectively, IF 1.349 - Q3)[1]. These papers were 28 times cited (27 times without self-citations) and the H-index 4 was reached. In the list of publication in the Thesis (Page 117), only five publications are listed (the paper in Sensors is missing).

The Thesis is divided into the following chapters: Summary, List of abbreviations and symbols, Introduction: Targets of dissertation, Chapters 1-4. (Voltammetric determination of ethylvanillin and methylvanillin; Voltammetric determination of caffeine and vitamin B6; Voltammetric determination of taurine after derivatization reaction; and Voltammetric determination of propafenone in pharmaceutical dosage form), Summary of presented work and Appendixes I-IV, and Conclusions. Each of Chapters 1-4 is composed from theoretical part, biological significance of analytes and their electrochemical activities, theory of used electrodes, and is completed by the list of used literature (references).

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[1] The used quartiles are based on Web of Science (Claritive) data, which in the case of chemical journals differ significantly from Qais-based quartiles recalculated in an unknown way and used by the Czech Academy of Sciences.

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The review of this work is partly facilitated because this Thesis is based on several articles published in impact factor journals and each of them was therefore reviewed by two or three (foreign) reviewers and most of the possible problems should be (I was convinced) fixed or clarified.
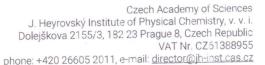
Overall, chapters 1-4 provide readers detailed theoretical information about molecules investigated, their chemical properties, and methods used (about 70 pages). On the other hand, the results achieved by the author were summarized and commented on 10 pages only. I would appreciate a reverse ratio. The theoretical part is too extensive and not so important for a reader, while the result part is too brief.

I have a few questions and comments, which should be discussed during the Thesis defense:

1) Would it be possible to determine the oxidative products caffeine, i.e., paraxanthine, theobromine, and theophylline using the developed electrochemical method? Would it be possible to differentiate these individual metabolites?

2) Page 59, line 31: Voltammetry was not developed by prof. Heyrovsky. Could the author explain the difference between of polarography and voltammetry? Does the author know, when polarography and voltammetry were invented?

3) Page 76, figure 11: In the case of EVA, the couple of peaks at about +0.2 V can be seen. However, these peaks are missing in the case of MVA. Could not be these signals used to recognize the MVA from EVA using this voltammetric method?

4) Is the application of more complicated electrode surface modifications reasonable? (NH_2fMWCNTs/GCE vs. NH_2fMWCNTs/GCE/AgNPs vs. NH_2fMWCNTs/GCE/QD – considering preparation time, regeneration, stability, reproducibility, differences in yielded results, etc.).

5) In all attached papers, there are determined concentrations of selected analytes in foodstuffs, drinks, or in pharmaceutical dosage forms, i.e., in relatively simple or not so complex matrices. Would it be possible to apply the developed methods and constructed electrodes to determine the studied analytes and their metabolites in more complicated matrices as body fluids etc.?
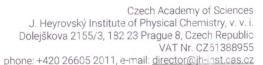
Other comments:

- Page 60, line 5-8: It is strange that in the sentence "The commonly used electrodes for voltammetric methods" mercury and amalgam electrodes are missing!!!

- Page 77, Page 79, Page 83, etc.: calibration curves:

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- o Confidence intervals of slopes end intercepts are missing.
- o Are the intercepts statistically significant (on chosen level of significance, e.g. $\alpha=0.05$)?
- o Was the number of given significant digits tested?

- Fig. 11 vs. Fig. 13: The ratio of slopes of MVA to EVA is about 6, however, according to the Fig. 11, the ratio of peak heights of MVA and EVA of equal concentrations is about 2. How to explain it?

- Page 78, line 8: "Satisfactory obtained results" – what does it mean?

- Page 79, Fig. 15: How the peak heights were evaluated in the case of both peaks (from the base line, from the onset to the end, zero level)?

- Page 79, Fig. 15: *"In presented work, experiments based on GCE/Nafion® modification for simultaneous determination of CA and VB6 have provided only one oxidation peak in 0.1 M BRB of pH 4.5 at peak potential values +0.882 and +1.349 V vs. ref., respectively (Fog.14)."* The peak potentials are incorrect. According to inset in Figure 15 and in correspondence with Fig. 1 in *"Farag A. S., Pravcová K., Česlová L., Vytřas K., Sýs M. (2019). Simultaneous Determination of Caffeine and Pyridoxine in Energy Drinks using Differential Pulse Voltammetry at Glassy Carbon Electrode Modified with Nafion®. Electroanalysis, 31, 1494–1499."*, the peak potentials of VB6 is about +0.8 V and of caffeine about 1.4 V. Nevertheless, the same mistake was published in this paper, page 1496, last three rows.

- What was the positive contribution of the manuscript *"Farag A. S., Pravcová K., Česlová L., Vytřas K., Sýs M. (2019). Simultaneous Determination of Caffeine and Pyridoxine in Energy Drinks using Differential Pulse Voltammetry at Glassy Carbon Electrode Modified with Nafion®. Electroanalysis, 31, 1494–1499."* if compared *LODs* for CA (367.4 µg/100 ml of drink in this paper with 0.8 µg/100 ml of drink in ref. [18] and linear ranges 63.1-600 µM in this Thesis vs. 0.1-7 µM in reference [18]) and for VB6 (37.9 µg/100 ml of drink in this Thesis with 1.7 µg/100 ml of drink in [31] and linear ranges 7.5-200 µM in this Thesis vs. 0.3-200 µM in reference [31])?

- Page 82: Confidence interval (on chosen level of significance, e.g. $\alpha=0.05$) should be used instead of the standard deviation.

- Page 84, Fig. 21: The calibration lines are confusing. The last point of the calibration line from 1 to 10 µM, which corresponds to 10 µM, exhibits the same height as the first point of the other calibration line, which corresponds to 20 µM. There is some error or it must

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be explained. The same problem I see in the Fig. 6 and in the corresponding equations (10) and (11) in the paper: *"Farag A. S., Bakirhan N. K., Švancara I., Ozkan S. A. **(2019)**. A new sensing platform based on $NH_2fMWCNTs$ for the determination of antiarrhythmic drug propafenone in pharmaceutical dosage forms. Journal of Pharmaceutical and Biomedical Analysis, 174, 534–540. "*

- *"Farag A. S., Klikarová J., Česlová L., Vytřas K., Sýs M. **(2019)**. Voltammetric determination of taurine in energy drinks after o-phthalaldehyde-ethanethiol derivatization. Talanta, 202, 486–493."*, Table 2:

  o Confidence intervals in the case of one-dimensional set of data (i.e., repeated analysis of the same sample) are calculated as: s.t(α, n-1)/sqrt(n) (if we suppose normal data distribution) (e.g. http://www.stat.yale.edu/Courses/1997-98/101/confint.htm or M. Meloun, J. Militký : Kompendium statistického zpracování dat, Metody a řešené úlohy, Academia Praha 2006, 985 stran, ISBN 80-200-1396-2, 3. vydání v nakladatelství Karolinum Praha 2013, ISBN 978-80-246-2196-8.)

  o It is necessary to consolidate the number of significant digits in results and in statistical uncertainty. It is usual to regulate the number of significant digits according to the corresponding confidence interval (usually) to 2 significant digits (see [Miller, J. N.; Miller, J. C., Statistics and Chemometrics for Analytical Chemistry. 2nd ed.; Pearson Education: Harlow, 2005.] or [Meloun, M.; Militky, J.; Forina, M., Chemometrics for Analytical Chemistry, Volume 1: PC-Aided Statistical Data Analysis, Volume 2: PC-Aided Regression and Related Methods. Ellis Horwood: Chichester, 1992; p 175.]).

  o Statistical comparisons using two sample t-tests (Equal sample sizes and variance, Equal or unequal sample sizes, similar variances or Equal or unequal sample sizes, unequal variances) or ANOVA test or t-test for paired samples and accepted conclusions are missing.

- The same errors and drawbacks can be found in *"Simultaneous Determination of Caffeine and Pyridoxine in Energy Drinks using Differential Pulse Voltammetry at Glassy Carbon Electrode Modified with Nafion®. Electroanalysis, 31, 1494–1499."*, Table 2.

- *Farag A. S., Sýs M., Hájek T., Vytřas K. **(2018)**. Voltammetric determination of ethylvanillin and methylvanillin sum at carbon paste electrode modified by sodium dodecyl sulfate in selected foodstuffs. Monatshefte für Chemie, 149, 1945–1953.*, Fig. 4:

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If there is no physical/chemical model of the investigated dependence, it is necessary to use lines to connect individual pairs of points.

Very minor comments:

- It is a bit misleading to express some results in mg/l and at the same time in µmol/l (*LOD* vs. analyte contents in samples) (in the case of most of analytes).

- In the whole text: The different possibilities of unit expressions are used: e.g. mol L^{-1} (Page 81), µM (Page 79), *mmol L^{1}* (Page 81), similarly mV and V (Page 81), etc.

- It is hardly believable that the authors confirmed "proofs" of the attached article in Journal of Pharmaceutical and Biomedical Analysis (e.g., page 538, there are many missing spaces, wrong formatted equations).

- Page 11, Fig. 4: Schematic illustrations for anionic and for zwitterionic surfactants are identical.

- Page 64, line 11: It should be "Electronic properties" instead of "Electronice properties". Nevertheless, correctly "Electrical" should be used. Moreover, line 12: "electrical conductivity" should be used instead of "electronic conductivity".

- Page 76, line 23, Legend for figure: Why two different mathematical forms of concentration units are used in the same line: „...5.0 × 10^{4} M MVA (dashed line), and 0.5 mM EVA..."

- Page 76, line 23, Legend for figure: I suppose that the concentration of MVA was "5.0 × 10^{-4} and not „...5.0 × 10^{4} M ..."

- Page 76: The abbreviation SDS was firstly used and introduced a few lines later (lines 3 vs. 29).

- Page 79, Fig. 15: Buffer and its pH value are missing.

- Page 85: The abbreviation $NH_2fMWCNT$ was not introduced in the text.

If I can judge, the submitted Thesis is transparently written. I appreciate the complexity of the work. It summarizes huge amount of theoretical and experimental work realized by the author (probably in close cooperation with his research team). Beside his own analytical work, the author reviewed in detail the electroanalytical techniques, the studied compounds, their importance environment, etc.

It is necessary to note that some parts of this thesis were published in impact journals. Therefore, it is no doubt about the high value and quality of the achieved results. The attached

comments, questions, and revealed errors should help to improve author's papers and outputs in future.

According to my opinion, it can be concluded that MSc. Farag proved in the Thesis his ability to solve scientific tasks, both theoretically and experimentally. All remarks, comments, and reminders can be only formal. I did not find any serious error which would prohibit the acceptation of this Thesis for awarding the doctor degree.

The presented Thesis brings new results and enlarges scientific knowledge in the chosen area. Quality of results is stressed by the acceptation and publication in high standard scientific journals. The presented Thesis fulfils all expected requirements. Therefore, according to my opinion, based on the submitted thesis, I recommend this thesis to be accepted and in the case of its successful defense, to award to MSc. Amir Shaaban Farag Mohamed Nawar the PhD. degree (Doctor of Philosophy).

Prague, August 12th, 2021

Prof. Ing. Tomas Navratil, PhD.
Dpt. of Electrochemistry at the Nanoscale
J. Heyrovsky Institute of Physical Chemistry of the
Czech Academy of Sciences
Dolejškova 3
182 23 Prague 8
Czech Republic

Report on the thesis

Thesis title: Electroanalytical methods in determination of selected biologically active compounds

PhD candidate: MSc. Amir Shaaban Farag Mohamed Nawar

Supervisor: Prof. Ing. Ivan Švancara, Dr.

Referee: RNDr. Jana Skopalová, Ph.D.

The thesis of MSc. Amir Shaaban Farag Mohamed Nawar submitted in July 2021 to Faculty of Chemical Engineering, University of Pardubice for the Ph.D. degree consists of 118 pages including list of abbreviations and symbols, lists of references and four appendixes. The thesis, written in English, is based on four papers (in Appendixes I-IV) published in the years 2018-2019 in journals with an impact factor: Monatshefte für Chemie, Electroanalysis, Talanta, and Journal of Pharmaceutical and Biomedical Analysis. The candidate is the first author in all four publications.

The targets of the thesis are presented in a concise introduction. The thesis is divided into five chapters. In the first chapter, devoted to the voltammetric determination of ethylvanillin and methylvanillin, the author summarizes the literature on the biological and electrochemical activity of vanillin and its synthetic derivative and pays attention to different types of surfactants and their use for the preparation of modified electrodes. The second chapter deals with the biological activity and electrochemical oxidation of caffeine and pyridoxine, and contains a literature review on the use of sulfonated tetrafluoroethylene based copolymer Nafion in electrochemistry. In the third chapter, the author deals with the voltammetric determination of amino acid taurine, its importance and biological activity, negative effects of some components of energy drinks and describes the use of derivatization in electrochemistry and other analytical techniques. Chapter 4 presents a theoretical introduction to voltammetric analysis of drugs, especially propafenone, and summarizes current knowledge of carbon nanotubes as electrode modifiers. Chapters 1-4 present a rich theoretical introduction summarizing current knowledge of studied biologically active substances, electrochemical sensors and modern approaches in electroanalysis. Some parts of these chapters containing basic information, such as carbon forms or types of surfactants, could serve as basic study literature for chemistry undergraduates. Chapter 5 is essential because it summarizes the results of the author's experimental work. It is written on 11 pages, incl. references, which is unfortunately somewhat disproportionate to the previous 70-page theoretical part (Chapters 1-4). In the one-page conclusion, the author briefly summarizes the results of his research work and its analytical significance.

The following comments on the thesis and questions are divided according to specific research topics. **Questions and comments written in bold should be discussed during the defense of the thesis.** The other comments below should help the author improve his scientific work in the future.

<u>Electroanalysis of vanillin</u>

1. **Modification of the working electrode surface with a surfactant seems to be elegant solution of passivation of carbon electrode surfaces caused by electrochemical reaction products. From a practical point of view, I would like to know, how and how often it is necessary to renew the SDS protective layer on the electrode, e.g. when analyzing one sample by the standard addition method.**

2. The calculated *LOD* and *LOQ* values need to be critically evaluated and, preferably, verified experimentally by measuring the signal for the calculated *LOD* and *LOQ* concentrations.

Especially if the calculated *LOD* is two orders of magnitude lower than the lowest concentration in the calibration series.

3. Page 77: The current and concentration units are not stated for the regression line equations. Furthermore, according to the slope values of regression lines, the sensor response is about 5-6 times more sensitive to methylvanillin (MVA) than to ethylvanillin (EVA). This must be considered when quantifying two substances in a mixture and expressing the results by the amount of one of them.

4. Page 78: It is written here: "Quantification of EVA and MVA sum in the foodstuffs samples was carried out by standard addition method due to low and <u>negative</u> intercept value...". For the use of the standard addition method, it is irrelevant whether the intercept of the calibration line has a positive or negative value. The intercept must be <u>statistically insignificant</u>. Then it can be omitted.

5. Appendix I, Table 1: Extremely low values $1 \times 10^{-5} - 8.2 \times 10^{-5}$ µmol/L of concentration range and LOD 2×10^{-6} µmol/L for Pd/PAF-6 sensor are most likely incorrect (10^{-5} µmol/L = 10^{-11} mol/L).

Electroanalysis of caffeine and vitamin B6

The advantages of Nafion as an electrode modifier for electroanalysis of positively charged substances have been known for a long time (as also reviewed in this thesis, pages 27-29). In presented work, these advantages were appropriately used for the simultaneous voltammetric determination of caffeine and pyridoxine in their mixture with the application potential in controlling the composition of energy drinks containing these two substances.

6. **Page 99: The effect of Nafion / GCE modification on the electrochemical behavior of caffeine and pyridoxine should be shown in Figure S1 (Appendix II). However, I did my best to find this figure on the Electroanalysis website, but unsuccessfully (as well as another fig. S2-S8). Could the author show voltammograms of the mixture of these substances on GCE and on GCE / Nafion electrodes?**

7. Page 78, Fig. 14: There is an error in the peak description – a peak at potential of 0.88 V belongs to pyridoxine and a peak at 1.4 V to caffeine.

Electroanalysis of taurine

This part of the work describes a newly developed method for voltammetric determination of the electroinactive amino acid taurine after its reaction with *o*-phthalaldehyde and ethanthiol providing an electroactive derivative of isoindole. This elegant derivatization reaction inspired by sample preparation procedures for analysis by spectral methods opens up the possibility of electrochemical analysis of a wide range of other electro-inactive amino acids.

8. Page 81: The abbreviation PITC (for phenyl isothiocyanate) is not explained in the text or in the List of abbreviations and symbols.

Electroanalysis of propafenone

The last topic of research presented in the thesis deals with the development of a voltammetric method for the determination of the antiarrhythmic drug propafenone (PPF) in a pharmaceutical dosage form using a GCE modified with amino-functionalized multiwalled carbon nanotubes. Electrochemical oxidation of the drug was described and used analytically for the first time. It would be interesting to build on the results and develop a method for the analysis of the major metabolite of PPF, i.e., 5-

hydroxypropafenone, phenolic structure of which suggests a different electrochemical behavior and opens the possibility to determine both substances simultaneously.

I consider this part of the thesis to be the most problematic, because it contains the greatest number of formal and factual shortcomings, which unfortunately also appear in the published article (Appendix IV).

9. Page 85, Fig. 22: There is a mistake in the reaction scheme. Symbols "-2e⁻, -2H⁺" must be above the upper arrow.

10. **Appendix IV, page 113, Fig. 3: Why is the peak of the cyan curve for pH 8 shifted to less positive potentials (E_p about 0.85 V), while the peak potentials at pH 7, 9 and 10 are almost constant at about 1 V? What was the course of the E_p – pH dependence?**

11. Page 114, paragraph 3.4.: The statement that "oxidation peak remarkably increased with slowing down the scan rate from 500 to 5 mV/s" does not correspond to the mentioned regression equations (5) and (6) in which the slope values are positive.

12. **Page 114, Fig. 5: Is there any explanation for the significant decrease in voltammetric peak current after PPF accumulation for 5 and 10 s compared to t_{acc} 0 s?**

13. Page 85, Fig. 21 and page 114, Fig. 6: There is no description of concentrations for individual DPAdV curves. The increments of higher concentrations obviously do not correspond to the points of the calibration curve in the inset. The peak current value obtained in 100 µM PPF solution for accumulation time 25 s was around 19 µA (Fig. 5) whereas current around 8 µA corresponds to the same concentration of PPF in calibration curve (Fig. 6).

14. Page 115, Table 1: Readers would appreciate an explanation of some of the statistical parameters (e.g. "percentage error (Er %)") and how they were calculated.

The thesis is written clearly, in good English. A more careful check of the final version could have prevented some typing errors (e.g., p. 13, fig. 4: there is the same scheme for anionic and a zwitterionic surfactant; p. 64: "electronice properties" instead of "electronic" or better in this case "electrical"; p. 76, capture for Fig. 11: "5.0 × 10⁴ M MVA" instead of "5.0 × 10⁻⁴ M MVA"; p. 84, the confused sentence: "However, the best and sensitive peak was obtained in the case of using of NH₂fMWCNTs /GCE; therefore, it was used as the most effective electrode modification for as the most effective electrode modification for building up a novel voltammetric method for its determination in pharmaceutical dosage form without any separation, evaporation, or otherwise difficult sample handling.further investigations (Fig. 20)."; etc.).

Let me conclude this report by stating that I do appreciate the large number of presented results of time-consuming experimental work. The thesis brings new and interesting knowledge of the use of voltammetric methods for the analysis of biologically active substances with application potential especially in the food industry and pharmacy. The above comments and suggestions aim to contribute to improve the quality of the author's scientific work in future.

In my opinion, the author has demonstrated his ability to work creatively in the field of research and I am convinced the thesis refereed meets the standard requirements for doctoral theses in the field of analytical chemistry. Therefore, I recommend it for the defense and further Ph.D. procedure.

Olomouc, August 11, 2021

RNDr. Jana Skopalová, Ph.D.
Department of Analytical Chemistry
Faculty of Science
Palacký University Olomouc