NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY POLITEHNICA BUCHAREST

FACULTY OF CHEMICAL ENGINEERING AND BIOTECHNOLOGIES DOCTORAL SCHOOL

DOCTORAL THESIS

RAPID SCREENING TESTS FOR THE EVALUATION OF THE QUALITY OF SEMISOLID PHARMACEUTICAL DOSAGE FORMS

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The doctoral thesis entitled “Rapid screening tests for the evaluation of the quality of semisolid pharmaceutical dosage forms” has an extension of 176 pages, includes 12 published articles, presents 56 figures and 39 tables and cites 78 bibliographic references. The content of the thesis with original numbering of the pages is:

Keywords: sensor, pharmaceutical analysis, semisolid dosage forms

1. Introduction

Electrochemical sensors developed for pharmaceutical analysis are of significant regulatory relevance. Complying with strict regulatory standards is essential in the pharmaceutical industry [34, 35]. As regulatory authorities, the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) prioritize the requirement for accurate and reliable analytical techniques to guarantee the safety and effectiveness of pharmaceuticals. Electrochemical sensors satisfy these requirements due to their sensitive and selective response, even in complex sample matrices. Employing these sensors ensures accuracy, reproducibility, and other essential analytical parameters, hence contributing to robust quality control processes. Pharmaceutical manufacturers can implement validated electrochemical sensor-based techniques to provide the integrity of drug production and quality control operations, enabling them to meet regulatory requirements successfully.

Another emerging concern is the environmental impact of pharmaceutical manufacturing, utilization, and disposal. Industrial, hospital, and household discharges may lead to water contamination, ecotoxicity, and the emergence of antibiotic resistance, while agricultural runoff and improper disposal worsen the problem [38–40]. Eco-friendly alternatives and regulations are needed to mitigate these environmental risks. Continuous drug monitoring is crucial for protecting aquatic ecosystems and human health. By focusing on drugs with a significant risk, and covering various locations and periods to evaluate variations, it aims to identify pollution sources, evaluate ecological risks, ensure regulatory compliance, and provide information to authorities to support adaptive management [50–52].

To address the increasing need for accurate analytical methods, this thesis proposes employing electrochemical sensors to determine topical drugs in both pharmaceutical and water samples. Due to their cost-efficiency, high selectivity, and sensitivity, electrochemical sensors can accurately detect and measure topical drugs even at trace-level concentrations, thus guaranteeing the safety and effectiveness of pharmaceutical products and preserving water quality [76–78]. The works presented in this thesis not only tackle the existing analytical challenges but also pave the way for significant progress in the field of electrochemical sensing, thereby contributing to higher-quality healthcare and a sustainable environment.

This thesis comprises literature studies that aim to identify current trends in determining drugs used in topical formulations, as well as a series of electrochemical sensors proposed for quantifying these drugs.

Accordingly, papers I-IV are review articles focused on the analytical performances of the latest electrochemical sensors proposed for determining various drugs commonly used in topical treatments.

Papers V-XII describe original works as following: paper V describes disposable sensors based on copper, carbon, and copper-carbon nanostructures deposited on paper substrates to determine three non-steroidal anti-inflammatory drugs simultaneously; paper VI presents an N-methylfulleropyrrolidine-based multimode sensor proposed for the determination of an antifungal azole, butoconazole; paper VII also introduces a multimode sensor integrated into a sensing platform used to quantify methylprednisolone, a glucocorticoid; paper VIII describes a voltammetric carbon paste electrode based on ZnO developed for the determination of betamethasone in real samples; paper IX explores the use of a calix arene and TiO_2 modified carbon paste electrode for the determination of nonivamide, a derivative of capsaicin, and papers X-XII are focused on a series of chemically modified screen-printed sensors utilized for investigating the simultaneous determination of betamethasone, a glucocorticoid, and other drugs such as vitamin D derivative calcipotriol (Paper X), and antibiotics fusidic acid (Paper XI) and gentamicin (Paper XII).

The newly developed sensors and platforms are highly reliable and can be used for purity tests, uniformity content tests, and for the assay of active compounds in surface water, given the fact that they can become pollutants with high risk for the population.

6. General conclusions

6.1. General conclusions

This thesis aims to develop and explore several electrochemical sensors that can be utilized to determine some drug molecules frequently found in topical dosage forms. Moreover, the sensors were applied to pharmaceutical and water real samples to study the effect of interfering molecules and the influence of the sample matrix. In addition to the need for robust, reliable, selective, and sensitive analysis methods in pharmaceutical quality control, the literature also points out the harmful effects caused by most of the examined molecules on the aquatic ecosystem. Therefore, the applicability of the proposed sensors was also assessed using real water samples collected from local rivers close to hospitals, where drug usage is more intense compared to other areas.

The first section of this thesis consists of a comprehensive literature study structured as review articles. These works explore and discuss various categories of electrochemical sensors. The evaluation of these methods considers their analytical performance, their applicability to real samples, as well as the costs associated with their construction, and other relevant factors. The literature study supports the experimental section by offering extensive insights into the field of electrochemical sensors for pharmaceutical molecules.

Three novel disposable stochastic sensors were obtained by modifying with maltodextrin nanostructured copper, carbon, and carbon-copper composite deposited on copy paper substrate. The sensors were employed to determine three NSAIDs (ibuprofen, ketoprofen, and flurbiprofen) and have successfully demonstrated their suitability for purity assessment and uniformity content testing.

A sensor based on a carbon paste electrode modified with a fulleropyrrolidine that can be utilized in two electrochemical methods (multimode sensor) was developed and applied to determine butoconazole. Furthermore, the electrooxidation mechanism of butoconazole was proposed for the first time, and its behavior at the working electrode was studied. The sensor exhibited very low limits of determination and high sensitivities in both modes while demonstrating significant reliability when employed in pharmaceutical samples. The sensor's features include its use in the pharmaceutical industry for quality control and uniformity content assay of topical formulations.

A valinomycin modified carbon paste electrode integrated into a multimode platform was developed and explored to identify and quantify methylprednisolone. The applicability of the platform was tested on pharmaceutical and water real samples, presenting accurate results that render it suitable for quality control of bulk methylprednisolone, uniformity content assay of dosage forms, and water quality monitoring. Moreover, the proposed platform may be utilized for on-site analysis and real-time data collection due to its portability and smartphone compatibility.

A novel stochastic sensor integrated into a detection platform was designed for the quantitative and qualitative analysis of nonivamide. The sensor was constructed by modifying a carbon paste electrode with calix[6]arene and TiO_2. The main feature of the proposed platform is its extensive on-site applications in the pharmaceutical industry (quality control of nonivamide and ensuring the content uniformity of topical dosage forms containing nonivamide) and water monitoring.

A multi-walled carbon nanotubes-gold nanoparticles screen-printed electrode was modified with calix[6]arene to develop a disposable stochastic sensing platform to simultaneously determine calcipotriol and betamethasone. The proposed platform achieved low quantification limits, wide

dynamic ranges, and high sensitivities. Accordingly, this platform is a sensitive, selective, robust, and reliable tool that may be useful for on-site pharmaceutical and water analysis.

Another disposable sensor developed within this thesis is based on a calix[4]arene modified polyaniline screen-printed carbon electrode. The sensor was integrated into a platform and tested for the simultaneous identification and determination of fusidic acid and betamethasone. Following the evaluation of the response characteristics and the platform's application on samples of cream and water, it was demonstrated that the platform is advantageous to performing rapid, on-site analysis suited for different types of industries or agencies.

Lastly, a boron-doped diamond electrode substrate was modified with a type of calix[4]arene to design a stochastic sensing platform for the simultaneous determination of betamethasone and gentamicin. The platform allows for on-site quality control of pharmaceutical goods and testing of betamethasone and gentamicin levels in water samples with minimal sample processing. Due to its cost-effectiveness and analytical performance, it has the potential to be preferred for rapid analysis.

Furthermore, all the previously introduced disposable sensors possess the benefit of preventing cross-contamination of samples from one another.

Therefore, all the electrochemical sensors proposed in this thesis exhibit numerous advantages compared to standard methods of analysis, as well as other electrochemical sensors. Hence, they serve as a more efficient option for the analysis of selected drug molecules, overall.

6.2. Original contributions

This thesis focuses on the development of stochastic, voltammetric, and multimode sensors of various types for the qualitative and quantitative analysis of drugs frequently found in topical dosage forms. The design of each sensor was customized to enhance specific characteristics, such as conductivity, selectivity, and sensitivity. Subsequently, they were studied to assess analytical parameters such as dynamic range, limit of detection or quantification, sensitivity, stability, accuracy, and other relevant factors. Moreover, in certain cases, interference studies were conducted. The sensors' applicability was determined using real samples of pharmaceutical products and water.

To provide a clear understanding of the results obtained using the proposed sensors, the calibration curve parameters were compared to those of other electrochemical sensors or other analytical methods identified in the literature, if available.

Throughout this thesis, multiple experiments lead to the development of novel analytical approaches. For example, for butoconazole, the electrochemical determination was studied for the first time, and its electrooxidation mechanism was also proposed. Furthermore, the simultaneous electrochemical determinations of betamethasone with calcipotriol, fusidic acid, and gentamicin were presented for the first time. All of these works contribute to the progress of knowledge and provide new opportunities for future exploration.

6.3. Future perspectives

Further extensive validation will be conducted for the proposed sensors to achieve national or international recognition and enable large-scale production. Moreover, the simultaneous determination of multiple drug molecules will be thoroughly investigated, potentially by adding new analytes. Thus, it is possible to further decrease costs and analysis duration.

Based on findings in literature studies, it has been revealed that the selected substances are present not only in pharmaceutical and water samples but also in other types of samples such as food

(vegetables and animal products), soil, and aquatic life. These types of samples will be included in future studies.

Appendix 1

PARTICIPATION TO NATIONAL AND INTERNATIONAL CONFERENCES

1. **Bianca-Maria Tuchiu**, Raluca-Ioana Stefan-van Staden, Jacobus Frederick van Staden, PS3-52. Calix[6]arene and TiO_2 modified reduced graphene oxide electrode-based portable stochastic platform for the determination of nonivamide from topical pharmaceutical dosage forms and water samples, Euroanalysis XXI, p. 327, Geneva, Switzerland, 27-31 August, 2023. (POSTER)
2. **Bianca-Maria Tuchiu**, Raluca-Ioana Stefan-van Staden, Jacobus Frederick van Staden, PS2-44. On-site simultaneous determination of calcipotriol and betamethasone in topical pharmaceutical formulations and surface water samples using an intelligent mini platform based on carbon nanotubes-gold nanoparticles screen-printed electrode modified with calix[6]arene, Euroanalysis XXI, p. 271-272, Geneva, Switzerland, 27-31 August, 2023. (POSTER)
3. Ruxandra-Maria Ilie-Mihai, Raluca-Ioana Stefan-van Staden, Alexandru Adrian Bratei, Damaris-Cristina Gheorghe, **Bianca-Maria Tuchiu**, OP9-5-3. DNA Mismatch Repair Assessment in Gastric and Colon Cancers Using Stochastic Microdisks, Euroanalysis XXI, Geneva, Switzerland, 27-31 August, 2023. (ORAL PRESENTATION)

Appendix 2

ARTICLES PUBLISHED IN ISI JOURNALS

Total IF = 40.2

REVIEW ARTICLES

IF = 10.9

The literature study section of this thesis is based on the following review articles:

I. **Țuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Recent Trends in Ibuprofen and Ketoprofen Electrochemical Quantification – A Review. Crit. Rev. Anal. Chem. **2024**, 54(1), 61–72. DOI: https://doi.org/10.1080/10408347.2022.2050348. **IF=5.0**

II. **Țuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Review—Electrochemical Sensors Used for the Determination of Some Antifungal Azoles. ECS Sens. Plus **2022**, 1(3), 030601. DOI: https://doi.org/10.1149/2754-2726/ac88e3.

III. **Țuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Mini-Review: Recent Innovations in Corticosteroid Determination Using Electrochemical Sensors. Anal. Lett. **2024**, 57(4), 665–680. DOI: https://doi.org/10.1080/00032719.2023.2220847. **IF=2.0**

IV. **Țuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Review—Novel Trends in the Determination of Pharmaceutical Compounds Commonly Found in Topical Treatments using Electrochemical Sensing Approaches. J. Electrochem. Soc. **2024**, 171, 047502. DOI: https://doi.org/10.1149/1945-7111/ad3a1e. **IF=3.9**

ORIGINAL ARTICLES

IF = 29.3

The experimental section of this thesis is based on the following original articles:

V. **Țuchiu, B.-M.**; Stefan-van Staden, R.-I.; Bădulescu, M.; van Staden, J.F. Disposable stochastic sensors for fast analysis of ibuprofen, ketoprofen, and flurbiprofen in their topical pharmaceutical formulations. J. Pharm. Biomed. Anal. **2022**, 215, 114758. DOI: https://doi.org/10.1016/j.jpba.2022.114758. **IF=3.4**

VI. **Țuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F.; Aboul-Enein, H.Y. N-Methylfulleropyrrolidine-Based Multimode Sensor for Determination of Butoconazole Nitrate. ACS Omega **2022**, 7(46), 42537–42544. DOI: https://doi.org/10.1021/acsomega.2c05904. **IF=4.1**

VII. Stefan-van Staden, R.-I.; **Tuchiu, B.-M.**; van Staden, J.F.; Sfirloaga, P. Multimode Detection Platform Based on 3D Integrated Sensor for Fast On-Site Assay of Methylprednisolone in Its Pharmaceutical Formulation and Surface Water Samples. J. Electrochem. Soc. **2023**, 170, 037516. DOI: https://doi.org/10.1149/1945-7111/acc42d. **IF=3.9**

VIII. **Tuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Sensitive Electrochemical Determination of Betamethasone in Daivobet and in Water Samples. U.P.B. Sci. Bull Series B. **2023**, 85(4), 169–176. **IF=0.5**

IX. **Tuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Stochastic platform based on calix[6]arene and TiO_2-modified reduced graphene oxide electrode for on-site determination of nonivamide in pharmaceutical and water samples. RSC Adv. **2023**, 13(26), 17628–17632. DOI: https://doi.org/10.1039/D3RA02363J. **IF=3.9**

X. **Tuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F.; Aboul-Enein, H.Y. Disposable Stochastic Platform for the Simultaneous Determination of Calcipotriol and Betamethasone in Pharmaceutical and Surface Water Samples. Chemosensors **2023**, 11(8), 446. DOI: https://doi.org/10.3390/chemosensors11080446. **IF=4.2**

XI. **Tuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Fast on-site simultaneous electroanalysis of fusidic acid and betamethasone in pharmaceuticals and water samples using novel stochastic platform. Electrochem. Commun. **2023**, 157, 107625. DOI: https://doi.org/10.1016/j.elecom.2023.107625. **IF=5.4**

XII. **Tuchiu, B.-M.**; Stefan-van Staden, R.-I.; van Staden, J.F. Intelligent miniplatform for on-site monitoring of water samples and pharmaceutical production of ointments based on betamethasone and gentamicin as active pharmaceutical ingredients. J. Electrochem. Soc. **2024**, 171(6), 067503. DOI: https://doi.org/10.1149/1945-7111/ad537e. **IF=3.9**

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