DOCTORAL THESIS SUMMARY

Investigation of the electrochemical behaviour of illicit substances and their redox pathways in the development of nanomaterial - modified platforms for decentralized analysis

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KEY WORDS: forensic drug analysis; electrochemical profiling; nanomaterials; portable platforms; wearable sensors; mass spectrometry.

INTRODUCTION

The market of illicit drugs continues to expand worldwide, the production, trafficking and abuse of these substances increasing, despite the continuous effort made by law enforcement agencies to disrupt this trend. This impacts society on many levels, from negative health consequences to environmental hazards and increased violence and criminal acts. Thus, continuous advancements in the field of forensic drug analysis are required to aid the fight against the spread of the illicit drugs market and its consequences.

The analytical methods currently employed in this field belong to one of the following two categories: presumptive tests or confirmatory tests. The presumptive tests are usually employed on-site and provide a fast screening of the presence or absence of an illicit drug (or a class of illicit drugs) and are used for the qualitative analysis of the samples, ideally enabling the exclusion of negative samples. However, these tests give a high number of false positive and false negative results.

An emerging alternative is represented by electrochemical techniques due to their excellent features including high sensitivity, possibility of miniaturization, low cost, and user friendliness, which make them a good choice for the development of portable and wearable devices.

In this context, the focus of the research performed within this doctoral thesis was the investigation of electrochemical techniques and various nanostructures for the screening of forensic samples in a decentralized manner.

STATE OF THE ART

The illicit drugs situation worldwide is unprecedented, displaying a tremendous increment in the spread and abuse of these substances, the yearly reports highlighting the persistent growth in the production, trafficking, and abuse of illicit drugs [1]. Globally, cocaine (COCA), amphetamine (AM) and methamphetamine (MA) were the main stimulants abused by most countries in 2021, while heroin was the main consumed narcotic [1], similar findings being reported in previous years [2,3].

The analytical methods currently employed for the analysis of suspicious cargos can be grouped into two main categories: laboratory-based techniques and portable devices and test. Within the first category fall the chromatographic techniques (e.g., liquid chromatography or gas chromatography) and capillary electrophoresis coupled to mass spectrometry, tandem mass spectrometry or photodiode array, as well as spectroscopic techniques such as Raman spectroscopy and Fourier-Transformed infrared (FTIR) spectroscopy [4]. Even though these methods are characterized by high analytical performance, they present some important drawbacks such as high cost, complicated operations, and lengthy analysis time [4,5].

The available portable devices and test are based on various methods, such as liquid chromatography coupled to mass spectrometry [6], Raman [7–10] and FTIR

spectroscopy [11,12], Ion Mobility Spectrometry [13–15], lateral flow assays [13,16] and colorimetry [17].

Electrochemical methods as well as nanotechnology have gained much interest in forensic analysis due to the valuable features these domains bring to the decentralized sensing field, such as affordability as well as excellent analytical capabilities [4]. For the electrochemical detection of illicit drugs, amperometric (chronoamperometry - CA, square wave voltammetry - SWV, differential pulse voltammetry - DPV and cyclic voltammetry - CV) and impedimetric (electrochemical impedance spectroscopy - EIS) techniques were mainly reported [4,18].

PERSONAL CONTRIBUTION

1. Aim and objectives of the thesis

The aim of the present work was the development of electrochemical strategies for the direct detection of illicit drugs in a decentralized manner, two main directions being followed within this work, namely the screening of suspicious samples encountered by the law enforcement officers in-field using disposable screen-printed electrodes (SPEs), and the sensing of illicit drugs in biological fluids using wearable electrochemical platforms.

For all studies, three general objectives were set and systematically investigated: (i) electrochemical characterisation of the targets and platforms, (ii) analytical characterisation of the optimized method, and (iii) evaluation of the analytical performance of the method in real samples.

2. General methodology

Disposable graphite SPEs (G-SPEs) were used as portable electrochemical platforms for the screening of confiscated samples, while microneedle patches were prepared and used as a wearable electrochemical platform.

CV and SVW were employed for the investigation of the electrochemical behaviour of the targets, while the electrochemical characterisation of the platforms (where the case) was performed by CV and EIS.

The analytical characterisation of the electrochemical methods was performed by evaluating the analytical figures of merit (linear range, limit of quantification - LOQ, limit of detection - LOD, and sensitivity), selectivity, and reproducibility. The applicability of the optimized electrochemical strategies for decentralized analysis was evaluated by testing real samples consisting of either confiscated forensic samples or artificial interstitial fluid (AISF).

3. Study 1 – Investigation of the Voltammetric Behaviour of methamphetamine for Its Fast Detection in Confiscated Samples Using a Portable Device

3.1. Introduction

MA is an amphetamine-type substance (ATS) synthetic drug [19], with central nervous system stimulant properties [20] and classified as a Schedule II controlled substance [21,22]. The most recent World Drug Report [23] showed that MA was the predominant amphetamine seized on the illicit drug market, while the European Monitoring Centre for Drugs and Drug Addiction reported in the same year that MA was the most widely used synthetic psychoactive drug in the world [24].

3.2. Objectives

The aim of this study was to assess the suitability of electrochemical methods for decentralized analysis of suspicious samples containing MA on G-SPEs. In this regard, several objectives were set and systematically assessed, such as the investigation of the electrochemical profile of MA, the elucidation of the redox pathways, the analytical characterisation of the method, and the evaluation of its analytical performance.

3.3. Materials and methods

Three electrochemical techniques were employed throughout this study, namely CV, SWV and CA for the assessment of the MA redox behaviour, the investigation of the MA electrochemical profile and, respectively, for the generation of the MA electro-oxidative products, which were analysed using LC-QTOF-MS.

The influence of several parameters such as the buffer pH, the sampling method and the presence of several adulterants on the MA electrochemical signal was assessed. Finally, a testing method was proposed based on two sampling strategies, which was applied for the analysis of seized samples.

3.4. Results and discussions

The pH study revealed that at values below pH 9 no electrochemical signal was registered, but an oxidation peak was obtained in the range from pH 9 to pH 12, revealing the electro-oxidation of MA, specifically of the secondary amine moiety from MA structure. Moreover, the increase of pH generated a cathodic shift of the MA peak potential and an increase of the current intensity. The redox pathways elucidation revealed the oxidation of MA to AM for low concentration solutions, and the formation of dimers for high concentration solutions.

For the qualitative analysis of MA two windows of detection corresponding to two concentration ranges were defined, while for the quantitative assessment of MA, a linear range between 50 and 2500 μM, with a LOD of 101.3 μM were obtained. The selectivity of the method was assessed against other illicit drugs and common adulterants/cutting agents. Using the proposed sampling strategies, any challenges posed by the investigated interferents were overcome.

Finally, the performance of the electrochemical method was evaluated on 34 seized samples, excellent values for the validation parameters being obtained, displaying a performance similar or superior to two other portable devices usually employed in forensic analysis.

3.5. Conclusions

Within this study the potential of SWV as a rapid electrochemical technique for decentralized screening of seized samples was displayed, focusing on detecting MA. The electrochemical approach exhibited excellent analytical performance, comparable to other portable devices [25].

4. Study 2 – Assessment of the Electrochemical Response of Cocaine and MDMA on Nanomaterial-Based Platforms

4.1. Introduction

COCA, a tropane alkaloid, and MDMA, the psychoactive component in Ecstasy [20], were used recreationally by 21 million and 20 million users worldwide [2], respectively. Their abuse often leads to central nervous system stimulation, producing euphoria, hallucinations, and cognitive enhancements, as well as cardiovascular anomalies, cognitive impairment, convulsions, insomnia, and paranoia. Besides, illicit drug are frequently adulterated, with various substances such as starch, sugars (such as lactose or mannitol), caffeine, paracetamol, or levamisole [4].

4.2. Objectives

The present study aimed to assess the potential of nanostructures, including metallic nanoparticles and carbon nanomaterials in the development of electrochemical strategies to be used for on-site forensic analysis of suspicious samples, having as targets COCA and MDMA. Thus, the evaluation of the influences of three parameters on the electrochemical profiles of the target analytes was investigated: (i) the composition of the platform, (ii) the pH of the electrolytic media, and (iii) the presence of commonly encountered adulterants / cutting agents in binary mixtures with the targets. The characterisation of the method was further performed, including the electrochemical characterisation of the platforms and the evaluation of the analytical performance in both standard solutions and confiscated samples.

4.3. Materials and methods

CV and EIS were employed for the electrochemical characterization of the platforms. SWV was the selected for the electrochemical profiling of the targets on SPEs modified with metallic (i.e., gold - AuNPs and platinum nanoparticles - PtNPs) and carbonaceous (i.e., graphene - GPH and multi-walled carbon nanotubes - MWCNTs) nanostructures and the evaluation of the analytical performance of the method, including the analytical figures of merit, the selectivity and reproducibility studies, and the real samples analysis.

4.4. Results and discussions

The optimisation step revealed that pH 12 and the SPEs modified with carbon-based nanomaterials would be better for the electrochemical detection of COCA and

MDMA since in all cases the peak potential registered a cathodic shift, which is advantageous for an electrochemical detection since a lower potential value can be translated into a lower risk of interferents. The characterisation of the selected platforms revealed that the nanomaterials-based SPEs presented higher porosity, higher surface area, higher conductivity and an improved electron transfer rate.

For the qualitative analysis, windows of detection corresponding to the electrochemical profiles of the two targets were defined, while for their quantitative assessment, wide linear ranges being obtained, with LODs of 57.76 μM for COCA and 24.0 μM for MDMA. The selectivity studies showed that using the MWCNTs-SPEs the two chosen targets could be detected in the presence of all tested interferents. Thus, the optimized method (pH 12 and the MWCNTs-SPEs) was employed for the analysis of 172 confiscated samples, exhibiting good analytical performance.

4.5. Conclusions

In this study, the impact of metallic nanoparticles and carbon nanomaterials on the electrochemical signal of COCA and MDMA was successfully evaluated in different pH environments. Optimal platforms and pH conditions were identified for the electrochemical detection of these psychoactive substances, both individually and in the presence of common adulterants found in seized samples. The GPH/MWCNT-based platforms demonstrated superior performance at pH 12 compared to unmodified G-SPEs or those modified with AuNPs and PtNPs. Additionally, MWCNTs-SPEs proved more effective for the identification of COCA and MDMA in equimolar binary mixtures, making this platform optimal for the analysis of these illicit drugs. The validation results indicated that the electrochemical device could significantly enhance the decentralized screening of suspicious samples [26].

5. Study 3 – Chemometric-Aid Optimisation of the Electrochemical Detection of Synthetic Cathinones on Nanomaterial-Based Platforms

5.1. Introduction

Synthetic cathinones (SCs) are structurally related to cathinone, a psychostimulant compound found in *Catha edulis* [27]. SCs exhibit AM- and COCA-like effects, functioning as central nervous system stimulants, leading to their use for adulteration of other illicit drugs or even as replacements for traditional drugs [20]. The dynamic nature of the SCs market, with numerous new compounds emerging continuously, poses a worldwide concern [19]. In the EU, SCs were the most frequently seized NPSs in 2020 [19], highlighting the need for rapid development of screening tests and analytical methods to detect this class of illicit drugs, especially given the potential presence of adulterants or other illicit substances in the samples.

5.2. Objectives

This study aimed to develop a rapid, decentralized method for detecting four synthetic cathinones: *N*-ethylhexedrone, 3-chloromethcathinone, 4-chloroeth-cathinone, and α-pyrrolidinovalerophenone (PVP) using SPEs modified with carbonaceous nanomaterials. The main objectives were the electrochemical

characterization and profiling of the targets, the elucidation of their oxidative pathways, the identification of the optimal analysis conditions and the evaluation of the analytical performance of the optimized method.

5.3. Materials and methods

As in the previous studies, CV and SWV were used for the electrochemical characterisation and profiling of the targets on SPE, while the elucidation of their oxidative pathways was performed via ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

Chemometric tools were used to identify the optimal analysis conditions considering platform type (G, GPH, and MWCNT) and electrolyte pH (PBS of pH 7, 9.5, and 12). The method's analytical performance was determined, after which the method was applied to screen confiscated samples for the mentioned drugs and selected adulterants (procaine, levamisole, caffeine, and creatine).

5.4. Results and discussions

The electrochemical characterisation of the targets in different conditions showed that a basic pH and the use of nanomaterials induced similar effects on the electrochemical profiles of the targets, namely a cathodic shift of the peak potential and an increase of the current intensity. The redox pathways elucidation indicated the oxidation of PVP at the pyrrolidine nucleus, while for the other three SCs an oxidative dealkylation of the secondary amine was observed.

The main findings of the chemometric processing of the electrochemical data indicated that a more alkaline pH and the nanomaterial-based platforms, especially GPH-SPEs, were optimal for SCs detection. Thus, pH 12 and the GPH-SPEs were chosen as optimal experimental conditions for the detection of the four SCs. The optimized method was evaluated in standard solutions and detection windows for the qualitative analysis of the four SCs were proposed, with LODs 14.54 to 45.94 μM.

The results obtained with the electrochemical method for the screening of confiscated samples were slightly inferior to the other two portable devices (i.e., Raman and FTIR spectrometers) in terms of specificity and accuracy, and superior to those obtained with the Raman device in terms of sensitivity, reflecting the important contribution of the electrochemical methods to the field of forensic drug analysis.

5.5. Conclusions

The SWV voltametric characterization under different conditions showed that the four SCs had oxidation peaks ranging from 0.52 V to 1.05 V. Chemometric analysis guided method optimization, identifying GPH-SPEs and an alkaline pH as ideal conditions. The optimized method demonstrated wide linear ranges and suitable LODs for the screening of confiscated samples [28].

6. Study 4 – Wearable Microneedle Sensor for the Electrochemical Screening and Monitoring of MDMA in Interstitial Fluid

6.1. Introduction

Apart from the illicit abuse of MDMA, clinical studies have explored its use in treating psychiatric conditions, highlighting the importance of wearable devices for MDMA identification and monitoring in biological fluids [4]. The (potentially) portable MDMA detection has been achieved in biological samples such as serum, urine, saliva, or fingerprints using different techniques such as surface-enhanced Raman spectroscopy and surface plasmon resonance [29]. Electrochemical methods have also been used for MDMA detection in various matrices, including serum, urine, and saliva[30–34]. In this context, microneedle (MN) arrays offer a promising platform for minimally invasive monitoring of various target species within the interstitial fluid (ISF).

6.2. Objectives

In this study the aim was the exploration of the potential of the electrochemical techniques combined with the use of microneedle arrays in the development of a wearable sensor for MDMA sensing in interstitial fluid.

6.3. Materials and methods

An electrochemical platform was constructed on a microneedle array by filling the MNs with conductive pastes of graphite for the counter and working electrodes, and of Ag/AgCl for the reference electrode. The obtained platform was than attached to screen printed connection, allowing its insertion into a portable potentiostat which can be controlled and powered by a smartphone. The obtained wearable sensor can be attached to the arm of a patient, pierce the skin with the MNs and reach the interstitial fluid, allowing the analysis of substances which pass from blood in this matrix.

The methodology of the study included three main steps, namely the optimization of the platform and adsorption time, the performance evaluation in buffer, including calibration and selectivity studies, and the performance evaluation in AISF, including calibration, stability and reversibility studies.

6.4. Results and discussions

The optimization of the platform was conducted by evaluating various nanostructures, both metallic nanoparticles and carbonaceous nanomaterials, the best performance being obtained on single-walled carbon nanotubes (SWCNTs) and reduced graphene oxide (rGO). These platforms were further investigated for the establishment of the absorption time, concluding that SWCNTs and 2.5’ were optimal.

The performance evaluation in buffer revealed that the SWCNTs functionalized platform allowed the detection of MDMA with a low LOD (under 2 uM). This platform was then used in the selectivity study for the analysis of binary mixtures of MDMA with biofluids components and illicit products compounds, including other illicit drugs, their metabolites, and common adulterants/cutting agents. Most of the recovery values obtained were above 87%. Afterwards, the performance of the optimized platform was

evaluated in AISF, a similar response to the analysis in buffer being obtained. To assess the mechanical robustness of the platform, a parafilm piercing test was performed, followed by the evaluation of the performance at room temperature as well as at 33^0C. In the first case, the signal response was similar to the one obtained before the parafilm piercing test, while at 33^0C an increase of the response was observed. Finally, the reversibility and stability of the response were evaluated using the optimized method, values below 7% of the standard deviation being obtained.

6.5. Conclusions

A wearable MN sensor functionalized with SWCNTs was developed of which performance was evaluated in both buffer and AISF, excellent analytical parameters being obtained [35].

7. General discussions

In this section, the results for the electrochemical strategies presented in the first three studies are integrated and discussed together for all the investigated illicit drugs. The detection windows for their identification were integrated in one protocol for the analysis of a suspicious sample which was applied for the interpretation of all results obtained for the electrochemical screening of the 215 seized samples. The electrochemical method presented good overall results, exhibiting better analytical performance in terms of both sensitivity and accuracy compared to the portable Raman device and an overall analytical performance inferior to that of the FTIR device. Nevertheless, the developed electrochemical strategy can bring significant improvements to the field of illicit drugs detection in a decentralized manner.

8. General conclusions

In this thesis the investigation of electrochemical techniques as fast and portable methods for decentralized testing in forensic drug analysis was carried out. The aim of developing fast and simple strategies for illicit drugs detection was achieved, both for the screening of suspicious samples encountered by the law enforcement officers in-field using disposable SPE, and for the detection and monitoring of MDMA in interstitial fluid using a wearable electrochemical platform.

9. The originality and innovative contributions of the thesis

The originality of this thesis consists in the development of novel electrochemical strategies applicable to forensic drug analysis in both seized samples and biological fluids (i.e., ISF). Throughout this work, novel nanomaterials-based platforms (GPH, MWCNTs and SWCNTs) were successfully employed for the enhancement of analytical performance of the sensing methods.

Importantly, the performance of the developed strategies for the suspicious samples screening was assessed in real samples and compared with commonly employed portable devices, building towards the implementation and validation of the electrochemical methods for on-site illicit drugs analysis.

Overall, the research conducted for this thesis contributes to the constant efforts of the research community, LEAs, and forensic laboratories for the disruption of the illicit drugs trafficking and abuse, bringing the advantages of electrochemical methods to the field of forensic analysis and increasing the safety and security of our society.

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REZUMATUL TEZEI DE DOCTORAT

Investigarea comportamentului electrochimic și a căilor redox ale unor substanțe ilicite în dezvoltarea unor platforme modificate cu de nanomateriale pentru analiză descentralizată

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CUVINTE CHEIE: analiză criminalistică de droguri; profilare electrochimică; nanomateriale; platforme portabile; senzori purtabili; spectrometrie de masa.

INTRODUCERE

Piaţa drogurilor ilicite continuă să se extindă la nivel mondial, producţia, traficul şi abuzul acestor substanţe crescând, în ciuda efortului continuu depus de organele de lege pentru a perturba această tendinţă. Acest lucru afectează societatea în mai multe moduri, avaând consecinţe negative asupra sănătăţii şi asupra mediului mediului, crescând în acelaşi timp violenţa şi actele criminale. Astfel, sunt necesare progrese continue în domeniul analizei criminalistice a drogurilor pentru a ajuta lupta împotriva răspândirii pieţei ilicite de droguri şi a consecinţelor acesteia.

Metodele analitice utilizate în prezent în acest domeniu aparţin uneia dintre următoarele două categorii: teste prezumtive sau teste de confirmare. Testele prezumtive sunt de obicei utilizate la faţa locului şi oferă o verificare rapidă a prezenţei sau absenţei unui drog ilicit (sau a unei clase de droguri ilicite) şi sunt utilizate pentru analiza calitativă a probelor, în mod ideal permiţând excluderea probelor negative. Cu toate acestea, aceste teste generează un număr mare de rezultate fals pozitive şi fals negative.

O alternativă în curs de dezvoltare este reprezentată de tehnicile electrochimice datorită caracteristicilor lor excelente, inclusiv sensibilitatea ridicată, posibilitatea de miniaturizare, costul scăzut şi uşurinţa de utilizare a acestora, ceea ce le face o alegere bună pentru dezvoltarea dispozitivelor portabile şi purtabile.

În acest context, centrul cercetării efectuate în cadrul acestei teze de doctorat a fost investigarea tehnicilor electrochimice şi a unor nanostructuri pentru analiza de probe criminalistice în mod descentralizat.

STADIUL ACTUAL AL CUNOAŞTERII

Situaţia drogurilor ilicite la nivel mondial este fără precedent, indicând o creştere extraordinară a răspândirii şi abuzului acestor substanţe, rapoartele anuale subliniind creşterea persistentă a producţiei, traficului şi abuzului de droguri ilicite [1]. La nivel global, cocaina (COCA), amfetamina (AM) şi metamfetamina (MA) au fost principalele droguri stimulente abuzate de majoritatea ţărilor în 2021, în timp ce heroina a fost principalul narcotic consumat [1], constatări similare fiind raportate în anii precedenţi [2,3].

Metodele analitice utilizate în prezent pentru analiza mărfurilor suspecte pot fi grupate în două categorii principale: tehnici de laborator şi teste pe teren. În prima categorie se încadrează tehnicile cromatografice (de exemplu, cromatografia lichidă sau cromatografia de gaze) şi electroforeza capilară cuplată cu spectrometria de masă, spectrometria de masă în tandem sau matricea de fotodiode, precum şi tehnicile spectroscopice precum spectroscopia Raman şi spectroscopia în infraroşu cu transformantă Fourier (FTIR) [4]. Chiar dacă aceste metode sunt caracterizate de performanţe analitice ridicate, ele prezintă unele dezavantaje importante, precum costul ridicat, operaţiunile complicate şi timpul lung de analiză [4,5].

Teste pe teren disponibile se bazează pe diferite metode, cum ar fi cromatografia lichidă cuplată cu spectrometria de masă [6], spectroscopie Raman [7–10]şi FTIR [11,12], spectrometria de mobilitate ionică [13–15], testele în flux lateral [13,16] şi colorimetria [17].

Metodele electrochimice, precum şi nanotehnologia au câştigat mult interes în analiza criminalistică datorită avantajelor pe care aceste domenii le aduc detecţiei descentralizate, precum accesibilitatea şi capacităţile analitice excelente [4]. Pentru detecţia electrochimică a drogurilor ilicite, au fost raportate în principal metode amperometrice (cronoamperometria - CA, voltametria cu undă pătrată - SWV, voltametrie puls diferenţială - DPV şi voltametria ciclică - CV) şi metode impedimetrice (spectroscopia de impedanţă electrochimică - EIS) [4,18].

CONTRIBUŢIA PERSONALĂ

1. Scopul şi obiectivele tezei

Scopul prezentei lucrări a fost elaborarea de strategii electrochimice pentru detecţia directă a drogurilor ilicite în mod descentralizat, în cadrul acestei lucrări fiind urmate două direcţii principale şi anume analiza probelor suspecte întâlnite de oamenii legii pe teren folosind electrozi serigrafiaţi (SPE) de unică folosinţă şi detecţia drogurilor ilicite în fluide biologice utilizând platforme electrochimice purtabile.

Pentru toate studiile au fost stabilite şi investigate sistematic trei obiective generale: (i) caracterizarea electrochimică a ţintelor şi platformelor, (ii) caracterizarea analitică a metodei optimizate şi (iii) evaluarea performanţei analitice a metodei în probe reale.

2. Metodologie generală

SPEs de grafit de unică folosinţă (G-SPEs) au fost folosiţi ca platforme electrochimice portabile pentru analiza probelor confiscate, în timp ce plasturi cu microace au fost dezvoltaţi şi utilizaţi ca platformă electrochimică purtabilă.

CV şi SVW au fost folosite pentru investigarea comportamentului electrochimic al ţintelor, în timp ce caracterizarea electrochimică a platformelor (unde a fost cazul) a fost efectuată prin CV şi EIS.

Caracterizarea analitică a strategiilor electrochimice dezvoltate a fost realizată prin evaluarea performanţelor analitice (domeniu liniar, limită de cuantificare - LOQ, limită de detecţie - LOD şi sensibilitate), selectivitate şi reproductibilitate. Aplicabilitatea strategiilor electrochimice optimizate pentru analiza descentralizată a fost evaluată prin testarea probelor reale constând fie în probe criminalistice confiscate, fie în fluid interstiţial artificial (AISF).

3. Studiul 1 – Investigarea Comportamentului Voltametric al Metamfetaminei pentru Detecţia sa Rapidă în Probe Confiscate Folosind un Dispozitiv Portabil

3.1. Introducere

MA este o substanţă de sinteză de tip amfetaminic (ATS) [19], cu proprietăţi de stimulare a sistemului nervos central [20] şi clasificată ca substanţă controlată din Tabelul II [21,22]. Cel mai recent Raport Mondial asupra drogurilor [23] a arătat că MA a fost amfetamina predominantă capturată pe piaţa ilegală a drogurilor, în timp ce Centrul European de Monitorizare a Drogurilor şi Dependenţei de Droguri a raportat în acelaşi an că MA este cel mai utilizat drog psihoactiv sintetic din lume. [24].

3.2. Obiective

Scopul acestui studiu a fost de a evalua potenţialul metodelor electrochimice pentru analiza descentralizată a probelor suspecte care conţin MA pe G-SPE. În acest sens, au fost stabilite şi evaluate sistematic mai multe obiective, cum ar fi investigarea profilului electrochimic al MA, elucidarea căilor redox, caracterizarea analitică a metodei şi evaluarea performanţei sale analitice.

3.3. Materiale şi metode

Pe parcursul acestui studiu au fost folosite trei tehnici electrochimice, şi anume CV, SWV şi CA pentru evaluarea comportamentului redox al MA, investigarea profilului electrochimic al MA şi, respectiv, pentru generarea produşilor electro-oxidativi ai MA, care au fost analizaţi folosind LC-QTOF-MS.

A fost evaluată influenţa mai multor parametri precum pH-ul tamponului, metoda de prelevare şi prezenţa mai multor adulteranţi asupra semnalului electrochimic al MA. În final, a fost propusă o metodă de testare bazată pe două strategii de eşantionare, care a fost aplicată pentru analiza probelor confiscate.

3.4. Rezultate şi dusc̦ii

Studiul pH-ului a evidenţiat faptul că la valori sub pH 9 nu a fost înregistrat niciun semnal electrochimic, dar s-a obţinut un pic de oxidare în intervalul de pH de la pH 9 la pH 12, dezvăluind electro-oxidarea MA, la nivelul grupării aminei secundare din structura MA. Mai mult, creşterea pH-ului a generat o schimbare catodică a potenţialului de pic a MA şi o creştere a intensităţii curentului. Elucidarea căilor redox a evidenţiat oxidarea MA la AM pentru soluţii cu concentraţie scăzută şi formarea de dimeri pentru soluţii cu concentraţie mare.

Pentru analiza calitativă a MA au fost definite două ferestre de detecţie corespunzătoare pentru două intervale de concentraţie, în timp ce pentru evaluarea cantitativă a MA s-a obţinut un interval liniar între 50 şi 2500 µM, cu LOD de 101,3 µM. Selectivitatea metodei a fost evaluată faţă de alte droguri ilicite şi adulteranţi/agenţi de diluare frecvent întâlniţi. Folosind strategiile de eşantionare propuse, au fost depăşite provocările prezentate de interferenţii investigaţi.

În final, performanţa metodei electrochimice a fost evaluată pe 34 de probe capturate, obţinându-se valori excelente pentru parametrii de validare, afişând o

performanţă similară sau superioară altor două dispozitive portabile utilizate de obicei în analiza criminalistică..

3.5. Concluzii

În cadrul acestui studiu a fost prezentat potenţialul SWV ca tehnică electrochimică rapidă pentru screening-ul descentralizat a probelor confiscate conţinând MA. Abordarea electrochimică a prezentat performanţe analitice excelente, comparabile cu alte dispozitive portabile [25].

4. Studiul 2 – Evaluarea Răspunsului Electrochimic al Cocainei şi al MDMA pe Platforme Bazate pe Nanomateriale

4.1. Introducere

COCA, un alcaloid tropan, şi MDMA, componenta psihoactivă din Ecstasy [20], au fost folosite în scopuri recreaţionale de 21 de milioane şi, respectiv, 20 de milioane de utilizatori din întreaga lume [2]. Abuzul lor duce adesea la stimularea sistemului nervos central, producând euforie, halucinaţii şi activitate cognitivă superioară, precum şi anomalii cardiovasculare, tulburări cognitive, convulsii, insomnie şi paranoia. În plus, drogurile ilicite sunt frecvent falsificate, cu diverse substanţe precum amidonul, zaharurile (cum ar fi lactoza sau manitolul), cofeina, paracetamolul sau levamisolul [4].

4.2. Obiective

Scopul prezentului studiu a fost evaluarea potenţialului nanostructurilor, inclusiv nanoparticule metalice şi nanomateriale de carbon, în dezvoltarea unor strategii electrochimice care să fie utilizate pentru analiza criminalistică la faţa locului a probelor suspecte, având ca ţinte COCA şi MDMA. Astfel, a fost investigată evaluarea influenţelor a trei parametri asupra profilurilor electrochimice ale analiţilor ţintă: (i) compoziţia platformei, (ii) pH-ul mediului electrolitic şi (iii) prezenţa adulteranţilor/ agenţilor de diluare întâlniţi frecvent în amestecuri binare cu ţintele. Ulterior a fost efectuată caracterizarea metodei, inclusiv caracterizarea electrochimică a platformelor şi evaluarea performanţei analitice atât în soluţii standard, cât şi în probele confiscate.

4.3. Materiale şi metode

CV şi EIS au fost folosite pentru caracterizarea electrochimică a platformelor. SWV a fost selectată pentru profilarea electrochimică a ţintelor pe SPEs modificaţi cu nanostructuri metalice (nanoparticule de aur - AuNP-uri şi nanoparticule de platină - PtNP-uri) şi pe bază de carbon (grafena - GPH şi nanotuburi de carbon multi-strat - MWCNT), şi pentru evaluarea performanţei analitice a metodei, inclusiv studii de selectivitate şi de reproductibilitate, precum şi analiza probelor reale.

4.4. Rezultate şi duscuţii

Etapa de optimizare a indicat faptul că pH-ul 12 şi SPE modificaţi cu nanomateriale pe bază de carbon ar fi de ales pentru detectarea electrochimică a COCA şi MDMA, deoarece în toate cazurile potenţialul de oxidare a înregistrat o deplasare catodică, ceea ce este avantajos pentru o detecţie electrochimică, deoarece un potenţial mai mic poate fi tradus într-un risc mai mic de interferenţi. Caracterizarea

platformelor selectate a arătat că SPE pe bază de nanomateriale au prezentat porozitate mai mare, suprafață mai mare, conductivitate mai mare și o rată de transfer electronic îmbunătățită.

Pentru analiza calitativă au fost definite ferestre de detecție corespunzătoare profilurilor electrochimice ale celor două ținte, în timp ce pentru evaluarea cantitativă a acestora s-au obținut intervale de liniaritate largi, cu LOD de 57,76 μM pentru COCA și 24,0 μM pentru MDMA. Studiile de selectivitate au arătat că folosind MWCNTs-SPE cele două ținte alese ar putea fi detectate în prezența tuturor interferenților testați. Astfel, metoda optimizată (pH 12 și MWCNTs-SPEs) a fost utilizată pentru analiza a 172 de probe confiscate, prezentând performanțe analitice bune.

4.5. Concluzii

În acest studiu, impactul nanoparticulelor metalice și al nanomaterialelor de carbon asupra semnalului electrochimic al COCA și MDMA a fost evaluat cu succes în diferite medii de pH. Au fost identificate platforme și condiții optime de pH pentru detectarea electrochimică a acestor substanțe psihoactive, atât individual, cât și în prezența adulteranților frecvent întâlniți în probele confiscate. Platformele bazate pe GPH/MWCNT au demonstrat performanțe superioare la pH 12 în comparație cu G-SPE nemodificați sau cu cei modificați cu AuNPs și PtNPs. În plus, MWCNTs-SPEs s-au dovedit mai eficienți pentru identificarea COCA și MDMA în amestecuri binare echimolare, făcând această platformă optimă pentru analiza acestor droguri ilicite. Rezultatele validării au indicat că dispozitivul electrochimic ar putea îmbunătăți semnificativ analiza descentralizată a probelor suspecte [26].

5. Studiul 3 – Optimizarea Chimiometrică a Detecției Electrochimice a Catinonelor Sintetice pe Platforme Bazate pe Nanomateriale

5.1. Introducere

Catinonele sintetice (SCs) sunt înrudite structural cu catinona, un compus psihostimulant găsit în *Catha edulis* [27]. SCs prezintă efecte asemănătoare AM și COCA, funcționând ca stimulenți ai sistemului nervos central, ducând la utilizarea lor pentru alterarea altor droguri ilicite sau chiar ca înlocuitori pentru drofurile tradiționale [20]. Natura dinamică a pieței de SCs, cu numeroași compuși noi care apar în mod continuu, reprezintă o preocupare la nivel mondial [19]. În UE, SCs au fost cele mai frecvent confiscate substanțe psihoactive în 2020 [19], subliniind necesitatea dezvoltării rapide a de teste și metode analitice pentru depistarea acestei clase de droguri ilicite, în special având în vedere prezența potențială a adulteranților sau a altor substanțe ilicite în probe.

5.2. Obiective

Acest studiu și-a propus dezvoltarea unei metodei rapide, descentralizate pentru detecția a patru catinone sintetice: N-etilhexedronă, 3-clorometcatinonă, 4-cloroetcatinonă și α-pirolidinovalerofenonă (PVP) folosind SPEs modificați cu nanomateriale pe bază de carbon. Principalele obiective au fost caracterizarea

electrochimică a ţintelor ţintelor, elucidarea căilor lor oxidative, identificarea condiţiilor optime de analiză şi evaluarea performanţei analitice a metodei optimizate.

5.3. Materiale şi metode

La fel ca în studiile anterioare, CV şi SWV au fost utilizate pentru caracterizarea electrochimică a ţintelor pe SPE, în timp ce elucidarea căilor lor oxidative a fost efectuată prin cromatografie lichidă de ultra-înaltă performanţă cuplată cu spectrometrie de masă în tandem (UPLC-MS/MS).).

Instrumentele chemometrice au fost utilizate pentru a identifica condiţiile optime de analiză, luând în considerare tipul de platformă (G, GPH şi MWCNT) şi pH-ul electrolitului (PBS de pH 7, 9,5 şi 12). A fost determinată performanţa analitică a metodei, iar ulterior metoda a fost aplicată pentru analiza probelor confiscate şi pentru adulteranţii selectaţi (procaină, levamisol, cofeină şi creatină).

5.4. Rezultate şi duscuţii

Caracterizarea electrochimică a ţintelor în diferite condiţii a arătat faptul că un pH bazic şi utilizarea nanomaterialelor au indus efecte similare asupra profilurilor electrochimice ale ţintelor, şi anume o deplasare catodică a potenţialului de oxidare şi o creştere a intensităţii curentului. Elucidarea căilor redox a indicat oxidarea PVP la nivelul nucleului pirolidinic, în timp ce pentru celelalte trei SC a fost observată o dezalchilare oxidativă a aminei secundare.

Principalele constatări ale prelucrării chimiometrice a datelor electrochimice au indicat că un pH mai alcalin şi platformele bazate pe nanomateriale, în special GPH-SPEs, au fost optime pentru detectarea SCs. Astfel, pH-ul 12 şi GPH-SPEs au fost alese ca parametri experimentali optimi pentru detectarea celor patru SC. Metoda optimizată a fost evaluată în soluţii standard şi au fost propuse ferestre de detecţie pentru analiza calitativă a celor patru SC, cu LOD 14,54 până la 45,94 μM.

Rezultatele obţinute cu metoda electrochimică pentru analiza probelor confiscate au fost uşor inferioare celorlalte două dispozitive portabile (i.e., spectrometre Raman şi FTIR) din punct de vedere al specificităţii şi acurateţei, dar superioare celor obţinute cu dispozitivul Raman din punct de vedere al sensibilităţii, reflectând contribuţia importantă a metodelor electrochimice în domeniul analizei criminalistice a drogurilor.

5.5. Concluzii

Caracterizarea voltametrică a SCs în diferite condiţii a arătat că patru substanţe au avut potenţiale de oxidare cuprinse între 0,52 V şi 1,05 V. Analiza chemiometrică a ghidat optimizarea metodei, identificând GPH-SPE şi un pH alcalin ca parametri ideali pentru detecţia electrochimică. Metoda optimizată a demonstrat intervale de liniaritate largi şi LOD adecvate pentru examinarea probelor confiscate [28].

6. Studiul 4 – Senzor purtabil cu microace pentru detecția electrochimic și monitorizarea MDMA în fluidul interstițial

6.1. Introducere

Pe lângă abuzul ilicit al MDMA, studiile clinice au explorat utilizarea acestuia în tratarea afecțiunilor psihiatrice, subliniind importanța dispozitivelor purtabile pentru identificarea și monitorizarea MDMA în fluide biologice [4]. Detecția (potențial) portabilă a MDMA a fost realizată în probe biologice precum ser, urina, saliva sau amprente folosind diferite tehnici, cum ar fi spectroscopia Raman amplificată de suprafață și rezonanța la suprafață a plasmonilor [29]. Metodele electrochimice au fost, de asemenea, utilizate pentru detectarea MDMA în diferite probe biologice, inclusiv ser, urină și salivă [30–34]. În acest context, platformele de microace (MN) oferă o alternativă promițătoare pentru monitorizarea minim invazivă a diferitelor specii țintă din fluidul interstițial (ISF).

6.2. Obiective

Scopul acestui studiu a fost explorarea potențialului tehnicilor electrochimice combinate cu utilizarea platformelor de microace în dezvoltarea unui senzor purtabil pentru detecția MDMA în fluid interstițial.

6.3. Materiale și metode

O platformă electrochimică a fost construită pe o platformă de microace prin umplerea microacelor cu paste conductoare de grafit pentru contraelectrod și electrodul de lucru, și de Ag/AgCl pentru electrodul de referință. Platforma obținută a fost apoi atașată unor conexiuni serigrafiate, permițând introducerea într-un potențiostat portabil care poate fi controlat și alimentat prin intermediul unui telefon mobil. Senzorul purtabil obținut poate fi atașat de brațul unui pacient, străpungând pielea prin intermediul microacelor și ajungând în lichidul interstițial, permițând astfel analiza substanțelor care trec din sânge în această matrice.

Metodologia studiului a inclus trei etape principale, respectiv optimizarea platformei și a timpului de adsorbție, evaluarea performanței în tampon fosfat salin, incluzând studii de calibrare și selectivitate, și evaluarea performanței în lichid interstițial artificial, incluzând studii de calibrare, stabilitate și reversibilitate.

6.4. Rezultate și discuții

Optimizarea platformei a fost realizată prin evaluarea diferitelor nanostructuri, atât nanoparticule metalice, cât și nanomateriale carbonice, cele mai bune performanțe obținându-se pe nanotuburi de carbon cu uni-strat (SWCNT) și oxid de grafen redus (rGO). Aceste platforme au fost investigate în continuare pentru stabilirea timpului de absorbție, concluzionând că SWCNT și 2,5' sunt optime.

Evaluarea performanței în tampon fosfat a indicat faptul că platforma funcționalizată cu SWCNT a permis detecția MDMA cu o LOD scăzută (sub 2 uM). Această platformă a fost apoi utilizată în studiul de selectivitate pentru analiza amestecurilor binare de MDMA cu componente din biofluide și compuși prezenți în produse ilicite, inclusiv alte droguri ilicite, metaboliții acestora și adulteranți/agenți de

diluare frecvent întâlniţi. Majoritatea valorilor de recuperare obţinute au fost peste 87%. Ulterior, performanţa platformei optimizate a fost evaluată în lichid interstiţial artificial, obţinându-se un răspuns similar analizei în tampon fosfat. Pentru a evalua robusteţea mecanică a platformei, a fost efectuat un test de perforare a parafilmului, urmat de evaluarea performanţei la temperatura camerei precum şi la 33^0C. În primul caz, răspunsul semnalului a fost similar cu cel obţinut înainte de testul de perforare a parafilmului, în timp ce la 33^0C s-a observat o creştere a răspunsului electrochimic. În final, reversibilitatea şi stabilitatea răspunsului electrochimic au fost evaluate prin metoda optimizată, obţinându-se valori sub 7% pentru abaterea standard.

6.5. Concluzii

A fost dezvoltat un senzor purtabil cu microace funcţionalizate cu SWCNTs a cărui performanţă a fost evaluată atât în tampon fosfat cât şi în lichid interstiţial artificial, obţinându-se parametri analitici excelenţi [35].

7. General discussions

În această secţiune, rezultatele pentru strategiile electrochimice prezentate în primele trei studii sunt integrate şi discutate împreună pentru toate drogurile ilicite investigate. Ferestrele de detecţie pentru identificarea lor au fost integrate într-un protocol de analiză a unei probe suspecte care a fost aplicat pentru interpretarea tuturor rezultatelor obţinute pentru analiza electrochimică a celor 215 probe confiscate. Metoda electrochimică a prezentat rezultate bune, prezentând performanţe analitice mai bune atât în ceea ce priveşte sensibilitatea, cât şi acurateţea, comparativ cu dispozitivul Raman portabil, şi o performanţă analitică generală inferioară celei a dispozitivului FTIR. Cu toate acestea, strategia electrochimică dezvoltată poate aduce îmbunătăţiri semnificative în domeniul detecţiei drogurilor ilicite într-o manieră descentralizată.

8. General conclusions

În această teză a fost realizată investigarea tehnicilor electrochimice ca metode rapide şi portabile de testare descentralizată în analiza criminalistică a drogurilor. Scopul dezvoltării unor strategii rapide şi simple de detecţie a drogurilor ilicite a fost atins, atât pentru analiza probelor suspecte întâlnite de oamenii legii pe teren folosind SPE de unică folosinţă, cât şi pentru detecşia şi monitorizarea MDMA în fluid interstiţial folosind un senzor electrochimic purtabil.

9. The originality and innovative contributions of the thesis

Originalitatea acestei teze constă în dezvoltarea de noi strategii electrochimice aplicabile analizei criminalistice a drogurilor atât în probe confiscate, cât şi în fluide biologice (ISF). Pe parcursul acestei lucrări, noi platforme bazate pe nanomateriale (GPH, MWCNT şi SWCNT) au fost folosite cu succes pentru îmbunătăţirea performanţei analitice a metodelor de detecţie.

Performanţa strategiilor dezvoltate pentru analiza probelor suspecte a fost evaluată în probe reale şi comparată cu dispozitive portabile utilizate în mod obişnuit,

avansând implementarea și validarea metodelor electrochimice pentru analiza drogurilor ilicite pe teren.

În ansamblu, cercetările efectuate pentru această teză contribuie la eforturile constante ale comunității de cercetare, ale forțelor de lege și ale laboratoarelor de criminalistică pentru perturbarea traficului și abuzului ilicit de droguri, aducând avantajele metodelor electrochimice în domeniul analizei criminalistice și sporind siguranța. și securitatea societății noastre.

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DOCTORAATSPROEFSCHRIFT SAMENVATTING

Onderzoek naar het elektrochemisch gedrag van illegale stoffen en hun redoxroutes bij de ontwikkeling van nanomateriaal-gemodificeerde platformen voor gedecentraliseerde analyse

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SLEUTELWOORDEN: forensische druganalyse; elektrochemisch profileren; nanomaterialen; draagbare platformen; draagbare sensoren; massaspectrometrie.

INLEIDING

De markt voor illegale drugs blijft wereldwijd groeien, waarbij de productie, smokkel en misbruik van deze stoffen toenemen, ondanks de voortdurende inspanningen van wetshandhavingsinstanties om deze trend te verstoren. Dit heeft gevolgen voor de samenleving op verschillende niveaus, van negatieve gezondheidseffecten tot milieurisico's en een toename van geweld en criminele activiteiten. Daarom zijn voortdurende ontwikkelingen op het gebied van forensische drugsanalyse vereist om de strijd tegen de verspreiding van de illegale drugsmarkt en de gevolgen ervan te ondersteunen.

De analytische methoden die momenteel in dit vakgebied worden gebruikt, behoren tot een van de volgende twee categorieën: eerste lijn tests (vermoedens) of tweede lijn tests (bevestiging). De vermoedelijke tests worden meestal ter plaatse gebruikt en bieden een snelle screening van de aan- of afwezigheid van een illegale drug (of een klasse van illegale drugs). Ze worden gebruikt voor de kwalitatieve analyse van de stalen, bij voorkeur met uitsluiting van negatieve stalen. Deze tests geven echter een groot aantal vals positieve en vals negatieve resultaten.

Een opkomend alternatief wordt vertegenwoordigd door elektrochemische technieken vanwege hun uitstekende eigenschappen, waaronder hoge gevoeligheid, mogelijkheid tot miniaturisatie, lage kosten en gebruiksvriendelijkheid, waardoor ze een goede keuze zijn voor de ontwikkeling van draagbare apparaten. In deze context richtte het onderzoek, binnen dit proefschrift uitgevoerd, zich op het onderzoek naar elektrochemische technieken en verschillende nanostructuren voor de screening van forensische stalen op gedecentraliseerde wijze.

STAND VAN ZAKEN

De situatie van illegale drugs wereldwijd is ongekend, echter met een enorme toename van de verspreiding en het misbruik van deze stoffen, waarbij de jaarlijkse rapporten wijzen op aanhoudende groei in de productie, smokkel en misbruik van illegale drugs [1]. Wereldwijd werden cocaïne (COCA), amfetamine (AM) en methamfetamine (MA) in 2021 het meest misbruikt door de meeste landen, terwijl heroïne het meest geconsumeerde narcoticum was [1]. Vergelijkbare bevindingen werden in voorgaande jaren gerapporteerd [2,3].

De analytische methoden die momenteel worden gebruikt voor de analyse van verdachte ladingen kunnen worden gegroepeerd in twee hoofdcategorieën: op laboratorium gebaseerde technieken enerzijds en draagbare apparaten en tests anderzijds. Binnen de eerste categorie vallen chromatografische technieken (bijvoorbeeld vloeistofchromatografie of gaschromatografie) en capillaire elektroforese gekoppeld aan massaspectrometrie, tandemmassaspectrometrie of fotodiode-array, evenals spectroscopische technieken zoals Ramanspectroscopie en Fourier-getransformeerde infraroodspectroscopie. [4]. Hoewel deze methoden worden

gekenmerkt door een hoge analytische prestatie, hebben ze enkele belangrijke nadelen zoals hoge kosten, gecompliceerde procedures en een lange analyse tijd.[4,5].

De beschikbare draagbare apparaten en tests zijn gebaseerd op verschillende methoden, zoals vloeistofchromatografie gekoppeld aan massaspectrometrie [6], Ramanspectroscopie [7–10], infraroodspectroscopie met Fourier-transformatie [11,12], ionmobiliteitsspectrometrie [13–15], laterale stroomtests [13,16] en colorimetrie. [17].

Elektrochemische methoden, evenals nanotechnologie, hebben veel interesse gekregen in forensische analyse vanwege de waardevolle eigenschappen die deze domeinen brengen naar het gedecentraliseerde sensing-veld, zoals betaalbaarheid en uitstekende analytische performantie [4]. Voor de elektrochemische detectie van illegale drugs werden voornamelijk amperometrische (chronoamperometrie - CA, vierkante golf voltammetrie - SWV, differentiële pulsvoltammetrie - DPV en cyclische voltammetrie - CV) en impedimetrische (elektrochemische impedantiespectroscopie - EIS) technieken gerapporteerd [4,18].

PERSOONLIJKE BIJDRAGE

1. Doel van het proefschrift

Het doel van dit werk was de ontwikkeling van elektrochemische strategieën voor de directe detectie van illegale drugs op gedecentraliseerde wijze, waarbij binnen dit werk twee hoofdrichtingen werden gevolgd, namelijk de screening van verdachte stalen die door wetshandhavingsambtenaren op het veld worden aangetroffen met behulp van wegwerpbare zeefdruk-elektroden (SPE's), en de detectie van illegale drugs in biologische vloeistoffen met behulp van draagbare elektrochemische platformen.

Voor alle studies werden drie algemene doelstellingen vastgesteld en systematisch onderzocht: (i) elektrochemische karakterisering van de doelenmoleculen, (ii) analytische karakterisering van de geoptimaliseerde methode, en (iii) evaluatie van de analytische prestaties van de methode in reële stalen.

2. Algemene methodologie

Wegwerpbare grafiet-SPE's (G-SPE's) werden gebruikt als draagbare elektrochemische sensorstrips voor de screening van in beslag genomen stalen, terwijl microneedle-patches werden bereid en gebruikt als een draagbaar elektrochemisch platform.

CV en SWV werden gebruikt voor het onderzoek van het elektrochemische gedrag van de doelen, terwijl de elektrochemische karakterisering van de platforms (indien aanwezig) werd uitgevoerd door CV en EIS.

De analytische karakterisering van de elektrochemische methoden werd uitgevoerd door de evaluatie van de analytische kenmerken (lineair bereik, limiet van kwantificering - LOQ, limiet van detectie - LOD en gevoeligheid), selectiviteit en reproduceerbaarheid. De toepasbaarheid van de geoptimaliseerde elektrochemische

strategieën voor gedecentraliseerde analyse werd geëvalueerd door reële stalen te testen, bestaande uit in beslag genomen forensische stalen of kunstmatige interstitiële vloeistof (AISF)..

3. Studie 1 - Onderzoek Naar Het Voltammetrische Gedrag Van MA Voor Snelle Detectie In In Beslag Genomen Stalen Met Behulp Van Een Draagbaar Apparaat

3.1. Inleiding

MA is een synthetische amfetamine-type stof (ATS) [19], met stimulerende eigenschappen van het centrale zenuwstelsel [20] en geclassificeerd als een gecontroleerde stof van klasse II [21,22]. Het meest recente World Drug Report [23] toonde aan dat MA de overheersende amfetamine was die in beslag werd genomen op de illegale drugsmarkt, terwijl het European Monitoring Centre for Drugs and Drug Addiction in hetzelfde jaar meldde dat MA de meest gebruikte synthetische psychoactieve drug ter wereld was [24].

3.2. Doelstellingen

Het doel van deze studie was om de geschiktheid van elektrochemische methoden voor gedecentraliseerde analyse van verdachte monsters die MA bevatten op G-SPE's te beoordelen. Het elektrochemische profiel van MA, de verduidelijking van de redoxpaden, de analytische karakterisering van de methode en de evaluatie van de analytische prestaties werden bestudeerd.

3.3. Materialen en methoden

Drie elektrochemische technieken werden in deze studie gebruikt, namelijk CV, SWV en CA voor de beoordeling van het MA-redoxgedrag, het onderzoek naar het MA-elektrochemische profiel en voor de generatie van de MA-elektro-oxidatieve producten, die werden geanalyseerd met LC-QTOF-MS.

De invloed van verschillende parameters zoals de pH van de buffer, de monstername-methode en de aanwezigheid van verschillende verontreinigingen op het MA-elektrochemische signaal werden beoordeeld. Ten slotte werd een testmethode voorgesteld op basis van twee monsternamestrategieën, die werd toegepast voor de analyse van in beslag genomen stalen.

3.4. Resultaten en discussies

Het pH-onderzoek toonde aan dat bij waarden onder pH 9 geen elektrochemisch signaal werd geregistreerd, maar een oxidatiepiek werd verkregen in het bereik van pH 9 tot pH 12, waarbij de elektro-oxidatie van MA werd onthuld, specifiek van de secundaire aminegroep in de MA-structuur. Bovendien leidde een verhoging van de pH tot een kathodische verschuiving van het MA-piekpotentieel en een toename van de stroomintensiteit. De verduidelijking van de redoxpaden toonde de oxidatie van MA naar AM voor oplossingen met lage concentratie en de vorming van dimeren voor oplossingen met hoge concentratie.

Voor de kwalitatieve analyse van MA werden twee detectievensters gedefinieerd die overeenkomen met twee concentratiebereiken, terwijl voor de

kwantitatieve beoordeling van MA een lineair bereik tussen 50 en 2500 µM werd verkregen, met een LOD van 101,3 µM. De selectiviteit van de methode werd beoordeeld ten opzichte van andere illegale drugs en gangbare verontreinigingen/snijmiddelen. Met behulp van de voorgestelde monsternamestrategieën werden eventuele uitdagingen die werden gesteld door de onderzochte interferenten, overwonnen.

Ten slotte werd de prestatie van de elektrochemische methode beoordeeld op 34 in beslag genomen stalen, waarbij uitstekende waarden werden verkregen voor de validatieparameters, die een prestatie weergaven die vergelijkbaar was of superieur was aan twee andere draagbare apparaten die doorgaans worden gebruikt in forensische analyse.

3.5. Conclusies

Binnen deze studie werd het potentieel van SWV als een snelle elektrochemische techniek voor de gedecentraliseerde screening van in beslag genomen monsters weergegeven, met de nadruk op het detecteren van MA. De elektrochemische benadering vertoonde uitstekende analytische prestaties, vergelijkbaar met andere draagbare apparaten [25].

4. Studie 2 - Beoordeling Van De Elektrochemische Respons Van Cocaïne En MDMA Op Op Nanomaterialen Gebaseerde Platforms

4.1. Inleiding

COCA, een tropanalkaloïde, en MDMA, het psychoactieve bestanddeel in Ecstasy[20], werden recreatief gebruikt door respectievelijk 21 miljoen en 20 miljoen gebruikers wereldwijd [2]. Hun misbruik leidt vaak tot stimulatie van het centrale zenuwstelsel, waardoor euforie, hallucinaties en cognitieve verbeteringen ontstaan, evenals cardiovasculaire afwijkingen, cognitieve stoornissen, convulsies, slapeloosheid en paranoia. Bovendien worden illegale drugs vaak versneden met verschillende stoffen zoals zetmeel, suikers (zoals lactose of mannitol), cafeïne, paracetamol of levamisol [4].

4.2. Doelstellingen

Het doel van deze studie was om het potentieel van nanostructuren, waaronder metallische nanopartikels en koolstofnanomaterialen, te beoordelen in de ontwikkeling van elektrochemische strategieën die kunnen worden gebruikt voor on-site forensische analyse van verdachte monsters met COCA en MDMA als doelwitten. Zo werden de invloeden van drie parameters op het elektrochemische gedrag van de doelanalyten onderzocht: (i) de samenstelling van het platform, (ii) de pH van het elektrolytische medium en (iii) de aanwezigheid van veelvoorkomende verontreinigingen of snijmiddelen in binaire mengsels met de doelwitten.

4.3. Materialen en methoden

CV en EIS werden gebruikt voor de elektrochemische karakterisering van de platforms. SWV werd geselecteerd voor het elektrochemisch profileren van de

doelwitten op SPE's die waren gemodificeerd met metallische (bijv. goud - AuNPs en platinanano-deeltjes - PtNPs) en koolstofachtige (bijv. grafeen - GPH en meerwandige koolstofnanobuizen - MWCNTs) nanostructuren, evenals voor de evaluatie van de analytische prestaties van de methode, inclusief studies naar analytische kenmerken, selectiviteit en reproduceerbaarheid, en de analyse van echte monsters.

4.4. Resultaten en discussies

De optimalisatiestap toonde aan dat pH 12 en de SPE's gemodificeerd met koolstofgebaseerde nanomaterialen beter geschikt waren voor de elektrochemische detectie van COCA en MDMA, omdat in alle gevallen het piekpotentieel een kathodische verschuiving vertoonde, wat gunstig is voor elektrochemische detectie, aangezien een lagere potentiewaarde kan worden vertaald naar een lager interferentierisico. De karakterisering van de geselecteerde platforms onthulde dat de op nanomaterialen gebaseerde SPE's een hogere porositeit, een groter oppervlak, een hogere geleidbaarheid en een verbeterde elektronentransportsnelheid vertoonden.

Voor de kwalitatieve analyse werden detectievensters gedefinieerd die overeenkwamen met de elektrochemische profielen van de twee doelmoleculen, terwijl voor hun kwantitatieve beoordeling brede lineaire bereiken werden verkregen, met LOD's van 57,76 µM voor COCA en 24,0 µM voor MDMA. De selectiviteitsstudies toonden aan dat met behulp van de MWCNTs-SPE's de twee gekozen doelwitten konden worden gedetecteerd in aanwezigheid van alle geteste interferenten. Zo werd de geoptimaliseerde methode (pH 12 en de MWCNTs-SPE's) gebruikt voor de analyse van 172 in beslag genomen monsters, waarbij goede analytische prestaties werden vertoond.

4.5. Conclusies

In deze studie werd met succes de impact van metallische nanopartikels en koolstofnanomaterialen op het elektrochemische signaal van COCA en MDMA geëvalueerd in verschillende pH-omgevingen. Optimale platformen en pH-omstandigheden werden geïdentificeerd voor de elektrochemische detectie van deze psychoactieve stoffen, zowel afzonderlijk als in aanwezigheid van veelvoorkomende versnijdingsmiddelen in in beslag genomen monsters. De GPH/MWCNT-gebaseerde platformen vertoonden superieure prestaties bij pH 12 in vergelijking met niet-gemodificeerde G-SPE's of die gemodificeerd waren met AuNPs en PtNPs. Bovendien bleken MWCNTs-SPE's effectiever te zijn voor de identificatie van COCA en MDMA in equimolaire binaire mengsels, waardoor dit platform optimaal is voor de analyse van deze illegale drugs. De validatieresultaten gaven aan dat het elektrochemische apparaat de gedecentraliseerde screening van verdachte monsters aanzienlijk kon verbeteren [26].

5. Studie 3 - Chemometrisch-Geoptimaliseerde Elektrochemische Detectie Van Synthetische Cathinonen Op Nanomaterialen Gebaseerde Platforms

5.1. Inleiding

Synthetische cathinonen (SC's) zijn structureel verwant aan cathinone, een psychostimulerende stof die wordt aangetroffen in *Catha edulis* [27]. SC's vertonen effecten vergelijkbaar met die van AM- en COCA-achtige stoffen, functionerend als centrale zenuwstelselstimulerende middelen, wat heeft geleid tot hun gebruik bij het versnijden van andere illegale drugs of zelfs als vervanging voor traditionele drugs [20]. De dynamische aard van de SC's-markt, met talloze nieuwe verbindingen die voortdurend opkomen, vormt wereldwijd een zorg [19]. In de EU waren SC's in 2020 de meest in beslag genomen NPS's [19], wat de noodzaak benadrukt om snel screeningstests en analytische methoden te ontwikkelen voor de detectie van deze klasse illegale drugs, vooral gezien de mogelijke aanwezigheid van versnijdingsmiddelen of andere illegale stoffen in de monsters.

5.2. Doelstellingen

Deze studie had tot doel een snelle, gedecentraliseerde methode te ontwikkelen voor de detectie van vier synthetische cathinonen: N-ethylhexedron, 3-chloromethcathinon, 4-chloroeth-cathinon en α-pyrrolidinovalerofenon (PVP) met behulp van SPE's gemodificeerd met koolstofachtige nanomaterialen. De belangrijkste doelstellingen waren de elektrochemische karakterisering en profilering van de doelwitten, de verduidelijking van hun oxidatieve paden, de identificatie van de optimale analyseomstandigheden en de evaluatie van de analytische prestaties van de geoptimaliseerde methode.

5.3. Materialen en methoden

Zoals in de eerdere studies werden CV en SWV gebruikt voor de elektrochemische karakterisering en profilering van de doelmoleculen op SPE, terwijl de verduidelijking van hun oxidatieve paden werd uitgevoerd via ultra-high performance liquid chromatography gekoppeld aan tandem massaspectrometrie (UPLC-MS/MS).

Chemometrische methoden werden toegepast om de elektrochemische respons van de vier SC's te optimaliseren. De selectiviteit van de elektrochemische methode werd geëvalueerd ten opzichte van interferenten zoals andere illegale drugs en snijmiddelen, en verschillende matrices.

5.4. Resultaten en discussies

De elektrochemische karakterisering van de doelmoleculen in verschillende omstandigheden toonde aan dat een basisch pH en het gebruik van nanomaterialen vergelijkbare effecten hadden op de elektrochemische profielen van de doelmoleculen, namelijk een kathodische verschuiving van het piekpotentieel en een toename van de stroomintensiteit. De verheldering van de redoxroutes wees op de oxidatie van PVP bij de pyrrolidinekern, terwijl voor de andere drie SC's een oxidatieve dealkylatie van de secundaire amine werd waargenomen.

De belangrijkste bevindingen van de chemometrische verwerking van de elektrochemische gegevens gaven aan dat een meer alkalische pH en de op nanomaterialen gebaseerde platforms, vooral GPH-SPE's, optimaal waren voor de detectie van SC's. Zo werden pH 12 en de GPH-SPE's gekozen als optimale experimentele omstandigheden voor de detectie van de vier SC's. De geoptimaliseerde methode werd geëvalueerd in standaardoplossingen en er werden detectievensters voor de kwalitatieve analyse van de vier SC's voorgesteld, met LOD's van 14,54 tot 45,94 μM.

De resultaten verkregen met de elektrochemische methode voor de screening van in beslag genomen monsters waren enigszins inferieur aan die van de andere twee draagbare apparaten (d.w.z. Raman- en FTIR-spectrometers) in termen van specificiteit en nauwkeurigheid, en superieur aan de resultaten verkregen met het Raman-apparaat in termen van gevoeligheid. , wat de belangrijke bijdrage van de elektrochemische methoden op het gebied van forensische geneesmiddelenanalyse weerspiegelt.

5.5. Conclusies

De voltametrische SWV-karakterisering onder verschillende omstandigheden toonde aan dat de vier SC's oxidatiepieken hadden variërend van 0,52 V tot 1,05 V. Door chemometrische analyse geleide methode-optimalisatie, waarbij GPH-SPE's en een alkalische pH als ideale omstandigheden werden geïdentificeerd. De geoptimaliseerde methode demonstreerde brede lineaire bereiken en geschikte LOD's voor de screening van in beslag genomen monsters [28].

6. Studie 4 - Draagbare Microneedle Sensor Voor De Elektrochemische Screening En Monitoring Van MDMA In Interstitiële Vloeistof

6.1. Inleiding

Naast het illegale misbruik van MDMA hebben klinische studies zijn gebruik onderzocht voor de behandeling van psychiatrische aandoeningen, waarbij het belang van draagbare apparaten voor MDMA-detectie en -monitoring in biologische vloeistoffen wordt benadrukt [4]. Draagbare MDMA-detectie is bereikt in biologische monsters zoals serum, urine, speeksel of vingerafdrukken met behulp van verschillende technieken zoals surface enhanced Raman-spectroscopie en oppervlakteplasmonresonantie [29]. Elektrochemische methoden zijn ook gebruikt voor MDMA-detectie in verschillende matrices, waaronder serum, urine en speeksel [30–34]. In dit verband bieden microneedle (MN) arrays een veelbelovend platform voor minimaal invasieve monitoring van verschillende doelsoorten in interstitiële vloeistof (ISF).

6.2. Doelstellingen

In deze studie was het doel de verkenning van het potentieel van elektrochemische technieken in combinatie met het gebruik van microneedle-arrays bij de ontwikkeling van een draagbare sensor voor MDMA-detectie in interstitiële vloeistof.

6.3. Materialen en methoden

Een elektrochemisch platform werd geconstrueerd op een microneedle-array door de MN's te vullen met geleidende pasta's van grafiet voor de tegen- en werk-elektroden, en van Ag/AgCl voor de referentie-elektrode. Het verkregen platform werd vervolgens bevestigd aan een zeefdrukverbinding, waardoor het kon worden ingebracht in een draagbare potentiostaat die kan worden bediend en van stroom kan worden voorzien door een smartphone. De verkregen draagbare sensor kan aan de arm van een patiënt worden bevestigd, de huid doorboren met de MN's en de interstitiële vloeistof bereiken, waardoor de analyse van stoffen die vanuit het bloed in deze matrix overgaan, mogelijk wordt.

De methodologie van de studie omvatte drie hoofdstappen, namelijk de optimalisatie van het platform en de adsorptietijd, de prestatie-evaluatie in buffer, inclusief kalibratie- en selectiviteitsstudies, en de prestatie-evaluatie in AISF, inclusief kalibratie-, stabiliteits- en omkeerbaarheidsstudies.

6.4. Resultaten en discussies

De optimalisatie van het platform werd uitgevoerd door verschillende nanostructuren te evalueren, zowel metalen nanopartikels als koolstofhoudende nanomaterialen, waarbij de beste prestaties werden verkregen met single-walled carbon nanotubes (SWCNTs) en gereduceerd grafeenoxide (rGO). Deze platformen werden verder onderzocht voor de vaststelling van de adsorptietijd, waarbij werd geconcludeerd dat SWCNTs en 2,5 minuten optimaal waren.

De prestatie-evaluatie in buffer toonde aan dat het SWCNTs-gefunctionaliseerde platform de detectie van MDMA mogelijk maakte over een breder concentratiebereik, met een lage LOD (minder dan 2 uM). Dit platform werd vervolgens gebruikt in de selectiviteitsstudie voor de analyse van binaire mengsels van MDMA met componenten van biovloeistoffen en verbindingen van illegale producten, waaronder andere illegale drugs, hun metabolieten en veelvoorkomende adulteranten/snijmiddelen. De meeste recovery testen waren boven de 87%. Vervolgens werd de prestatie van het geoptimaliseerde platform geëvalueerd in AISF, waarbij een vergelijkbare respons werd verkregen als bij de analyse in buffer. Om de mechanische robuustheid van het platform te beoordelen, werd een parafilm-doorpriktest uitgevoerd, gevolgd door de evaluatie van de prestaties bij kamertemperatuur en bij 33^0C. In het eerste geval was de signaalrespons vergelijkbaar met die verkregen vóór de parafilm-doorpriktest, terwijl bij 33^0C een toename van de respons werd waargenomen. Tot slot werden de omkeerbaarheid en stabiliteit van de respons geëvalueerd met behulp van de geoptimaliseerde methode, waarbij waarden onder de 7% standaardafwijking werden verkregen.

6.5. Conclusies

Een draagbare MN-sensor gefunctionaliseerd met SWCNTs werd ontwikkeld en de prestaties ervan werden geëvalueerd in zowel buffer als AISF, waarbij uitstekende analytische parameters werden verkregen [35].

7. Algemene discussies

In dit gedeelte worden de resultaten van de elektrochemische strategieën uit de eerste drie studies geïntegreerd en besproken voor alle onderzochte illegale drugs. Detectievensters voor hun identificatie werden geïntegreerd in een protocol voor de analyse van verdachte monsters, dat werd toegepast voor de interpretatie van alle resultaten die werden verkregen voor de elektrochemische screening van de 215 in beslag genomen monsters. De elektrochemische methode vertoonde over het algemeen goede resultaten, met een betere analytische prestatie op het gebied van zowel gevoeligheid als nauwkeurigheid in vergelijking met het draagbare Raman-apparaat en over het algemene analytische prestatie inferieur aan die van het FTIR-apparaat. Desalniettemin kan de ontwikkelde elektrochemische strategie aanzienlijke verbeteringen brengen op het gebied van de detectie van illegale drugs op een gedecentraliseerde manier.

8. Algemene conclusies

In deze thesis werd onderzoek gedaan naar elektrochemische technieken als snelle en draagbare methoden voor gedecentraliseerde tests in forensische druganalyse. Het doel van het ontwikkelen van snelle en eenvoudige strategieën voor de detectie van illegale drugs werd bereikt, zowel voor het screenen van verdachte monsters die door wetshandhavingsinstanties ter plaatse worden aangetroffen met behulp van wegwerp-SPE, als voor de detectie en monitoring van MDMA in interstitiële vloeistof met behulp van een draagbaar elektrochemisch platform.

9. De Oorspronkelijkheid en Innovatieve Bijdragen van de thesis

De originaliteit van deze thesis ligt in de ontwikkeling van nieuwe elektrochemische strategieën die toepasbaar zijn in de forensische druganalyse, zowel in in beslag genomen monsters als in biologische vloeistoffen (bijvoorbeeld ISF). Gedurende dit project werden nieuwe nanomateriaal-gebaseerde platformen (GPH, MWCNTs en SWCNTs) met succes ontwikkeld om de analytische prestaties van de sensormethoden te verbeteren.

Belangrijk is dat de prestaties van de ontwikkelde strategieën voor het screenen van verdachte monsters zijn beoordeeld in reële stalen en zijn vergeleken met veelgebruikte draagbare apparaten, wat bijdraagt aan de implementatie en validatie van de elektrochemische methoden voor on-site analyse van illegale drugs.

Over het algemeen draagt het onderzoek bij aan de voortdurende inspanningen van de onderzoeksgemeenschap, wetshandhavingsinstanties en forensische laboratoria om de illegale drugshandel en -misbruik te bestrijden, en brengt het de voordelen van elektrochemische methoden naar het veld van forensische analyse, waarbij de veiligheid en beveiliging van onze samenleving worden verhoogd.

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