Development and Validation of Point-of-Care Deployable Sensors for the Rapid Detection of Emerging New Psychoactive Substances (NPS): N-ethylpentylone and MDPHP

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A thesis submitted in fulfilment of the requirements of the Manchester Metropolitan University for the degree of Master of Science (by Research)

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2020

Dedication

I would like to dedicate this thesis to my parents Aida and Shafaq Ali, alongside my grandfather Captain Abdul Qayyum Meer who would have been proud of the work I have done. My parents have shown me nothing but patience, support, and I will forever be grateful for their help to become the best version of myself.

Acknowledgments

Firstly, I would like to thank my supervisors Dr Oliver Sutcliffe and Dr Dale Brownson for their patience and support throughout my research. Dr Oliver Sutcliffe has played a pivotal role in my success and I would not have been able to complete my research without the support he has provided. I would like to thank the lab technicians and other colleagues, who have always taken the time to teach and help when needed.

On a more personal note, I would like to thank my parents and sisters, Hidayah and Hadiyqah, for the endless support they have provided myself throughout my masters. I would like to thank the people closest to me, particularly Farah Rai for playing a huge part in my support system and always being there when needed, and Ateeque Ahmad Din, who has motivated me continuously and supported me throughout my masters.

Abstract

The continuous synthesis of new psychoactive substances on the drug market highlights the requirement for a portable, rapid analytical tool with the ability to detect and quantify substances at low concentrations, whilst providing sensitive and reproducible results. Synthetic cathinone's are the second largest group of new psychoactive substances and are increasingly popular among drug abusers due to their stimulant effects and availability. In this study, the electrochemical detection of two synthetic cathinone's, 3',4'-methylenedioxy-α-pyrrolidinohexiophenone (MDPHP) and N-ethylpentylone (NEP) were studied using screen-printed graphene electrodes. In regards to cyclic voltammetry, the linear ranges were found to be 25 – 600 μg mL^{-1} in acetate buffer (0.1 M, pH 5.5) for NEP and 100 – 1000 μg mL^{-1} in acetate buffer (0.1 M, pH 5.5) for MDPHP.

Whilst using cyclic voltammetry in regards to the NEP, the linear ranges were found to be 25 – 600 μg mL^{-1} in acetate buffer (0.1 M, pH 5.5) using the control NEP and 25 – 500 μg mL^{-1} in acetate buffer (0.1 M, pH 5.5) using the street sample of NEP. The linear range for MDPHP was found to be 100 – 1000 μg mL^{-1} in acetate buffer (0.1 M, pH 5.5) and in spiked diluted human urine, for both the control and street sample. The corresponding limit of detection were calculated to be 0.046 μg mL^{-1} , 0.104 μg mL^{-1} , 0.195 μg mL^{-1} , 0.130 μg mL^{-1} and 0.205 μg mL^{-1} for the NEP control and street sample in acetate (0.1 M, pH 5.5) , and the MDPHP control and street sample in acetate (0.1 M, pH 5.5) and spiked diluted human urine, respectively. To increase sensitivity of the method, differential pulse voltammetry was utilized and the linear ranges were found to be 25 – 100 μg mL^{-1} for NEP and 100 – 1000 μg mL^{-1} for MDPHP. The corresponding limit of detection were calculated to be 0.130 μg mL^{-1} for NEP and 0.348 μg mL^{-1} for MDPHP. Quantification of MDPHP street samples were also recorded and the possible interference of common adulterants was tested using cyclic voltammetry. The electrochemical techniques studied show that the detection and quantification of synthetic cathinone's are viable and serves potential to develop as a portable analytical detector.

Abbreviations

CSEW – Crime Survey for England and Wales

CV – Cyclic voltammetry

DPV – Differential pulse voltammetry

EMCDDA – European Monitoring Centre for Drugs and Drug Addiction

MDMA - 3,4-Methylenedioxymethamphetamine

MDPHP - , 3',4'-methylenedioxy-α-pyrrolidinohexiophenone

MDPV- 3,4-methylenedioxypyrovalerone

NEP – N-ethylpentylone

NPS – New psychoactive substances

SPE – Screen-printed electrode

UNODC – United Nations Office on Drugs and Crime

WHO – World Health Organization

Contents

1. Introduction

1.1. Drug abuse

Around 271 million people across the globe used drugs in the previous year according to the 2018/19 World Drug Report by The United Nations Office on Drugs and Crime (UNODC)[1], which is now 30 per cent higher than in 2009. The continual increase in drug misuse globally shows an urgent need for international cooperation to develop efficient detection methods, prevent supply and work with heath and law enforcements to ensure the correct response to drug misuse. The Crime Survey for England and Wales (CSEW)[2] 2018/19 shows that in the United Kingdom itself around 1 in 11 (9.4%) adults aged 16-59 had taken a drug in the last year, which equates to around 3.2 million people, out of which around 3.7% had taken a Class A drug in the last year. Both figures show an upward trend in comparison to previous years and once again highlight an ongoing issue. The drug market has proved a difficult area to maintain control over given the diversification of substances available, especially the focus put onto the misuse of prescription drugs and the development of new psychoactive substances (NPS), also known as synthetic drugs, over the last decade. New psychoactive substances are structurally similar to existing illicit drugs and mimic the desired effects, however due to the usually higher potency and potential combination of unknown drugs to customers, there is a greater risk of fatalities and long-term effects. As new substances are constantly being synthesized and sold as alternatives to, or mixed with, controlled substances, there is a considerable challenge for prevention and treatment. The main factors that have increased popularity for NPS use is the low price and availability with the possibility to avoid using legally banned substances. Users believe that these substances are legal alternatives to the controlled drugs, hence commonly referred to as “legal highs”.

1.1.1. Types of Drugs

The UNODC defines new psychoactive substances as "substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat"[3]. Following this, new psychoactive substances have been categorised into seven key types[4]: hallucinogens, dissociative, opioids, cannabinoids, depressants, empathogens and stimulants. The 2019 World Dug Report by the United Nations Office on Drugs and Crime (UNODC)[5] highlights the increase of stimulant use: after cannabinoids, stimulants constitute the second most widely used group of drugs used across the globally, accounting for 68-million yearly users within the past year.

1.1.2. Drug Laws

There have been international responses to the continuous emergence of new psychoactive substances by the World Health Organization (WHO) Expert Committee on Drug Dependence (ECDD)[6], who carry out in-depth evaluations of reported psychoactive substances, in order to determine whether the substances should be placed under international control. The European Union also responded by creating the European Union Early Warning System (EWS)[7] which is operated by the EMCDDA and Europol, which allows for monitoring, detection, assessment and response to health and social threats by these substances. Many countries have specific new psychoactive substance related legislation including Austria (New Psychoactive Substances Act)[8], Ireland (Criminal Justice Psychoactive Substances Act 2010)[9] and New Zealand (The Psychoactive Substances Act 2013)[10].
The UK Misuse of Drugs Act (1971) was introduced to provide a legislative framework to prevent the misuse of controlled drugs by preventing manufacture, supply and possession. A complete ban was placed on possessing, importing and exporting controlled drugs unless otherwise stated by regulations. Controlled drugs can be divided into three classes: Class A, B and C. Examples of drugs and the penalties for each class are stated in table 1.

Table 1. Drug class system

Class	Drug	Penalties
Class A	• Heroin • Cocaine • Ecstasy (MDMA) • LSD • Methadone	Possession of drug: • Unlimited fine • Up to 7 years in prison Supply / Production • Unlimited fine • Up to life in prison
Class B	• Cannabis • Ketamine • Synthetic cannabinoids • Synthetic cathinones • Amphetamines	Possession of drug: • Unlimited fine • Up to 5 years in prison Supply / Production • Unlimited fine • Up to 14 years in prison
Class C	• Diazepam • Piperazines • Khat • Anabolic steroids	Possession of drug: • Unlimited fine • Up to 2 years in prison Supply / Production • Unlimited fine • Up to 14 years in prison

As stated above, although the manufacture, supply and possession of controlled drugs is prohibited unless permitted by regulations. The Misuse of Drugs Regulations (2001) defines the conditions that the supply and possession of controlled drugs is permitted and under which professional capacities. Controlled drugs are divided into five Schedules, which defines the requirements surrounding the possession, production, supply and prescription of the drugs. Details of each Schedule are shown in table 2.

Table 2. Schedule system

Schedule	Example of Drug	Requirements
1	• Ecstasy type drugs • Hallucinogenics • Cannabis • Raw opium	• A Home Office licence is required for production, supply or possession. • Controlled drugs supplied by a pharmacy must be recorded in a controlled drug register.
2	• Opiates (morphine) • Cannabis-based products • Cocaine • Heroin	• Possession and supply is allowed for pharmacists and others stated in the 2001 legislation • Controlled drug register is required
3	• Gabapentin • Tramadol • Pregabalin	• Safe custody requirements • Records in register is not required but invoices must be kept for 2 years
4	• Minimal control drugs • Midazolam • Anabolic steroids	• No requirements for controlled drug register records or safe custody
5	• Preparations of particular controlled drugs (e.g. morphine)	• Exempt from controlled drug requirements due to low strength of drug

Although the legislation put into place was useful and placed a ban over the supply/ possession of the controlled drugs, the emergence of psychoactive substances became increasingly popular in order to avoid the stated laws, as the substances were similar but not the exact controlled drug. Therefore, the UK Psychoactive Substances Act came into effect on 26th May 2016 and made it an offence to produce, supply, and offer to supply, possess with intent to supply, import or export psychoactive substances with a maximum seven years' imprisonment penalty[11].

1.1.3. Cathinones

Cathinone is a naturally occurring alkaloid found in the leaves of the khat plant (Catha edulis) found in North East Africa and the Arabian Peninsula. Peter Forskal first discovered the shrub in the eighteenth century and methcathinone was the first synthetic cathinone to be synthesized in 1928[12], followed by mephedrone which was first mentioned in 1929 in the Bulletin de la Societe Chimique de France[13]. Abuse of these substances was not reported till the early 21st century, when in 2007 mephedrone was the first synthetic cathinone to be detected by European Authorities in 2007 and then in 28 European countries in 2010. Synthetic cathinones belong to the class psychostimulants, due to their stimulant nature they act on the central nervous system and increase alertness and can cause behavioural excitement[14]. They are often referred to by their street name of "bath salts" and are sold as stimulants via internet forums or "headshops", prior to the new psychoactive substance ban in 2016, under names such as "Flakka", "Ivory Wave", "Vanilla Sky" and "Cloud Nine"[15]. Reported effects include extreme paranoia, hallucinations, increased energy and aggressiveness[16]. Synthetic cathinones alongside synthetic cannabinoids makeup the largest groups of new psychoactive substances monitored by the EMCDDA and reflects the demand for stimulants in Europe.

1.1.4. N-ethylpentylone

N-Ethylpentylone (NEP) is a synthetic cathinone synthesized in the 1960's for pharmaceutical use and it first appeared in USA in 2014 and more prominently in Europe in 2016. The substance is used as an alternative to MDMA (commonly known as "ecstasy" or "molly") and majority of the time drug users are unaware of the contents as the tablets are marked with the same logo characteristics as MDMA. Although these tablets appear to be similar, NEP is three to four times more potent than MDMA, and leads to extreme negative effects including severe insomnia, paranoia, aggressiveness and drug-induced psychosis. Not only does it cause longer-lasting negative effects, there have been reports of fatalities due to the unexpected high potency of the substance globally, including two fatalities in Alabama in 2017 reported by Atherton et al[17] and one in Baltimore reported by Ikeji et al[18] in 2018. NEP has been detected in various pills over the past year, especially common in festivals in the UK: The Loop reported that yellow "Super Mario" pills were being circulated in Cornwall[19].

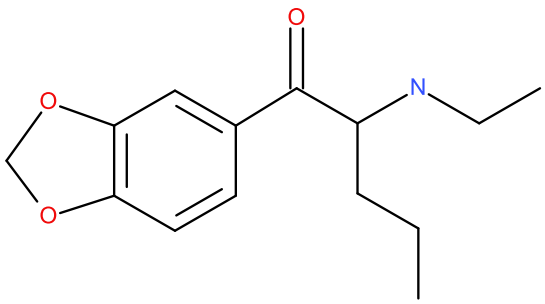


Figure 1. Structure of N-ethylpentylone

1.1.5. MDPHP

Another synthetic cathinone that has recently appeared on the UK drug market is 3',4'-methylenedioxy-α-pyrrolidinohexiophenone (MDPHP), shown in figure 2 , which is an analog of 3,4-methylenedioxypyrovalerone (MDPV), shown in figure 3, and differs by the addition of a single carbon to the alkyl side chain. MDPV was first developed in 1969 by a team at Boehringer Ingelheim[20] as a possible pharmaceutical drug for chronic fatigue and resurfaced as a drug of abuse in the early 21st century, with formal notification to the EMCDDA being made in 2008[21]. Similarly MDPHP was first synthesized in 1960 but only recently appeared as a recreational drug after MDPV was put under control of the Misuse of Drugs Act (1971) in 2010 as a Class B drug. Commonly known as “Monkey-Dust”, the synthetic cathinone received widespread media attention and was branded as “Zombie-Dust” or “Cannibal-Dust” as there are many reports claiming users’ experienced super-human strength and a zombie-like attraction to eat human faces. However, there is little evidence to support the claims that MDPHP is the reason for these effects, as many reports were proven incorrect and baseless. Due to limited history of use, there is difficulty in determining the long-term effects MDPHP may have; however, we can gain insight into the short-term effects by referring to case studies of use (later discussed) and reports from authorities. Reports of MDPHP gained increase in 2018 in the UK, particularly Staffordshire, as Staffordshire Police reported 950 calls related to the drug in a three-month time frame[22]. First-responders from the West Midlands Ambulance Service released a statement regarding their experience with MDPHP users, explaining that a variety of effects have been seen on individual patients,

including paranoia and lack of fear[23]. It can be concluded that MDPHP displays common stimulant effects, with some users reacting more severely.

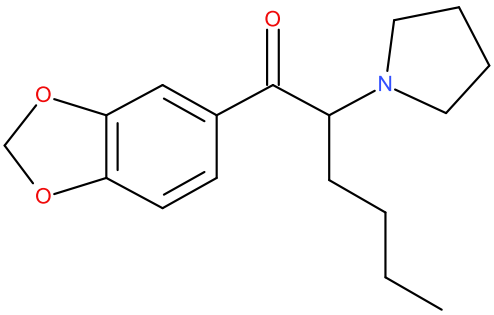


Figure 2. Structure of MDPHP

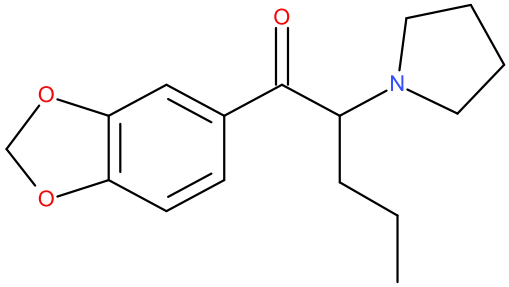


Figure 3. Structure of MDPV

1.2 Existing Detection Methods

The constant synthesis of new substances has highlighted an urgent need to develop detection and quantification methods that can also be used by first-responders or law enforcements: a number of researchers globally have used a range of analytical techniques to detect and characterize synthetic cathinones.

Liquid chromatography-high resolution mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are the most popular choices for the analysis of synthetic cathinone's. In 2019 Błażewicz et al.[24] identified and analytically characterized analytically characterized seven cathinone derivatives using liquid chromatography-high-resolution tandem mass spectrometry (LC-ESI-QTOF-MS), gas chromatography with mass spectrometry (GC-EI-MS) and nuclear magnetic resonance spectrometry (NMR). The detection of and characterization of the following synthetic cathinone's was reported: N-propylcathinone, 2,4-dimethylmethcathinone (2,4-DMMC), 2,4-dimethyl-a-pyrrolidinopropiophenone (2,4-DMPPP), 2,4-dimethylethcathinone (2,4-DMEC), 4-bromo-α-pyrrolidinopropiophenone (4-Br-PPP), 1-(2,3-dihydro-1H-inden-5-yl)-2-(pyrrolidin-1-yl)hexan-1-one (5-BPDi) and 2,4-dimethylisocathinone (4-iso-DMC). The premise of research was to focus primarily on using the given techniques to elucidate the synthetic cathinone derivatives structures, rather than developing a more sensitive method of detection than

previously reported. This research is useful for characterisation of a cathinone but does not improve existing methods to detect the cathinone's successfully. The techniques used in this research are all laboratory based so cannot be utilised in-the-field.

In 2015 Hong *et al*[25]. conducted a study that proposed a method using gas chromatography-mass spectrometry (GC-MS) to analyse and quantify the following six synthetic cathinone's in urine samples: mephedrone (4-MMC), methylone (bk-MDMA), butylone, ethylone, pentylone and methylenedioxypyrovalerone (MDPV). The results found that the limit of detection for the proposed method was 5 ng mL^{-1}, with the exception of MDPV, which was 20 ng $mL^{-1}$and the limit of quantification was 20 ng mL^{-1}, with the exception of MDPV, which was 50 ng mL^{-1}. The findings prove a valid detection method was developed, sensitive enough to detect a small concentration in a urine sample, which could be useful for forensic science laboratories analysing biological samples containing the synthetic cathinone's studied. Although the method was successful in the detection of the given synthetic cathinone's, the technique of GC-MS can only be used within a laboratory setting and does not provide quick in-the-field results. Further work in 2018 researched the discrimination of synthetic cathinone's by GC-MS and GC-MS/MS using cold electron ionization by Levitas *et al*[26] in which classical and cold electron ionization were compared. For the 35 synthetic cathinone's tested in this study there was a noticeable improvement in the molecular ion relative intensity and in many cases cold electron ionization yielded additional fragment ions compared to classical electron ionization. Other analytical techniques have been explored by researchers: in 2013 Mabbott *et al*[27] used surface enhanced Raman scattering (SERS) which again shows a technique that can successfully detect cathinone's in a laboratory based setting, but can not be used to produce quick, reliable results in-the-field.

A different analytical technique was explored by LaPointe *et al*[28] by using direct analysis in real time mass spectrometry (DART-MS) to characterize and analyse three synthetic cathinone's and three metabolites in urine without any sample preparations. The method of DART-MS analysis proved successful in detecting and characterizing the cathinones and its metabolites with speed and efficiency at clinically relevant levels (ng mL^{-1}) in urine. The detection of the synthetic cathinone's in urine without sample preparation is a great

development as this can be used to further explore the possibility of a sensor that can be used by frontline workers who deal with biological samples. However, the technique of DART-MS is laboratory based and will not be able to be used in-the-field as a portable sensor.

Traditional analytical techniques have clearly proved successful by many researchers, with the use of HPLC and GC-MS with LC-MS being the most common choice; however, law enforcements require a method that is adaptable to become a portable in-the-field device, producing the same or even better sensitivity to small concentrations. Electrochemistry is a branch of chemistry recently explored in regards to the detection of new psychoactive substances: it is an advantageous analytical tool as it has the potential to work as an in-the-field device given its fast and reliable response, as well its portability in comparison to an offsite laboratory method.

1.2.1 Electrochemistry

Electrochemistry studies the relationship between electron transfer and chemical change: electrochemical reactions involve the transfer of charge from a charged species across an electrode to a solution phase species. The energy of the charged species is dependent on the potential of the phase the species is in: a potential is set up across the two phase when a metal is partly immersed in an electrolyte i.e. an electrode/electrolyte interface (or a solution/electrode interface)[29]. As electrons move towards the equilibrium a net charge separation is developed and a potential difference is created across the interface of the two phases (at the solution/electrode interface) as the charge transfer occurs. The potential difference is measured by the use of a circuit consisting of a surface/electrode interface and a reference electrode that maintains a fixed potential difference. The electrochemical cell setup usually involves the use of three electrodes: a working electrode, a counter electrode and a reference electrode connected to the potentiostat, which controls the potential difference between the reference and counter electrode. The working electrode is where the reaction of interest occurs, which are commonly made of inert materials such as Au, Pt, glassy carbon and Ag etc. The counter electrode is a non-reactive high surface area electrode used to close the current circuit in the electrochemical cell, which is usually made of inert materials such as Pt, Au and Graphite. The reference electrode has a well-known electrode potential and is used as the point of reference for the potential control and

measurement[30]. Since the 1990s[31], screen-printing technology has produced inexpensive and highly reproducible single-use sensors, which is ideal for a portable in-the-field device. The application of this technology to electrochemical detection allows all three electrodes to be combined onto one surface to create a screen-printed electrode (SPE) which is portable, economical and disposable. Graphene has been explored as a highly promising material for electrochemical sensing and is used to make screen-printed graphene electrodes: graphene possesses a much larger specific surface area (2630m^2 g^{-1}) and an excellent electrical conductivity (7200 Sm^{-1}) compared with other carbon materials[32]. This is useful as a larger surface area is required for the counter electrode, so that it is higher than the area of the working electrode and will not be a limiting factor in the kinetics of the electrochemical process.

In 2012 by Krishnaiah *et al*[33] were the first researchers to report the electrochemical behaviour of a synthetic cathinone: the study focused on mephedrone in basic conditions using a mercury dropping electrode. An analytical range of 2.7 x 10^{-4} to 1.8 µg mL^{-1} with a detection limit of 2.2 x 10^{-3} µg mL^{-1} was reported. Although the study was successful in reporting the electrochemical behaviour of mephedrone, the use of mercury is not practical for a portable device, as it is considered a harmful chemical[34]. To further develop this work and explore the use of electrochemistry in the detection of synthetic cathinone's, Smith *et al*[35] researched the effect of scan rate of pH on the detection of methcathinone mephedrone and 4'-methyl-N-ethylcathinone (4-MEC). All three cathinone's were electrochemically detected using boron-doped diamond, glassy carbon and screen-printed graphite macroelectrodes in a range of buffers: limits of detection for methcathinone, mephedrone and 4'-methyl-N-ethylcathinone (4-MEC) were 44.5, 39.8 and 84.2 µg mL^{-1} respectively. The study shows for the first time the electrochemical detection of the cathinone class and proves that the electrochemical technique provides useful analytical parameters and may be further explored to develop a portable sensor.

1.2.2. Aims

This project will aim to detect and quantify both N-ethylpentylone and MDPHP electrochemically using techniques such as cyclic voltammetry and differential pulse voltammetry. Reference standards and corresponding street samples of N-ethylpentylone and MDPHP will be fully characterised using ^{1}H- and ^{13}C-NMR, gas chromatography-mass spectrometry (GC-MS) and infrared spectroscopy. Once characterised, an electrochemical detection method will be developed and validated (vs. GC-MS) for both N-ethylpentylone and MDPHP using cyclic voltammetry and differential pulse voltammetry. Once the methods have been validated, the street samples (provided by law enforcement agencies) will be utilised to determine the applicability of the optimised electrochemical method for the quantification of the two target analytes in real-world samples. As there is little research and information in regards to N-ethylpentylone, it is necessary that this thesis aims to successfully detect the drug and this can be used as the basis for future developments. The detection of MDPHP will be tested using electrochemical techniques, due to the high number of seized samples across the country it is apparent that is necessary to investigate the possibility that MDPHP may be detected in a biological sample using an electrochemical technique. If the detection of MDPHP is shown to be successful in a biological sample in this thesis, this will be further investigated and developed in the future. The primary focus of this thesis is to prove the successful detection of both drugs for the first time.

1.2.3. Electrochemical Properties

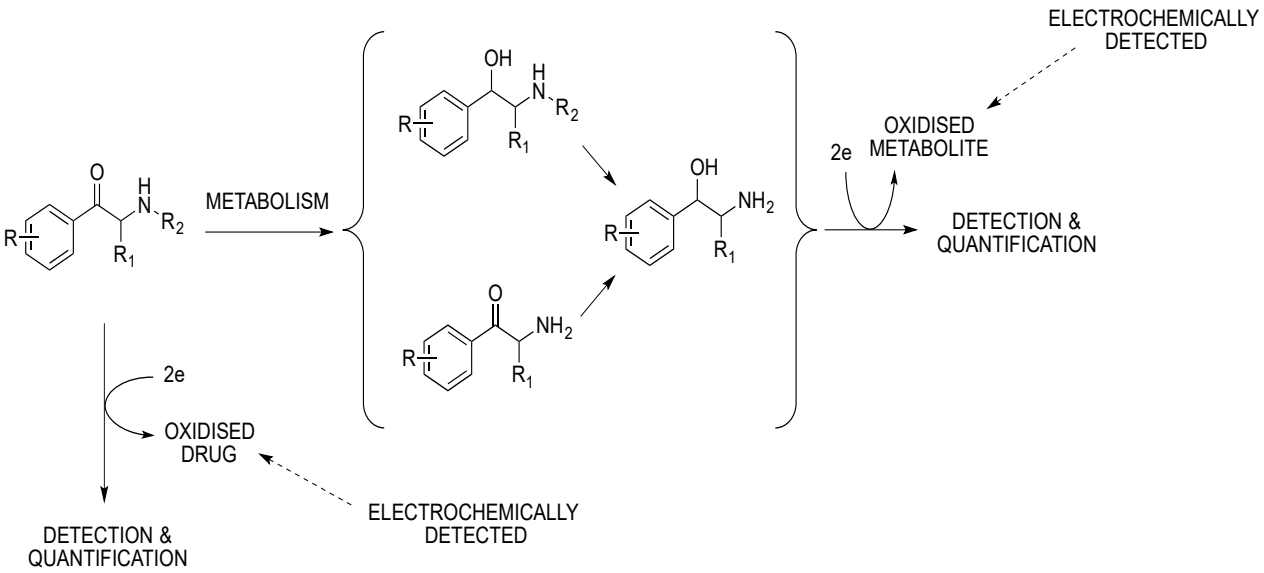


Figure 4. Electrochemical properties of a synthetic cathinone

Figure 4 shows the detailed electrochemical properties of a synthetic cathinone which outlines the no. of electrons transferred in the oxidation and reduction process. This is applicable to both N-ethylpentylone and MDPHP.

2. Experimental

2.1. General Details

All chemicals used were used as received from Sigma-Aldrich (Gillingham, U.K) and were of analytical grade. Solutions were prepared with deionized water of resistively no less than 18.2 Ω cm, unless otherwise stated, and were vigorously degassed with nitrogen to remove oxygen prior to analysis for a minimum of 40 minutes. Regarding the drugs used reference materials were either prepared in-house or obtained, under UK Home Office licence, by authorised personnel and in compliance with both the UK Misuse of Drugs Act (1971) and UK Misuse of Drugs Regulations (2001). Test samples (street samples) were provided by Greater Manchester Police (GMP) personnel, in accordance with the legislation and under the approved Memorandum of Understanding operating between the MANchester DRug Analysis and Knowledge Exchange (MANDRAKE) and GMP. All controlled/restricted materials were stored, transferred, used and destroyed in compliance with the UK Misuse of Drugs Act (1971) and UK Misuse of Drugs Regulations (2001). The voltammetric measurements were recorded using an 'Autolab PGSTAT 101' (Metrohm Autolab, The Netherlands) computer-controlled potentiostat with the Nova 2.0 software. Experiments were performed using screen-printed graphite macroelectrodes (SPEs) which have a 3mm diameter working area which were produced in-house with appropriate stencil designs using a DEK 248 screen-printing machine (DEK, Weymouth, U.K.). The SPE's were characterized and reported previously within literature[1]. The reproducibility of the batch within literature of screen printed electrodes were found to be 0.76%[36] relative standard deviation (RSD) using the $Ru(NH_3)^{2+/3+}$ redox probe in 1M KCL. The heterogeneous rate constant, k^o for the $Ru(NH_3)^{2+/3+}$ redox probe in 1M KCL was found to be $2.12 \times 10{-}3$ cm $^{s-1}$.

Gas Chromatography – Mass Spectrometry (GC-MS) analysis was performed on an Agilent 7890B GC coupled to an Agilent 5977B Mass Spectrometer (Agilent Technologies, Cheadle, UK). GC-MS parameters: Carrier Gas: He; Flow-rate: 1.2 mL/min Column: HP5-MS column (30 m x 0.25 mm x 0.25 µm); MS Range (Scan Mode): 40-550 m/z at 5.4 scans/sec. Injection Volume: 0.5 µL injection (1 mg/mL); Split Ratio: 50:1; Inlet & Transfer Line Temperature: 265°C. Temperature programme: Hold time of 3 minutes at 50°C, ramp at 30°C/min for 8 minutes, then hold for 6 minutes.

2.2. Determination of Method's

Prior to preparing solutions and recording electrochemical measurements using the NEP and MDPHP samples, the methods used were determined using previously stated methods from literature. The two methods used to determine parameters and ensure the system was working correctly are stated in the following sections.

2.2.1. Preparation of standard stock solutions and calibration plot solutions for determination of cyclic voltammetry method: mephedrone (4-MMC)

Four stock solutions of 1000 μg mL^{-1} mephedrone (4-MMC control) were made by weighing out 10 mg into 10mL volumetric flasks, and were made up to the mark with the following buffer solutions: acetate buffer pH 2, 5.5, 9 and 12. All solutions were diluted to achieve the following concentrations: 500, 400, 250,200,125 and 100 μg mL^{-1} of 4-MMC. 25 μg mL^{-1} of each respective solution was applied to the SPE, which was attached to the potentiostat. The cyclic voltammograms were recorded in the range of 0.2 to -1.6 V using a scan rate of 0.05 V/s. This was repeated three times per each pH buffer solution and concentration.

2.2.2. Preparation of standard stock solutions and calibration plot solutions for determination of differential pulse voltammetry method: absorbic acid

A stock solution of 1mM absorbic acid was prepared by weighing out 1 mg into a 10 mL volumetric flask, made up to the mark by pH 4 phosphate buffer solution. The SPE was fully immersed into the solution and differential pulse voltammograms were recorded using the following conditions: modulation amplitude: 0.07 V; modulation time 0.07 s; interval time: 0.7 s; step: -0.005 V and scan rate: 0.5 V/s.

2.3. MDPHP

A sample of control MDPHP and six street samples containing MDPHP were used for the following experiments, the street samples were labelled as: D15, D16, D19, T1D, T4B and T2C. A 2500 μg mL^{-1} stock solution of MDPHP (control) and (each street sample) were made by weighing out 25 mg respectively into 10 mL volumetric flasks and made up to the mark using 0.1 M acetate buffer at pH 5.5 (unless stated otherwise): this was made fresh for each of the following experiments. Each electrochemical measurement was repeated three times with a new SPE used each time. Based on previous literature[36], the expected optimum value

is between 250 μg mL^{-1} and 500 μg mL^{-1} for the detection of synthetic cathinone's. In order to ensure a linear measurable range, the greatest concentration tested will be 1000 μg mL^{-1} and this will be tested in reduced increments in order to determine the optimum concentration for the detection of MDPHP. In regards to the limit of detection, previous literature[18] shows the lowest concentration in a biological sample to be detected is 7 μg mL^{-1}, therefore concentrations lower than this will be tested to attempt to detect MDPHP at a lower concentration using electrochemical techniques.

2.3.1. Preparation of standard stock solutions and calibration plot solutions for cyclic voltammetry (CV): MDPHP Control Sample

Two MDPHP stock solutions were made by weighing out 8 mg and 5 mg in 1 mL vials, which was made up to the mark using 0.1 M acetate buffer pH 4.3, to make an 800 μg mL^{-1} and 500 μg mL^{-1} solution respectively. Both were serially diluted with 0.1 M acetate buffer pH 4.3 to the concentrations 800, 500, 400, 250, 200, 125, 100, 62.5, 50, 31.25, 25 and 10 μg mL^{-1} of MDPHP. 25 μg mL^{-1} of each respective solution was applied to the SPE, which was attached to the potentiostat. The cyclic voltammograms were recorded in the range 0 to -2 V, using 0.05 V/s. This process was repeated three times using three different pH acetate buffers: pH 5.5, pH 9 and pH 12.

2.3.2. Preparation of standard stock solutions and calibration plot solutions for differential pulse voltammetry (DPV): MDPHP Control Sample

5000 μl of the 2500 μg mL^{-1} stock solution of MDPHP (control) was put into a glass vial and then diluted to the following concentrations using 0.1 M acetate buffer pH 5.5: 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 μg mL^{-1}. The SPE was fully immersed into the solution and differential pulse voltammograms were recorded using the following conditions: modulation amplitude: 0.15 V; modulation time 0.15 s; interval time: 0.2 s; step: -0.005 V and scan rate: 0.025 V/s. This process was repeated three times.

2.3.3. Preparation of standard stock solutions and calibration plot solutions for cyclic voltammetry (CV): MDPHP street samples

5000 μl of the stock solution of MDPHP (street sample) was put into a glass vial and then diluted to the following concentrations: 1000, 900, 800, 700, 600, 500, 400, 300, 200 and

100 μg mL^{-1}. The SPE was fully immersed into the solution and cyclic voltammograms were recorded in the range of 0 to -2 V, using the 0.05 V/s. This process was repeated for each street sample, three times.

2.3.4. Preparation of standard stock solutions and calibration plot solutions for cyclic voltammetry (CV): MDPHP street samples spiked with adulterants

Three adulterants were tested in the following experiment: paracetamol, benzocaine and caffeine. 5000 μl of the 2500 μg mL^{-1} stock solution of MDPHP (street sample) was put into a glass vial and diluted to 500 μg mL^{-1} using the 0.1 M acetate buffer pH 5.5. 500 μg (5 mg) of one adulterant was added into the solution and the SPE was fully immersed into the solution: cyclic voltammograms were recorded in the range of 0 to -2 V, using scan rate 0.05 V/s. The adulterant was changed, to ensure each street sample was tested with each adulterant.

2.3.5. Preparation of standard stock solutions and calibration plots for cyclic voltammetry (CV): MDPHP in Urine Solution-Calibration and Limit of Detection

Calibration: A stock solution of urine was made by adding 5 mL of urine into a 100 mL flask and made up to the mark with 0.1 M acetate buffer. 5000 μL of the urine stock solution was put into a glass vial, 200 μL was taken out and 200 μL of the 2500 μg mL^{-1} stock solution of MDPHP (control) was put in to give a concentration of 100 μg mL^{-1}. This process was repeated to achieve concentrations of 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μg mL^{-1}. The SPE was fully immersed into the solution and cyclic voltammograms were recorded in the range of 0 to -2 V, using 0.05 V/s.

Limit of Detection: A new 50 μg mL^{-1} stock solution (labelled "R1") was made by pipetting 2 mL of the 2500 μg mL^{-1} MDPHP (control) stock solution into a 100 mL volumetric flask, which was made up to the mark with 0.1 M acetate buffer pH 5.5. 5000 μL of the urine stock solution was pipetted into a glass vial and 100 μL was taken out and 100 μL of the "R1" solution was added in to achieve a concentration of 1 μg mL^{-1}. This was repeated to achieve

concentrations, 2,3,4,5,6 ,7 and 8 μg mL^{-1}. The SPE was fully immersed into the solution and cyclic voltammograms were recorded in the range of 0 to -2 V, using 0.05 V/s.

2.3.6. Preparation of standard stock solutions and calibration plots for cyclic voltammetry (CV): MDPHP street sample quantification

A 2600 μg mL^{-1} stock solution of MDPHP (control) was made by adding 26 mg MDPHP (control) into a 10 mL volumetric flask, which was made up to the mark with pH 5.5 acetate buffer. 5000 μL of the solution was added to a glass vial and further diluted to achieve concentrations 2500, 2400, 2300, and 2000 μg mL^{-1}. The SPE was fully immersed into the solution and cyclic voltammograms were recorded in the range of 0 to -2 V, using 0.05 V/s. Next, 5000 μL of each 2500 μg mL^{-1} MDPHP (street sample) stock solution was pipetted into a separate glass vial and cyclic voltammograms were recorded in the range of 0 to -2 V, using 0.05 V/s. When making the 2500 μg mL^{-1} MDPHP (street sample) stock solutions for this experiment it was necessary to record the accurate mass of each street sample used in order to calculate the percentage quantification.

2.4. NEP

A sample of control NEP and four street samples containing NEP were used for the following experiments, the street samples were labelled as: SS1, SS2, SS3 and SS4. A 2500 μg mL^{-1} stock solution of NEP (control) and (each street sample) were made by weighing out 25 mg respectively into 10 mL volumetric flasks and made up to the mark using 0.1 M acetate buffer at pH 5.5 (unless stated otherwise): this was made fresh for each of the following experiments. Each electrochemical measurement was repeated three times with a new SPE used each time. Based on previous literature[36], the expected optimum value is between 250 μg mL^{-1} and 500 μg mL^{-1} for the detection of synthetic cathinone's. In order to ensure a linear measurable range, the greatest concentration tested will be 1000 μg mL^{-1} and this will be tested in reduced increments in order to determine the optimum concentration for the detection of NEP.

2.4.1. Preparation of standard stock solutions and calibration plots for cyclic voltammetry (CV); NEP Control Sample pH study

Seven solutions of NEP (control) were prepared by weighing out 0.005 g into 10 mL volumetric flasks, and were made up to the mark with the following buffer solutions: acetate buffer at pH 4.3, 5.5, 9 and 12 and phosphate buffer solution at pH 6, 7 and 8. 25 μg mL^{-1} of each respective solution was applied to the SPE, which was attached to the potentiostat. The cyclic voltammograms were recorded in the range 0 to -2 V, using 0.05 V/s. This was repeated three times per each pH buffer solution.

2.4.2. Preparation of standard stock solutions and calibration plots for cyclic voltammetry (CV): NEP Control Additions study

Four stock solutions of NEP (control) were made by weighing out 10 mg into 10 mL volumetric flaks, and were made up to the mark with the following buffer solutions: acetate buffer pH 4.3, 5.5, 9 and 12. Each solution was diluted to achieve the following concentrations: 1000, 500, 100, 50, 10, 5 and 1 μg mL^{-1}. 25 μg mL^{-1} of each respective solution was applied to the SPE, which was attached to the potentiostat. The cyclic voltammograms were recorded in the range 0 to -2 V, using 0.05 V/s. This was repeated three times per each pH buffer solution and concentration.

2.4.3. Preparation of standard stock solutions and calibration plots for cyclic voltammetry (CV); NEP Street sample

Two NEP stock solutions (per street sample) were made by weighing out 8 mg and 5 mg in 1 mL vials, which was made up to the mark using 0.1 M acetate buffer pH 5.5, to make an 800 μg mL^{-1} and 500 μg mL^{-1} solution respectively. Both were serially diluted with 0.1 M acetate buffer pH 5.5 to the concentrations 800, 500, 400, 250, 200, 125, 100, 62.5, 50, 31.25, 25 and 10 μg $mL^{-1}$of MDPHP. 25 μg $mL^{-1}$of each respective solution was applied to the SPE, which was attached to the potentiostat. The cyclic voltammograms were recorded in the range 0 to -2 V, using 0.05 V/s. This process was repeated four times to ensure each NEP street sample was tested.

2.4.4. Preparation of standard stock solutions and calibration plot solutions for differential pulse voltammetry (DPV): NEP control sample

Two NEP stock solutions (control) were made by weighing out 8 mg and 5 mg in 1 mL vials, which was made up to the mark using 0.1 M acetate buffer pH 5.5, to make an 800 μg mL^{-1} and 500 μg mL^{-1} solution respectively. Both were serially diluted with 0.1 M acetate buffer pH 5.5 to the concentrations 800, 500, 400, 250, 200, 125, 100, 62.5, 50, 31.25, 25 and 10 μg mL^{-1} of MDPHP. The SPE was fully immersed into the solution and differential pulse voltammograms were recorded using the following conditions: modulation amplitude: 0.17 V; modulation time 0.17 s; interval time: 0.25 s; step: -0.05 V and scan rate : 0.02 V/s. This process was repeated three times.

3. Discussion

3.1. Validation of System

In order to benchmark the system and ensure optimisation of the electrode, a scan rate study using ruthenium salt was performed by following methodology stated in previous literature. The effect of voltammetric scan rate on the size of peak current was analysed and as shown in Figure 5 (A), as the voltammetric scan rate was increased, the size of the peak current increased. This is due to the relationship between the diffusion layer and scan rate: a faster scan rate results in a shorter diffusion layer, which then provides a greater size of peak current. The scans also show a variation of peak position: as the voltammetric scan rate increases the peak potential becomes more positive, which suggests the process is electrochemically quasi-reversible.

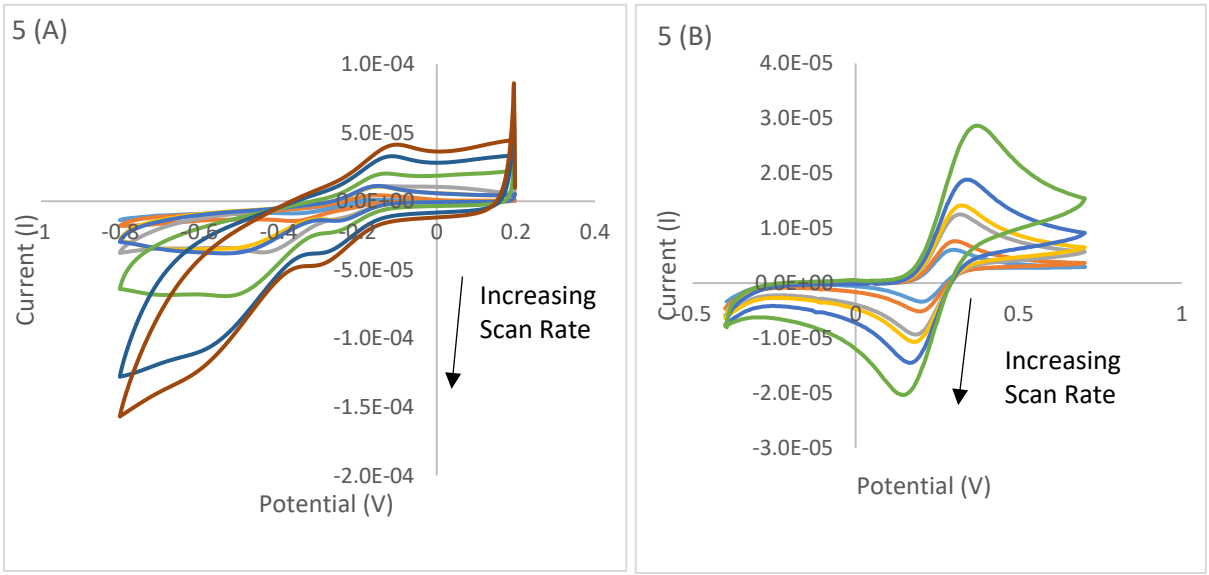


Figure 5 (A) Ruthenium scan rate study (0.005 V s^{-1} to 0.5 V $s^{-1)}$ showing the voltammetric responses of 1mM ruthenium in 1M KCl using screen-printed graphite macroelectrodes (vs. Ag/AgCl). Figure 5 (B) Potassium ferrocyanide(II) scan rate study (0.015 V s^{-1} to 0.5 V s^{-1}) showing the voltammetric responses of 1mM potassium ferrocyanide(II) in 0.1M KCl using screen-printed graphite macroelectrodes

To determine how fast the electron is transferring the difference between reduction and oxidation potentials was found and the heterogeneous rate constant,K^o, was calculated by applying the Nicholson method described in equation 1 and 2. The calculated K^ovalue is $2.12 \times 10^{-3}\mathrm{cm\ s}^{-1}$ which is similar to the literature value of 3.36 × 10^{-3} cm s−1[36], therefore it ensures that the system is working correctly and there is confidence in the system.
The area of the electrode that is reactive was calculated by performing a voltammetric scan rate study using potassium ferrocyanide (II) as shown in figure 5 (B) .

$$\mathrm{K^o} = \Psi(2.49 \times 10^{-6} \times \pi \times 0.1 \times (1859.802) \times 1)$$

Equation 1. Nicholson's Equation used to calculate the heterogeneous rate constant, K^o. $\Psi(\Delta Ep)$ is the peak to peak separation in mV and can be calculated using equation 2

$$\psi = (-0.6288 + 0.0021X)/(1 - 0.017X)$$

Equation 2. Peak to peak separation, which fits Nicholson's data

$$0.630237109\ (2.49 \times 10^{-6} \times \pi \times 0.1 \times (1859.802) \times 1) = 2.12 \times 10^{-3}\mathrm{cm\ s}^{-1}$$

Equation 3. Heterogeneous rate constant, K^o calculation

The Randles-Sevcik equation (equation 4) was used to calculate the area of the electrode based upon peak current, which was found to correspond to $0.013\mathrm{cm}^2$, the geometric area of the electrode is found by $A = \pi r^2 = 0.071\mathrm{cm}^2$. The calculated area is significantly less than the geometric area as the carbon ink contains polymers that are not reactive, which makes parts of the electrode unreactive. The Randles-Sevcik equation (equation 4) can also be used to describe how the peak current increases linearly with the square root of the scan

rate, which means the reaction is diffusional based. This means that if some of the analyte diffuses on the surface there will be excess, rather than the analyte adsorbing onto the surface of the electrode, which leads to deviation from linearity in the plot of peak current vs square root of scan rate.

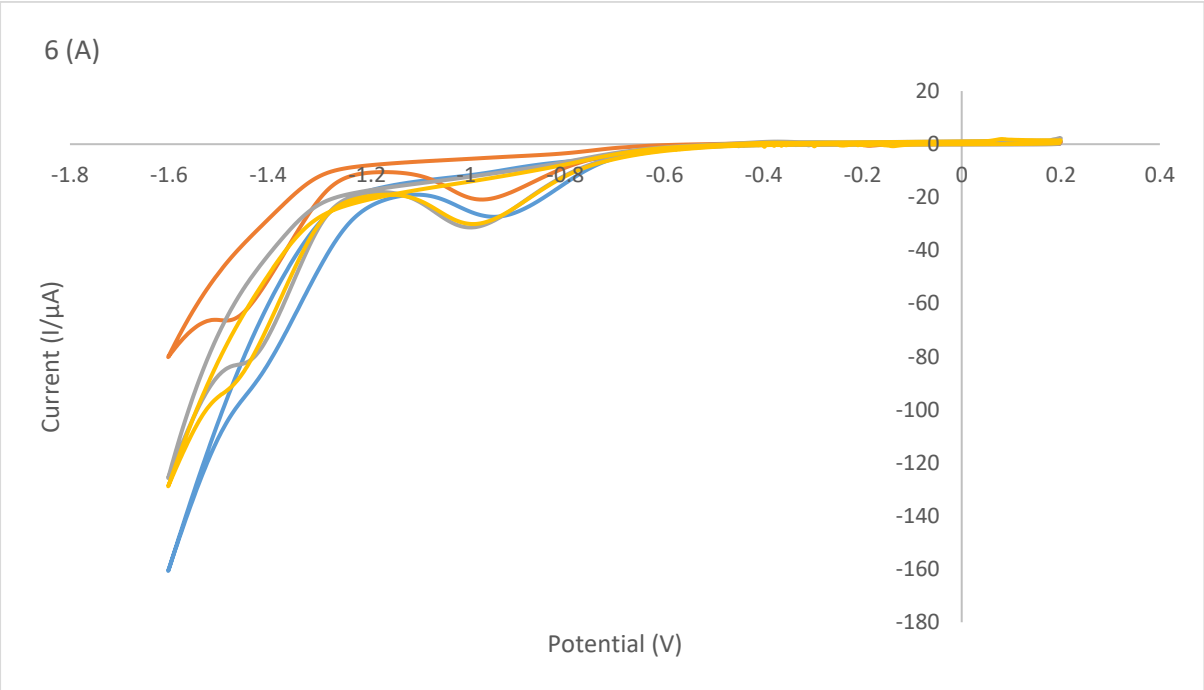
$$i_p = 0.446nFAC^0\left(\frac{nFvD_o}{RT}\right)^{1/2}$$

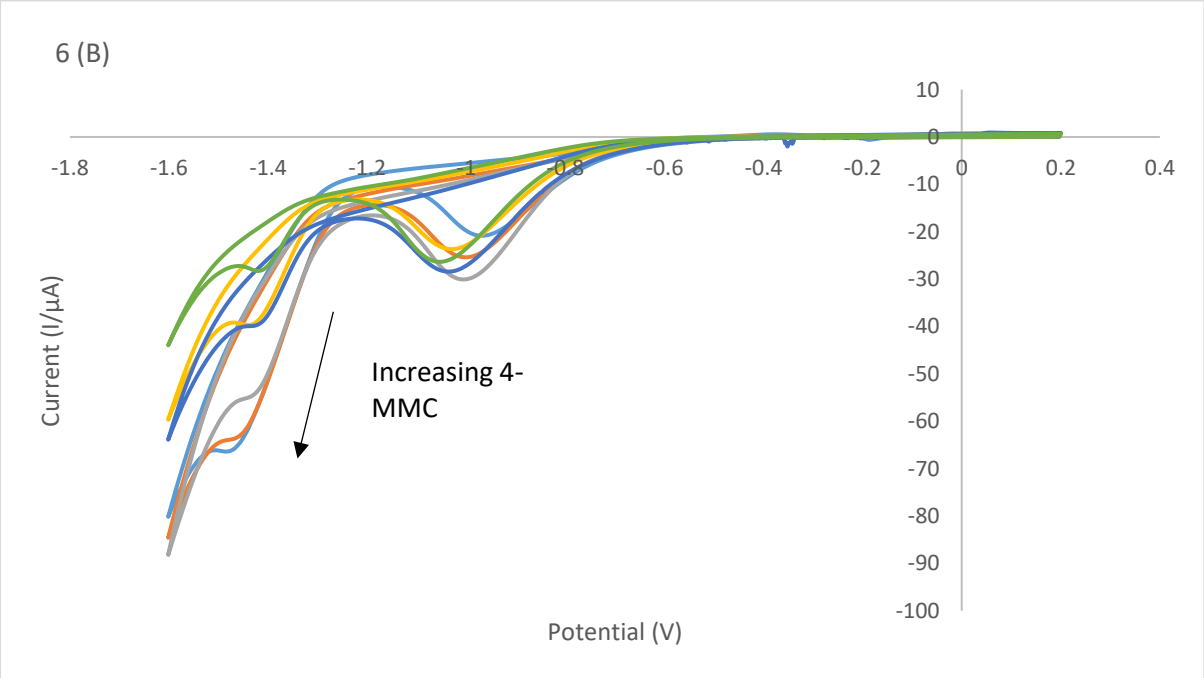
Equation 4. The Randles-Sevcik equation describes how peak current is related to scan rate and can also be used to calculate the area of the electrode and diffusion coefficients. Where, $\mathbf{i_p}$ is the peak current in amperes, A is the electrode area in cm^2, D_o is the diffusion coefficient in $cm^2\ s^{-1}$, C^0 is the concentration in mol cm^{-3}, and $\boldsymbol{v}$ is the sweep rate in $V\ s^{-1}$

As previous research has been conducted by Smith *et al* in regards to the electrochemical detection of mephedrone, which is a popular cathinone, the method used by the researchers was investigated to ascertain the relevance to the electrochemical detection of N-ethylpentylone.

The electrochemical reduction response of 500 μg mL^{-1} mephedrone in 0.1 M acetate buffer solution was studied over the pH range 2 – 12 as shown in figure 6 (A): the reduction peak is not apparent in an acidic environment (pH 2), but is well defined in a more neutral environment (pH 5.5). The peak shape and intensity of the peak is shown to change in different pH environments: the reduction peak is not apparent in an acidic environment (pH 2), and is the most defined and intense in a neutral environment (pH 5.5). The peak intensity decreases and becomes less defined in a basic environment (pH 9 and pH 12). Therefore, pH 5.5 is the optimum acetate buffer for the electrochemical reduction of mephedrone. Following this, figure 6 (B) shows the electrochemical response of a range between 500 – 100 μg mL^{-1} mephedrone in 0.1 M acetate buffer solution pH 5.5: the size of the peak current decreases proportionally to the decrease in concentration of mephedrone, which is also shown in figure 6 (C). This suggests that the peak detected is dependent on concentration. To ensure confidence in the method, the optimum pH and limit of detection was compared: the results show that pH 5.5 is the optimum pH and in the literature pH 4.3 was used, which is close in acidity therefore concurrent. The calculated limit of detection is

equal to 39.18 µg mL^{-1} which can be compared to the literature value of 39.8 µg mL^{-1} [36]. The sensitivity is equal to 2 x 10^{-8} µg mL^{-1}. As the results found in this investigation are similar to the results reported in the literature, the method is shown to be successful and can be used with confidence in the investigation of N-ethylpentylone.





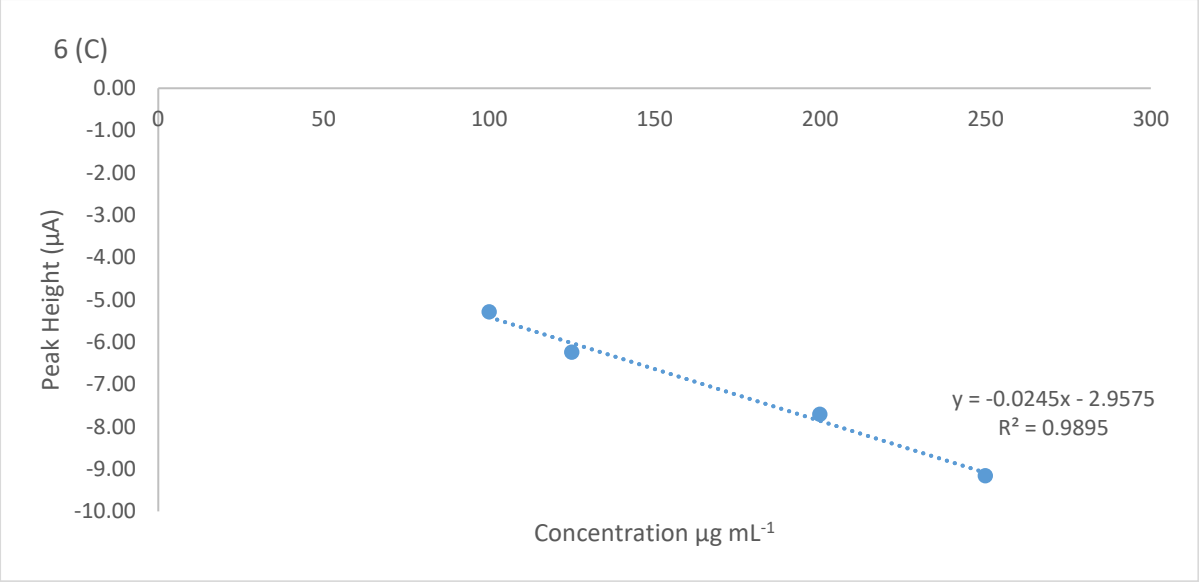


Figure 6 (A) Cyclic voltammograms showing the electrochemical response of 500 µg mL^{-1} mephedrone in 0.1 M acetate buffer at pH 2 (blue), 5.5 (orange), 9 (grey) and 12 (yellow) using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Cyclic voltammograms showing electrochemical response of 500 – 100 µg mL^{-1} mephedrone in 0.1 M acetate buffer solution pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (C) Linear calibration plot showing the relationship between concentration of mephedrone and the peak height within the linear range measurable.

3.2. N-ethylpentylone Results

To determine the optimum conditions for the electrochemical detection of N-ethylpentylone, the electrochemical reduction response was investigated in acetate and phosphate buffer solution (PBS) over a range of pH's and concentrations.

3.2.1. Control Sample in Acetate Buffer

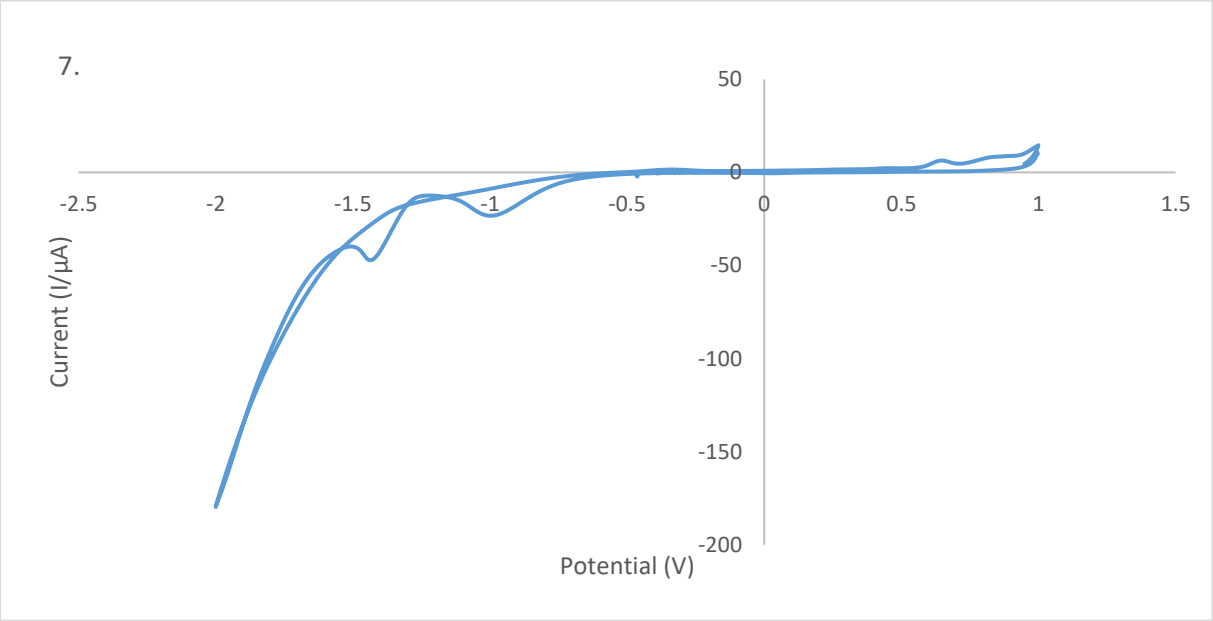
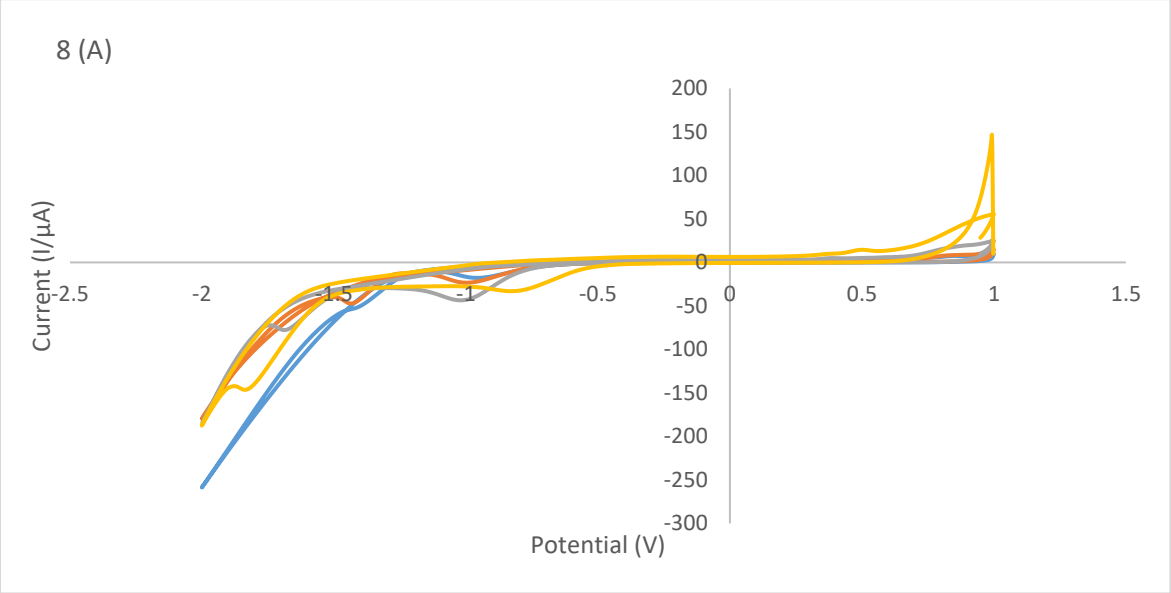


Figure 7. Cyclic voltammogram showing the electrochemical response of 500 µg mL^{-1} N-ethylpentylone in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

The electrochemical response of 500 µg mL^{-1} N-ethylpentylone in 0.1 M acetate buffer was studied at pH 5.5 as shown in figure 7 as pH 5.5 was previously determined as the optimum condition during validation of the system. A scan was performed over the range 1 to -2 V which shows a visible oxidation peak at ≈ 0.64 V and a reduction peak at ≈ -1.4V. There is also a visible residual oxygen peak at -1 V although the solution was thoroughly degassed using nitrogen. The reduction peak has a greater peak intensity in comparison to the oxidation peak, therefore the region in which the reduction peak is visible will be focused on

in future studies. As the chemical process is unknown, the process will be optimised by conducting a pH study.

3.2.2. pH Study



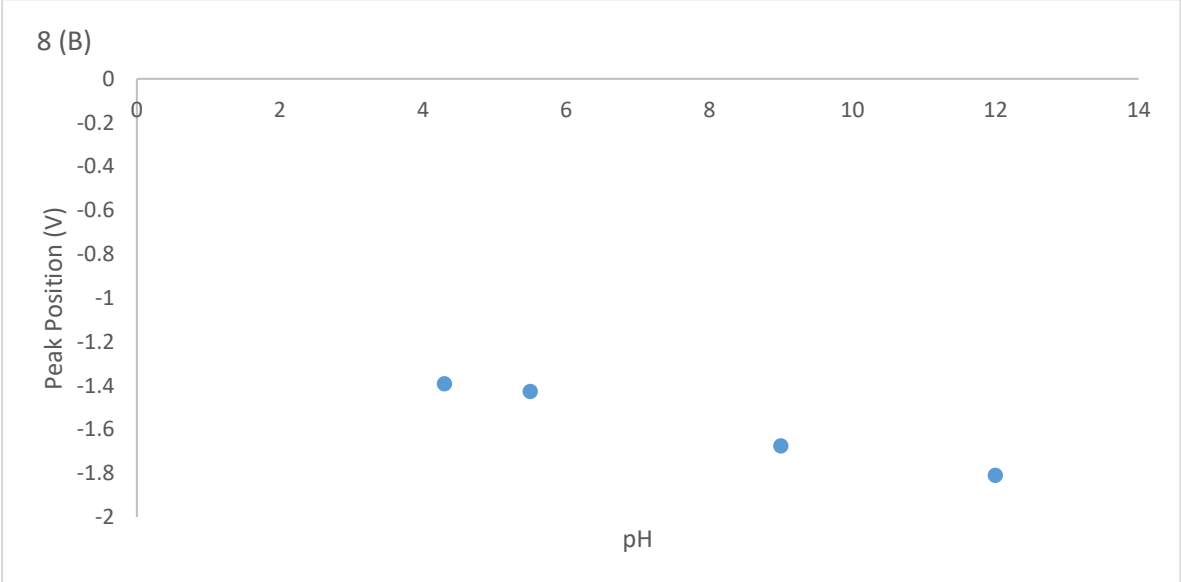
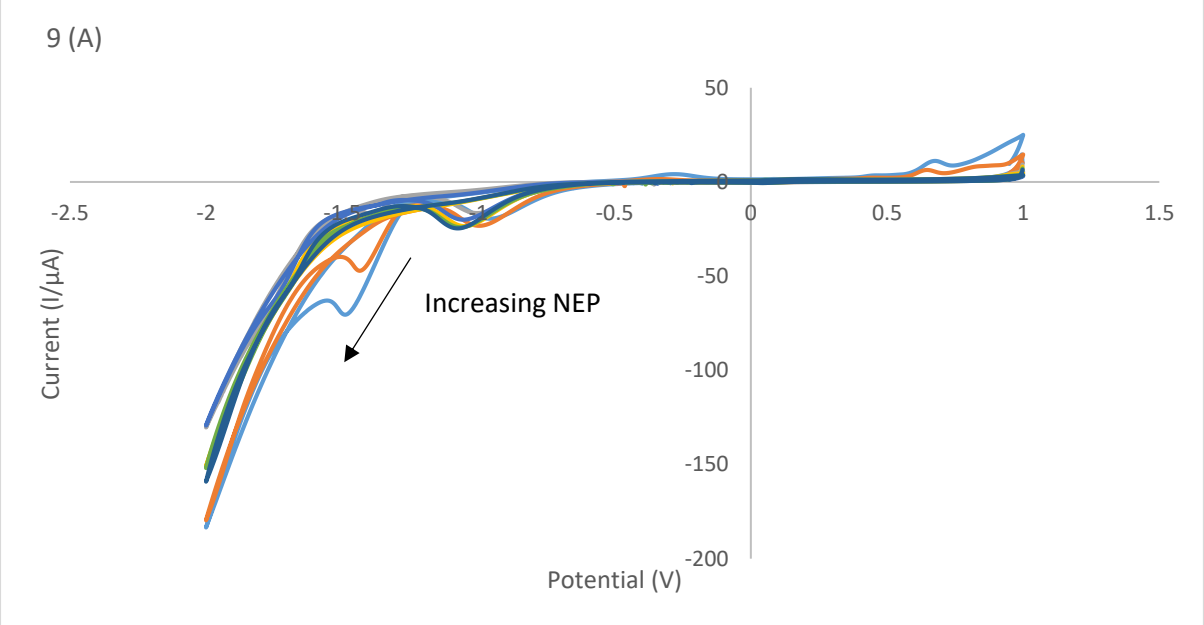


Figure 8 (A) Cyclic voltammograms showing the electrochemical response of 500 µg mL^{-1} N-ethylpentylone in 0.1 M acetate buffer at pH 4.3 (blue), 5.5(orange), 9(grey) and 12 (yellow) using screen-printed graphite macroelectrodes (vs. Ag/AgCl). Figure 8 (B) Plot showing peak position vs pH

It was observed in figure 8 (B) that the NEP peak position shifts to a more negative potential as the pH is increased to a more basic medium, indicating the electrochemical reduction of NEP is a pH dependent process. The change in pH may affect the electrochemical reduction of NEP, resulting in a difference in peak position, as the concentration H+ and OH- ions are altered in the various pH solutions. The peak currents show that pH 5.5 is the optimum pH for the detection of the reduction peak, therefore an additions study was conducted using the optimum pH. Fig 8 (B) shows a plot of peak position against pH, with a gradient of 57 mV, which is close to the theoretical value of 59mV in literature by Wu *et al* , indicating an electrochemical process involving an equal number of electrons and protons.

3.2.3. Additions Study

The effect of the N-ethylpentylone concentration on peak current was studied in the optimum pH 5.5 0.1 M acetate buffer, figure 9 (A) shows cyclic voltammograms produced and figure 9 (B) shows an overlay of peak current vs concentration over a range of pH's.



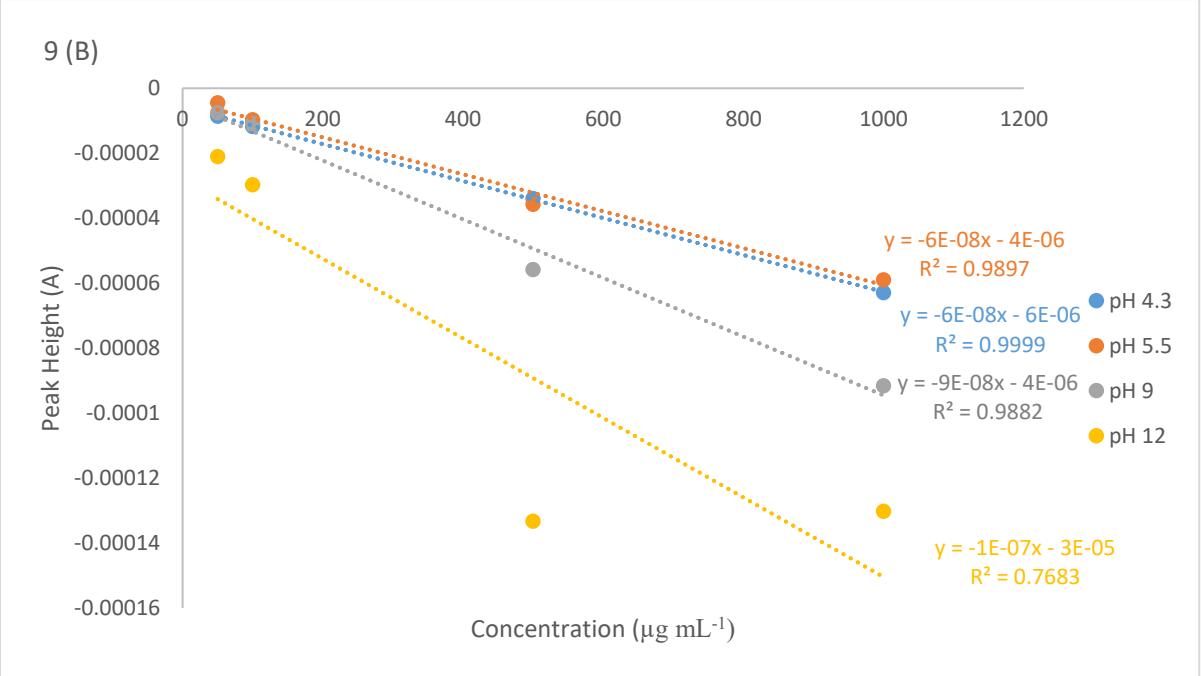
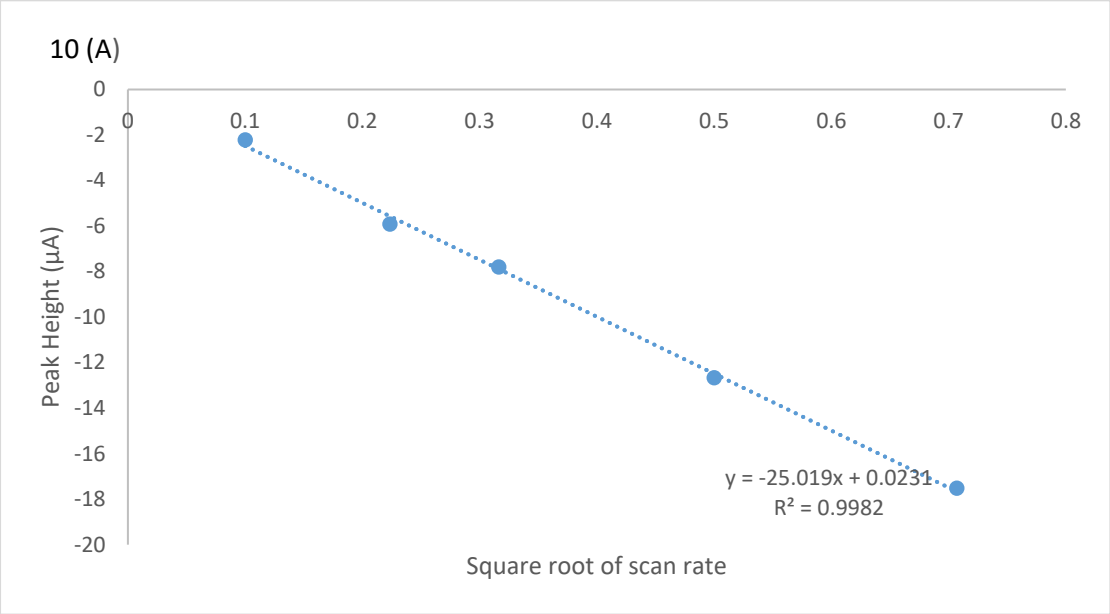


Figure 9 (A) Cyclic voltammograms showing the electrochemical response of 1 – 1000 µg mL^{-1} N-ethylpentylone in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Overlay of calibration plots showing peak height vs concentration of NEP in 0.1 M acetate buffer at pH 4.3, 5.5, 9 and 12.

Figure 9 (A) shows the cyclic voltammograms produced at each concentration and displays that the higher concentrations yielded higher reduction peak intensities. At the highest concentration of 1000 µg mL^{-1} the peak shape is less defined in comparison to the peak produced at 500 µg mL^{-1}. An overlay of calibration plots showing peak current vs concentration in figure 9 (B) shows that as the pH increases to a more basic medium, the peak current decreases. The change in calibration curves constructed at each pH can be explained by the change in cathinone stability. Previous work conducted by Tsujikawa *et al*[37], investigated the stability of five synthetic cathinone's over a range of pH and found that the drug stability increased as the pH decreased. This explains the results found at the most basic pH, 12, as the analyte most likely degraded in the basic media which results in poor peak currents in comparison to the peak currents in the optimal acidic media.

3.2.4. Scan Rate Study

The effect of scan rate upon the electrochemical reduction of 500 µg mL^{-1} NEP in pH 5.5 acetate buffer was investigated.



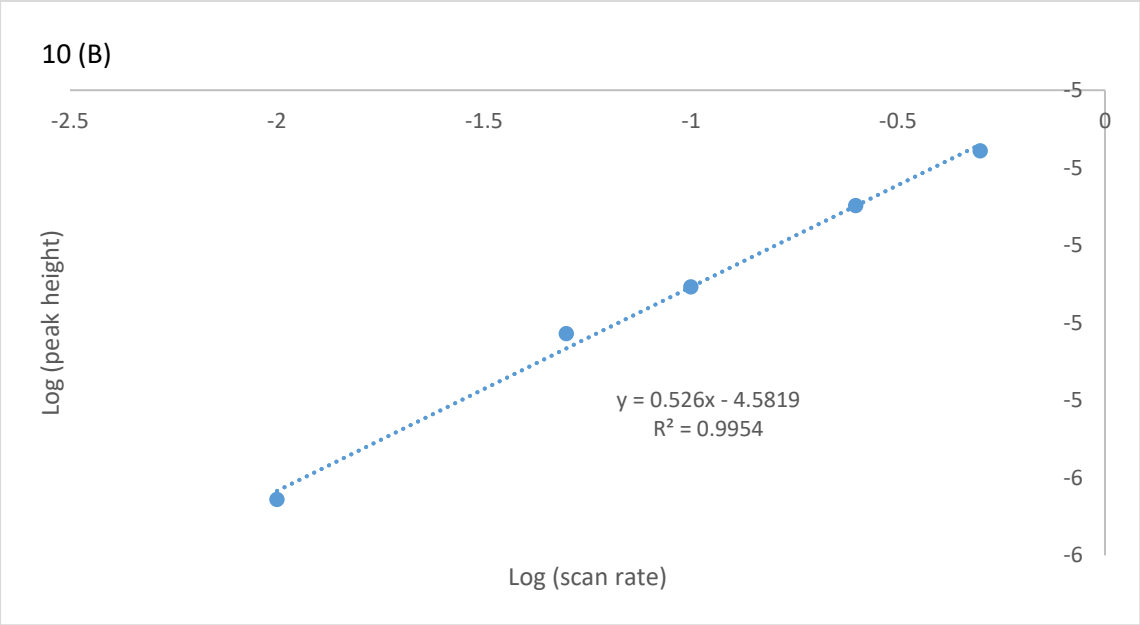


Figure 10(A). Calibration plot showing the relationship between Peak height and Square root of scan rate of 500 µg mL-1 MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Calibration plot showing relationship between Log (peak height) and Log (scan rate) of 500 µg mL^{-1} MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

A plot of 'peak height' against the square root of scan rate/ was found to be linear which indicates a diffusional process, as indicated with the following regression equation Ip/A = -2.502 x 10^{-5} A $(V\ s^{-1})^{1/2}$ + 2.31 x 10^{-8} A; R^2 = 0.998 (Figure 10 A). When plotting log peak height against log scan rate, a gradient close to 0.5 was found (Figure 10 B). A gradient of 0.5 also indicates a diffusional process, as indicated with the following regression equation log Ip / logA = 0.526 log A (log V s-1) – 4.582 log A; R^2 = 0.995. 5 points are used in the calibration plot as this is within the linear range measurable and effectively represents the trend.

3.2.5. PBS

The electrochemical response of 1-1000 µg mL^{-1} N-ethylpentylone in 0.1 M phosphate buffer solution was studied over the pH range 6 – 8 as shown in figure 11. It was observed that the NEP peak potential shifts towards a more negative potential and the peak intensity decreases when the pH is increased to a more basic environment. The electrochemical reduction of NEP is possible in all three mediums of PBS, but all peaks appear to have a background peak visible which results in a less defined peak shape.

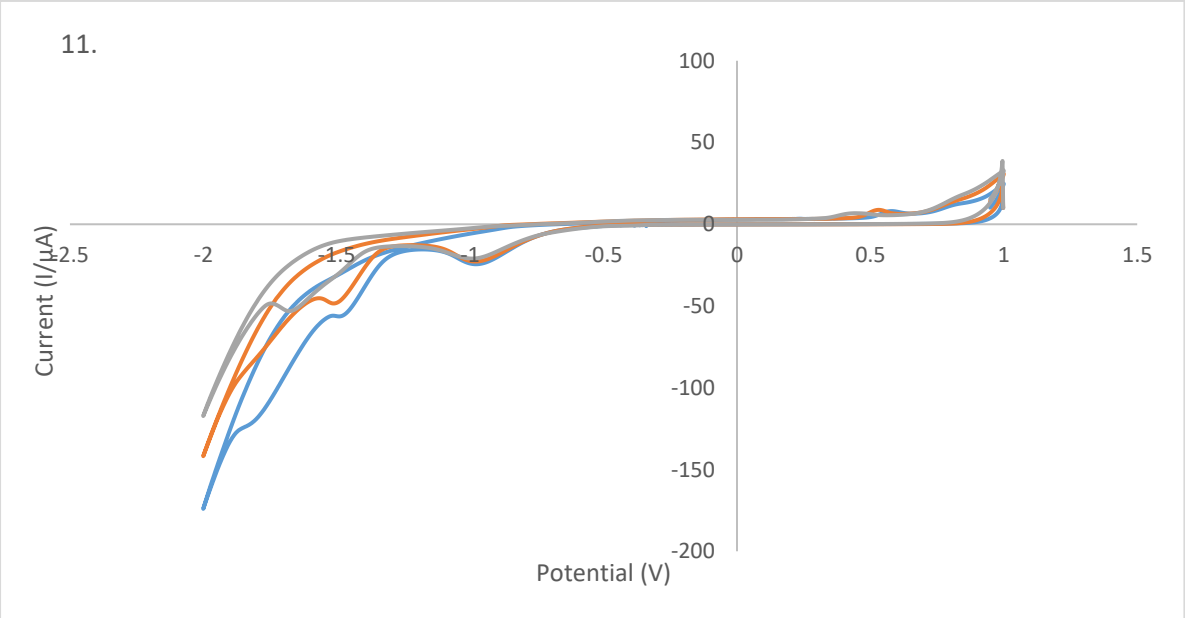


Figure 11. Cyclic voltammograms showing the electrochemical response of 500 µg mL^{-1} N-ethylpentylone in 0.1 M PBS at pH 6 (blue), 7 (orange) and 8 (grey) using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

3.2.6. Comparison of Electrochemical Response of NEP in acetate and PBS

The electrochemical reduction is shown to be viable in both acetate buffer and phosphate buffer, the most prominent peaks from both studies are compared in figure 12. Although pH 6 was the optimum environment in PBS, the peak shape is more defined and intense in pH 5.5 acetate buffer.

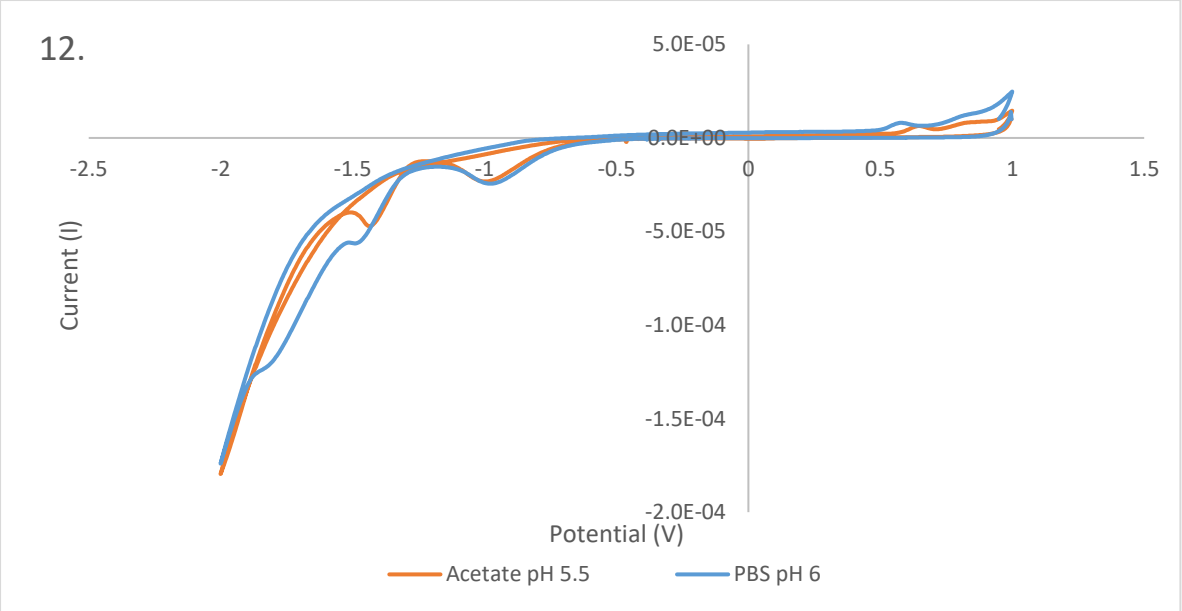


Figure 12. Comparison of cyclic voltammograms showing the electrochemical response of N-ethylpentylone in 0.1 M acetate buffer at pH 5.5 and 0.1 M PBS at pH 6 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

The reduction peak is visibly more defined and the peak intensity is greater in the pH 5.5 acetate buffer in comparison to the pH 6 PBS. Given the results from each study, it can be concluded that pH 5.5 acetate buffer is the optimum environment for the electrochemical reduction of NEP and will be used in further studies. There are also visible oxidation peaks in all the voltammetric responses, however the reduction peak was focused upon as the peak intensity was greater.

3.2.7. Detection of NEP (Street Samples) in acetate

The electrochemical responses of four different 500 μg mL^{-1} N-ethylpentylone Street Samples, (referred to as "SS1", "SS2", "SS3" and "SS4"), in 0.1 M acetate buffer pH 5.5 were studied using cyclic voltammetry. A set concentration and pH were used in this part of the study as the aim was to determine whether the street samples contained NEP and could be electrochemically reduced.

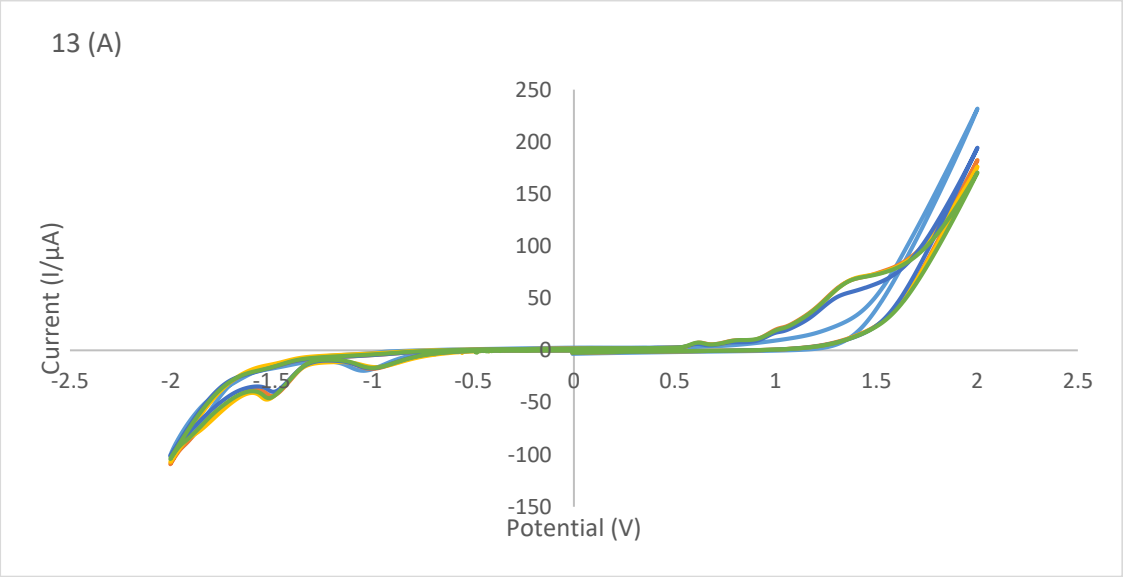


Figure 13 (A) Cyclic voltammograms showing the electrochemical response of N-ethylpentylone street samples (SS1(green), SS2(dark blue), SS3(yellow) and SS4(grey)), control sample (orange) and blank buffer (blue) in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

Figure 13 (A) shows that each street sample contains NEP as the electrochemical reduction peak appears at a similar potential as the control NEP sample. Electrochemical oxidation peaks appear in the positive potential region, however the reduction peaks will be focused upon as the peak intensities are greater. For the next part of the study, the electrochemical response of one street sample, SS1, in 0.1 M acetate buffer pH 5.5 was explored over a concentration range of 10 – 800 μg mL^{-1}. Figure 13 (B) shows a plot of NEP peak height (Ip/units) against concentration (μg mL^{-1}) which generates a linear plot between the range of 25 – 500 μg mL^{-1}.

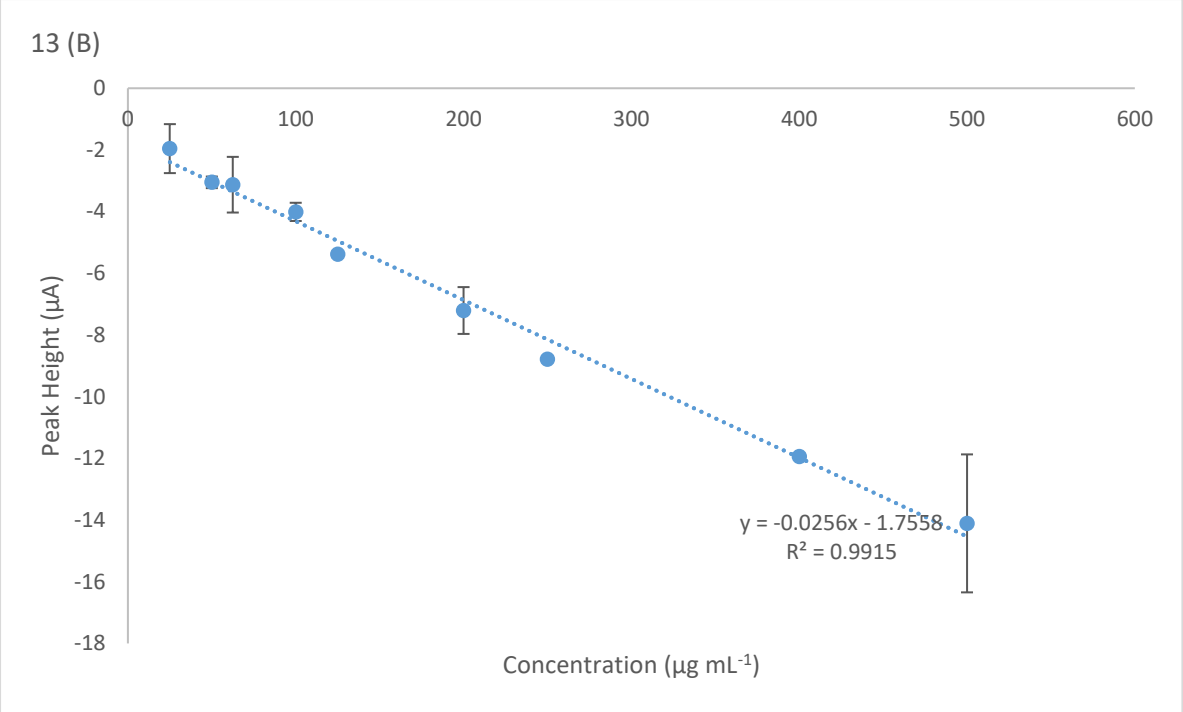


Figure 13 (B) Linear calibration plot of peak height (Ip) against concentration of N-ethylpentylone SS1. *N*=3.

3.2.8. NEP Spiked samples- adulterants

The selectivity of the method was tested in order to determine if commonly found adulterants in street samples affected the electrochemical response of NEP. Cyclic voltammograms were recorded of each street sample spiked with three common adulterants separately: paracetamol, caffeine and benzocaine. Cyclic voltammograms were recorded for each sample in the potential window 0 to -2 V to focus on the region that the electrochemical response of NEP was previously detected.

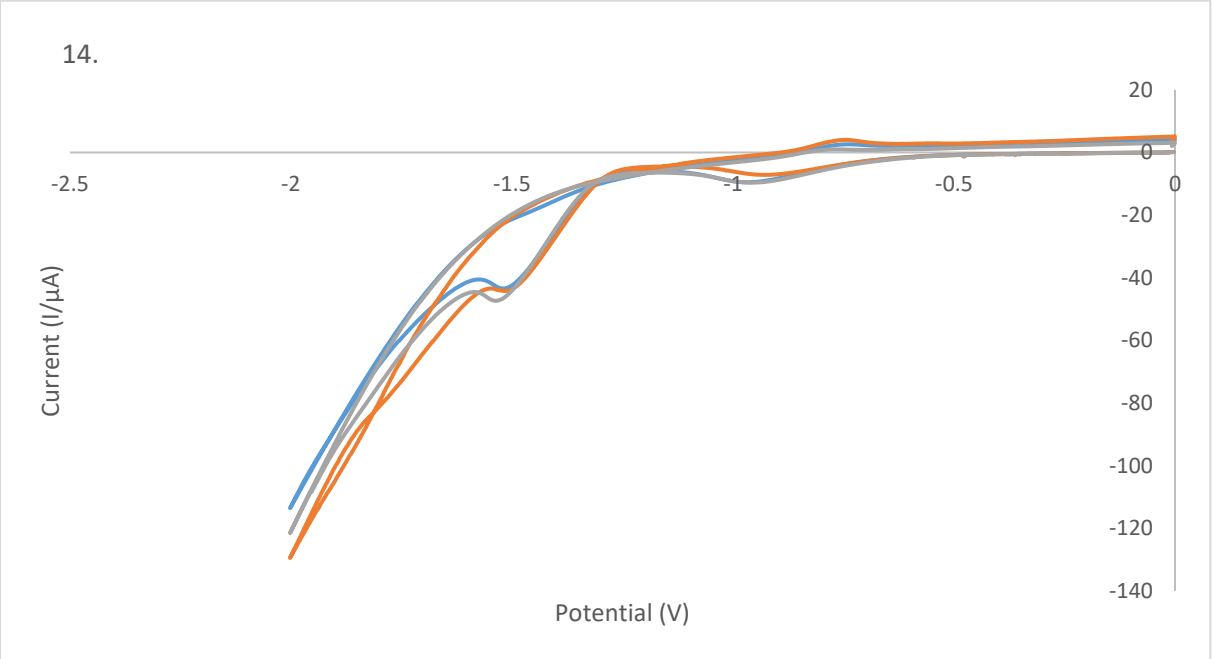
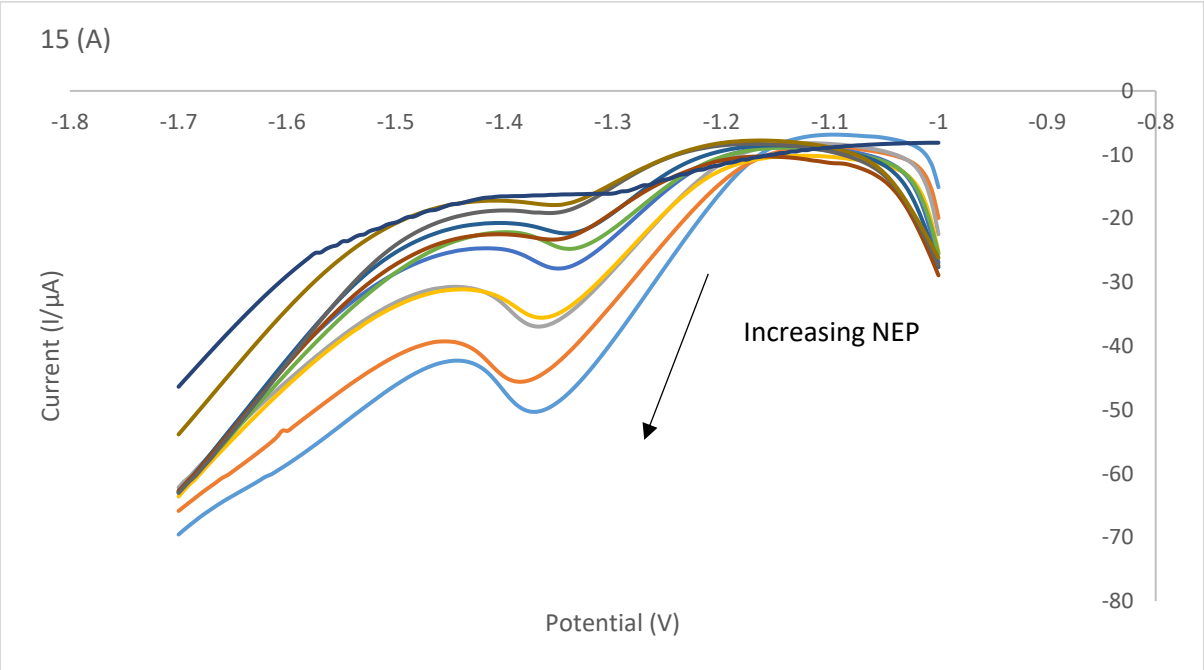


Figure 14. Cyclic voltammograms showing the electrochemical response of N-ethylpentylone SS1 spiked separately with 500µg of caffeine (blue), benzocaine (orange) and paracetamol (grey) in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

The voltammograms of the NEP street sample (SS1) spiked with each adulterant are shown in figure 14: each adulterant shows a small oxidation peak at approximately -0.75 V, which indicates that there will be little interference with the electrochemical response of the reduction peak of NEP at 1.5 V. The expected reduction peak of NEP is visible and the adulterants do not interfere with the peak potential or intensity.

3.2.9. Detection of NEP (Control) in Acetate using DPV

Differential pulse voltammetry (DPV) is an advantageous technique as it provides more sensitive results in comparison to cyclic voltammetry. DPV measures the difference between two currents and the modulation amplitude is kept constant which subtracts the contribution of the non-faradaic processes. Therefore, peaks are well-resolved and are typically sharper than peaks found using cyclic voltammetry. The electrochemical reduction of 25 – 500 µg mL^{-1} N-ethylpentylone (control) in 0.1 M acetate buffer pH 5.5 was studied using differential pulse voltammetry in attempt to improve sensitivity as reported by Elbardisy *et al*[38]. during the electrochemical sensing of mephedrone metabolites. Conditions were firstly optimised and the optimum conditions were found to be: modulation amplitude: 0.17 V; modulation time 0.17 s; interval time: 0.25 s; step: -0.05 V and scan rate: 0.02 V/s.



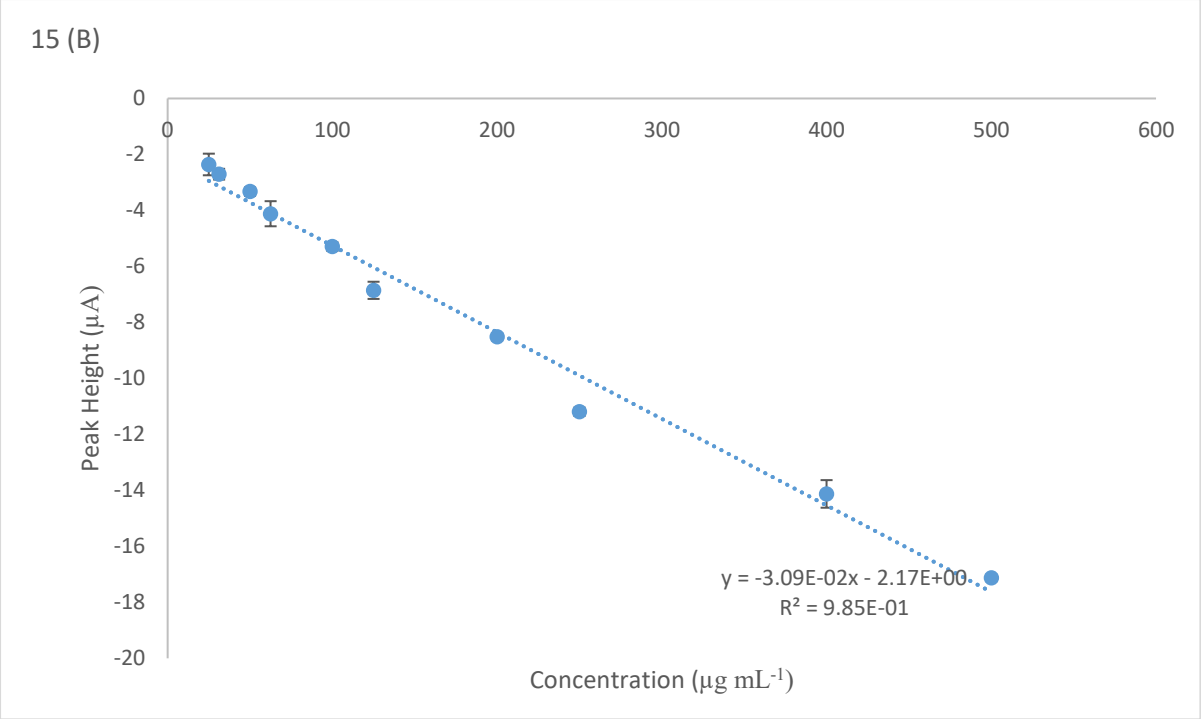


Figure 15 (A) Differential pulse voltammograms showing the electrochemical response of N-ethylpentylone in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Linear calibration plot of peak height against concentration of N-ethylpentylone. *N*=3.

Figure 15 (A) shows that as the concentration of NEP is increased, the peak intensity increases and the peak potential generally shifts very slightly to a more negative potential due to a larger concentration of NEP at the surface electrode. The peak shape becomes more defined as the concentration increases and is not a symmetrical curve as expected with the technique of DPV. In comparison to the peaks shown using CV in figure 9 (A), the peaks are more defined as expected. An asymmetrical peak is usually attributed to an irreversible reaction, however this is not concurrent with the findings using CV as the peaks generated suggests a reversible reaction. Figure 15 (B) shows a plot of peak height (Ip/µA) against concentration (µg mL^{-1}) generates a linear calibration plot between the range of 25 – 500 µg mL^{-1} with the R^2 value of 0.985. The Limit of Detection was calculated to be 0.130 µg mL^{-1} and the RSD is 5.85%.

3.2.10. Comparison of CV and DPV Findings

Both techniques of cyclic voltammetry and differential pulse voltammetry were compared in the electrochemical reduction of N-ethylpentylone.

The electrochemical responses recorded from the technique of cyclic voltammetry and differential pulse voltammetry were compared in order to ascertain the most sensitive method for the electrochemical reduction of MDPHP.

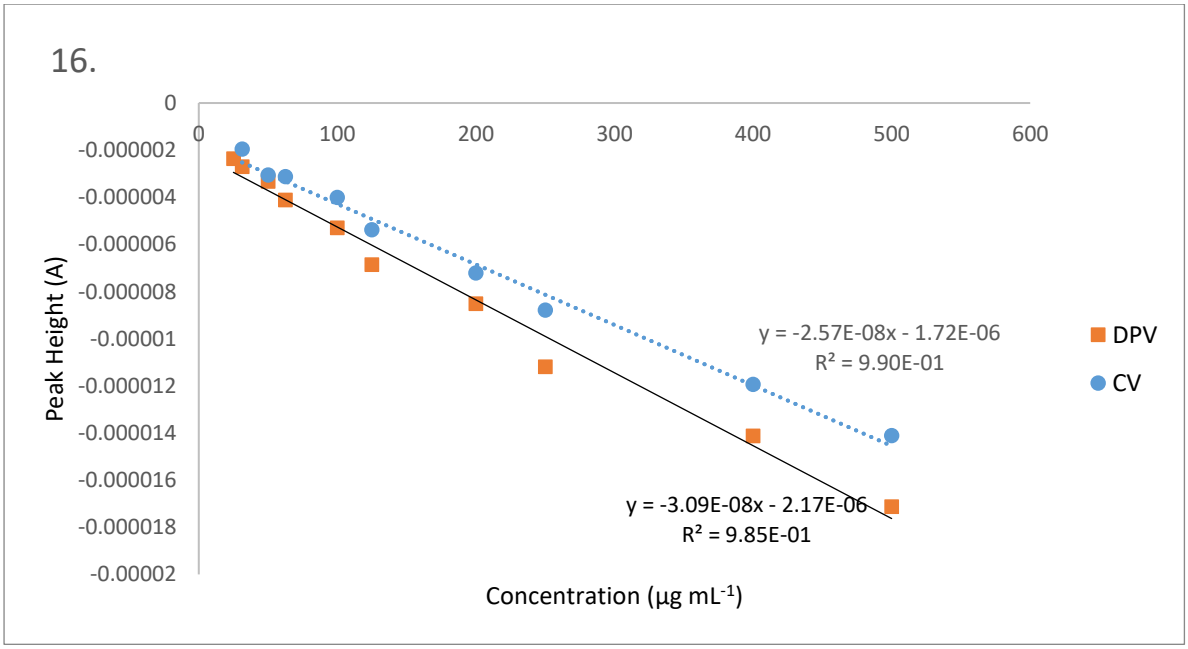


Figure 16. Comparison of linear calibration plots from cyclic voltammetry and differential pulse voltammetry techniques, showing peak height against concentration of N-ethylpentylone in 0.1 M acetate buffer.

Figure 16 shows a comparison of the linear calibration plots constructed for the NEP peak height (Ip/µA) against concentration (µg mL^{-1}), using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Using the standard error of the slope generated, the limit of detection was calculated to be 0.130 µg mL^{-1} and 0.104 µg mL^{-1} for DPV and CV respectively. According to literature, DPV is the more sensitive method which typically results in a lower limit of detection: the study conducted does not reflect this and shows a more linear

relationship between peak height and concentration, and a lower LOD. Therefore, cyclic voltammetry was the preferred technique of use for the remaining experiments.

3.2.11. Summary

N-ethylpentylone is shown to be detectable using cyclic voltammetry, in both PBS and acetate buffer over a range of pH's and it was determined that pH 5.5 acetate buffer was the optimum medium for the electrochemical response. Both control samples and street samples were successfully detected in linear ranges of 25 – 600 $\mu g\ mL^{-1}$ and 25 – 500 $\mu g\ mL^{-1}$ in acetate buffer (0.1 M, pH 5.5) respectively. The corresponding limit of detections in acetate buffer (0.1 M, pH 5.5) were calculated to be 0.046 $\mu g\ mL^{-1}$ (control sample) and 0.104 $\mu g\ mL^{-1}$ (street sample). The technique of differential pulse voltammetry was also successfully utilized to detect the control sample and the linear range was found to be 25 – 100 $\mu g\ mL^{-1}$ with a limit of detection of 0.130 $\mu g\ mL^{-1}$. In comparison, cyclic voltammetry was the preferred technique as there is a more linear relationship between peak height and concentration, and a lower LOD. The method of cyclic voltammetry was shown to be a selective method, as control samples were spiked with the three common adulterants, paracetamol, benzocaine and caffeine and were shown not to interfere with the expected electrochemical response of NEP. In regards to the electrochemical process, a scan rate study was conducted, which produced a gradient value of 0.5, indicating a diffusional process.

3.3. MDPHP Results

3.3.1. Detection of MDPHP (control) in Acetate using Cyclic Voltammetry (CV)

To determine the optimum conditions for the electrochemical detection of MDPHP, the electrochemical reduction response was investigated in acetate and phosphate buffer solution (PBS) over a range of pH's and concentrations

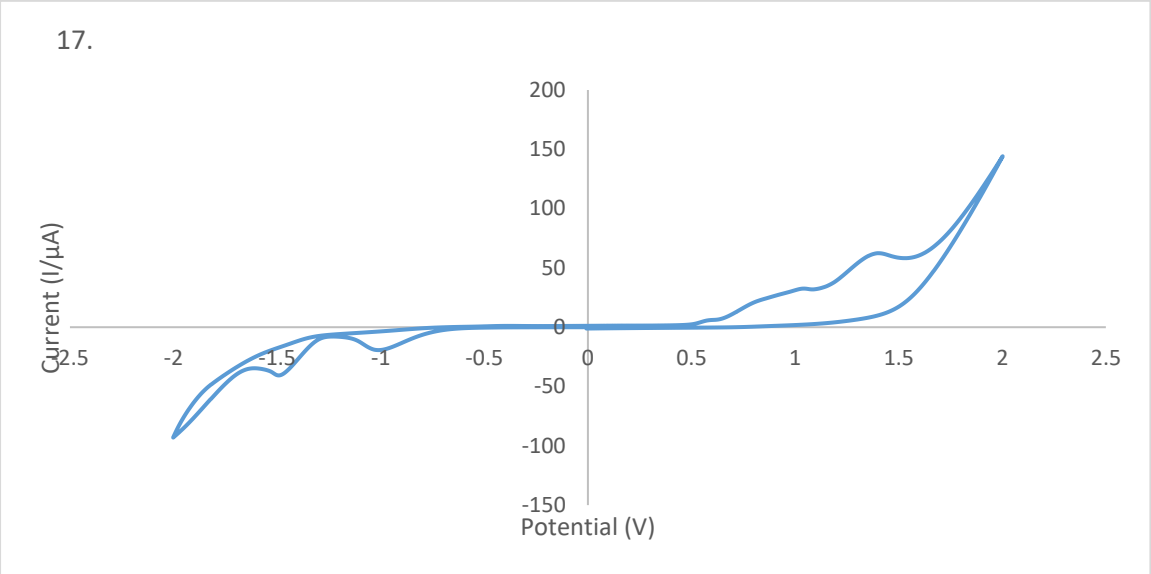
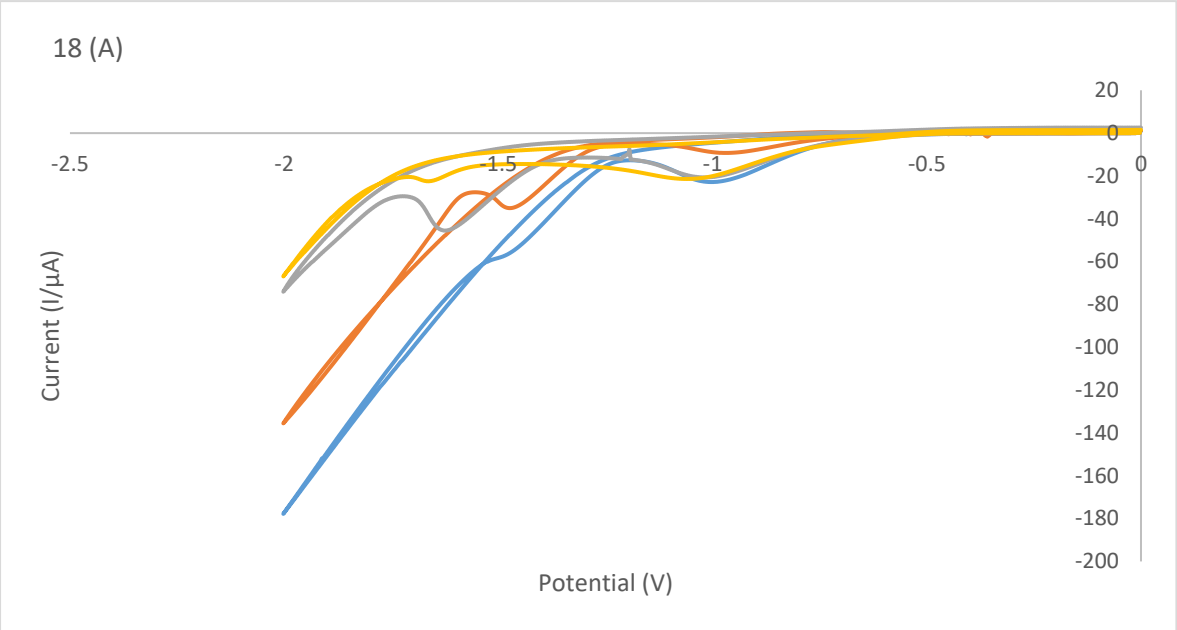


Figure 17. Cyclic voltammogram showing the electrochemical response of 500 µg mL^{-1} MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

The electrochemical response of 500 µg mL^{-1} MDPHP in 0.1 M acetate buffer was studied at pH 5.5 as shown in figure 17 as pH 5.5 was previously determined as the optimum condition during validation of the system. A scan was performed over the range 2 to -2 V which shows a visible oxidation peak at ≈ 0.64 V and a reduction peak at ≈ -1.4V. There is also a visible residual oxygen peak at -1 V although the solution was thoroughly degassed using nitrogen. The reduction peak has a greater peak intensity in comparison to the oxidation peak and is more defined in shape, therefore the region in which the reduction peak is visible will be focused on in future studies. As the chemical process is unknown, the process will be optimised by conducting a pH study.

3.3.2. pH study



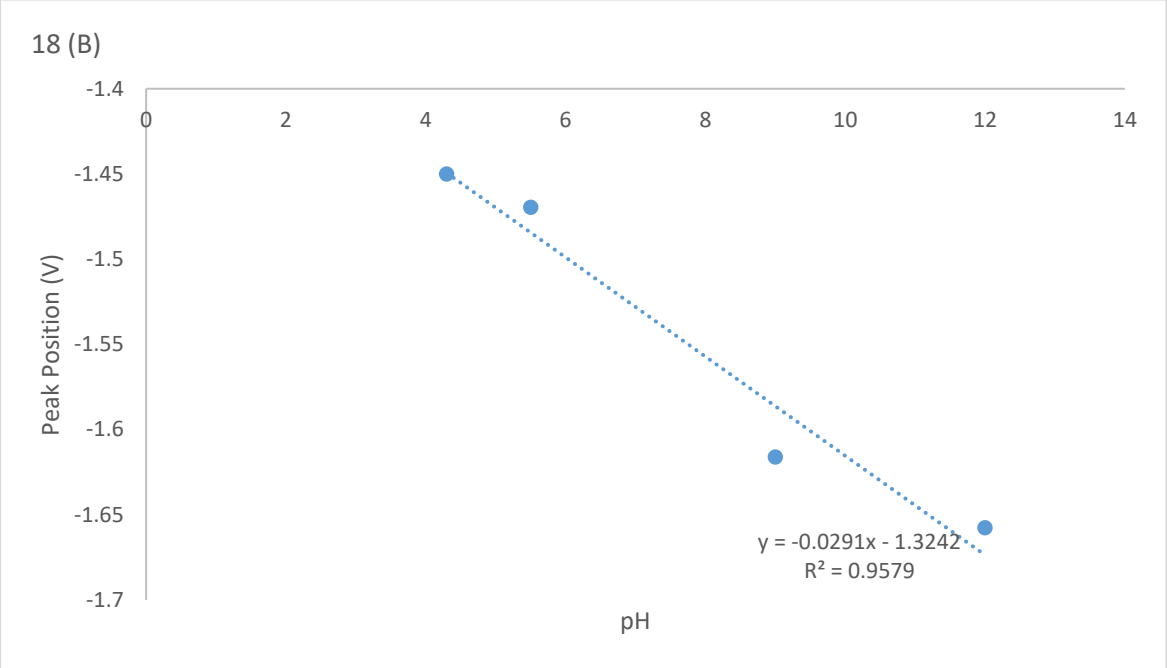
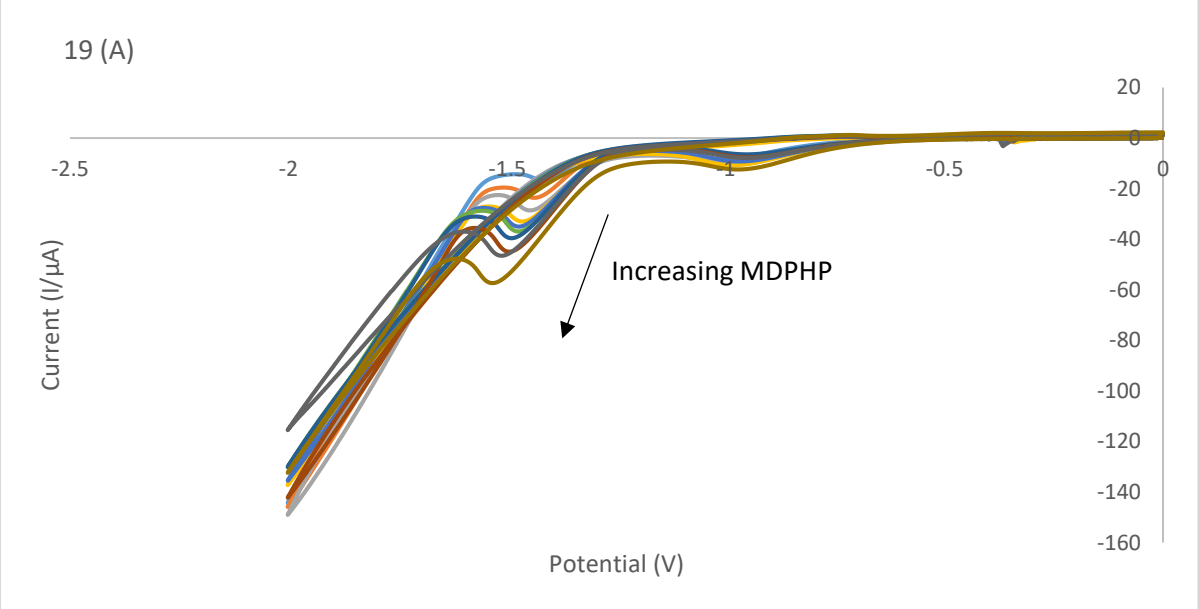


Figure 18 (A) Cyclic voltammograms showing the electrochemical response of 5000 µg mL^{-1} MDPHP in 0.1 M acetate buffer at pH 4.3 (blue), 5.5 (orange), 9 (grey) and 12 (yellow) using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Plot showing peak position vs pH

It was observed in figure 18 (A) that the MDPHP peak position shifts to a more negative potential as the pH is increased to a more basic medium, indicating the electrochemical reduction of NEP is a pH dependent process. The change in pH may affect the electrochemical reduction of MDPHP, resulting in a difference in peak position, as the concentration H+ and OH- ions are altered in the various pH solutions. The peak currents show that pH 5.5 is the optimum pH for the detection of the reduction peak, therefore an additions study was conducted using the optimum pH. Fig 18 (B) shows a plot of peak position against pH, with a gradient of 29 mV, which is similar to the value of 33mV in literature by Smith *et al*[36], indicating an electrochemical process involving double the number of electrons over that of protons.

3.3.3. Additions Study

The effect of the MDPHP concentration on peak current was studied in the optimum pH 5.5 0.1 M acetate buffer, figure 19 (A) shows cyclic voltammograms produced and figure 19 (B) shows an overlay of peak current vs concentration over a range of pH's.



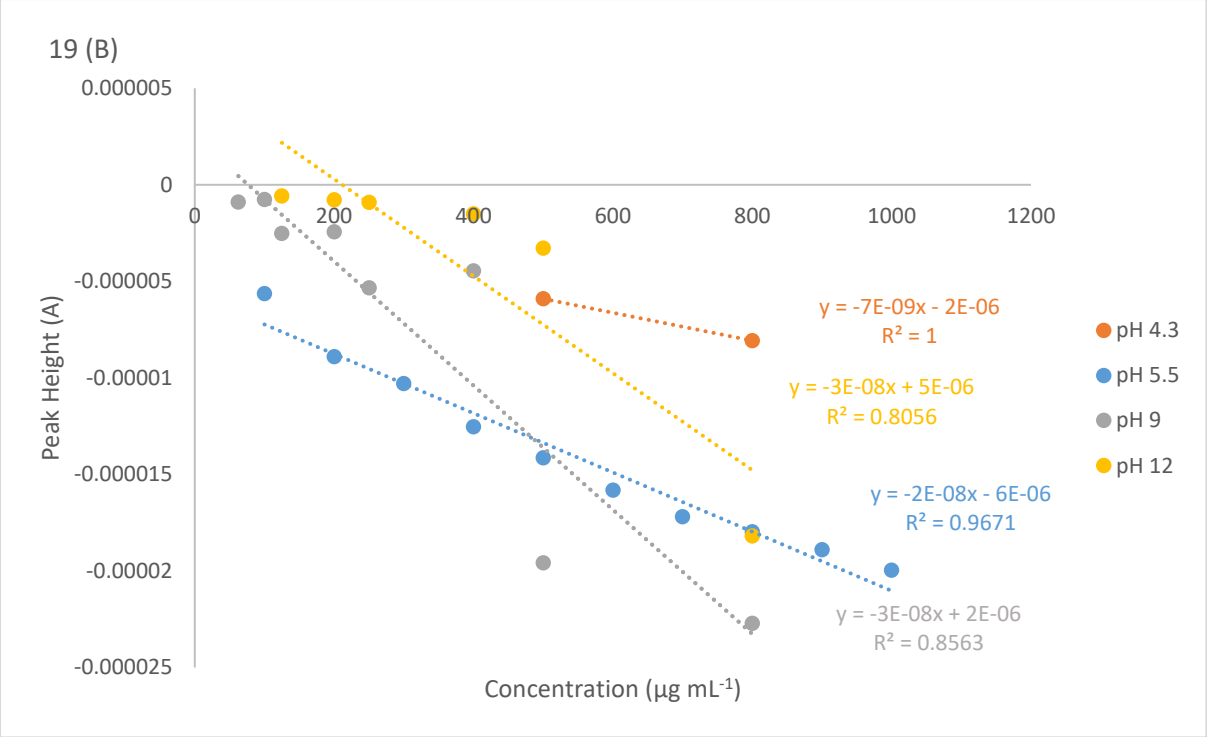


Figure 19 (A) Cyclic voltammograms showing the electrochemical response of 1 – 1000 µg mL^{-1} MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Overlay of calibration plots showing peak height vs concentration of MDPHP in 0.1 M acetate buffer at pH 4.3, 5.5, 9 and 12.

Figure 19 (A) shows the cyclic voltammograms produced at each concentration and displays that the higher concentrations yielded higher reduction peak intensities. At the highest concentration of 1000 µg mL^{-1} the peak shape is less defined in comparison to the peak produced at 500 µg mL^{-1}. An overlay of calibration plots showing peak current vs concentration in figure 19 (B) shows that as the pH increases to a more basic medium, the peak height decreases. The limit of detection was calculated to be 0.195 µg mL^{-1}.

3.3.4. Scan Rate Study

The effect of scan rate upon the electrochemical reduction of 500 µg mL^{-1} MDPHP in pH 5.5 acetate buffer was investigated.

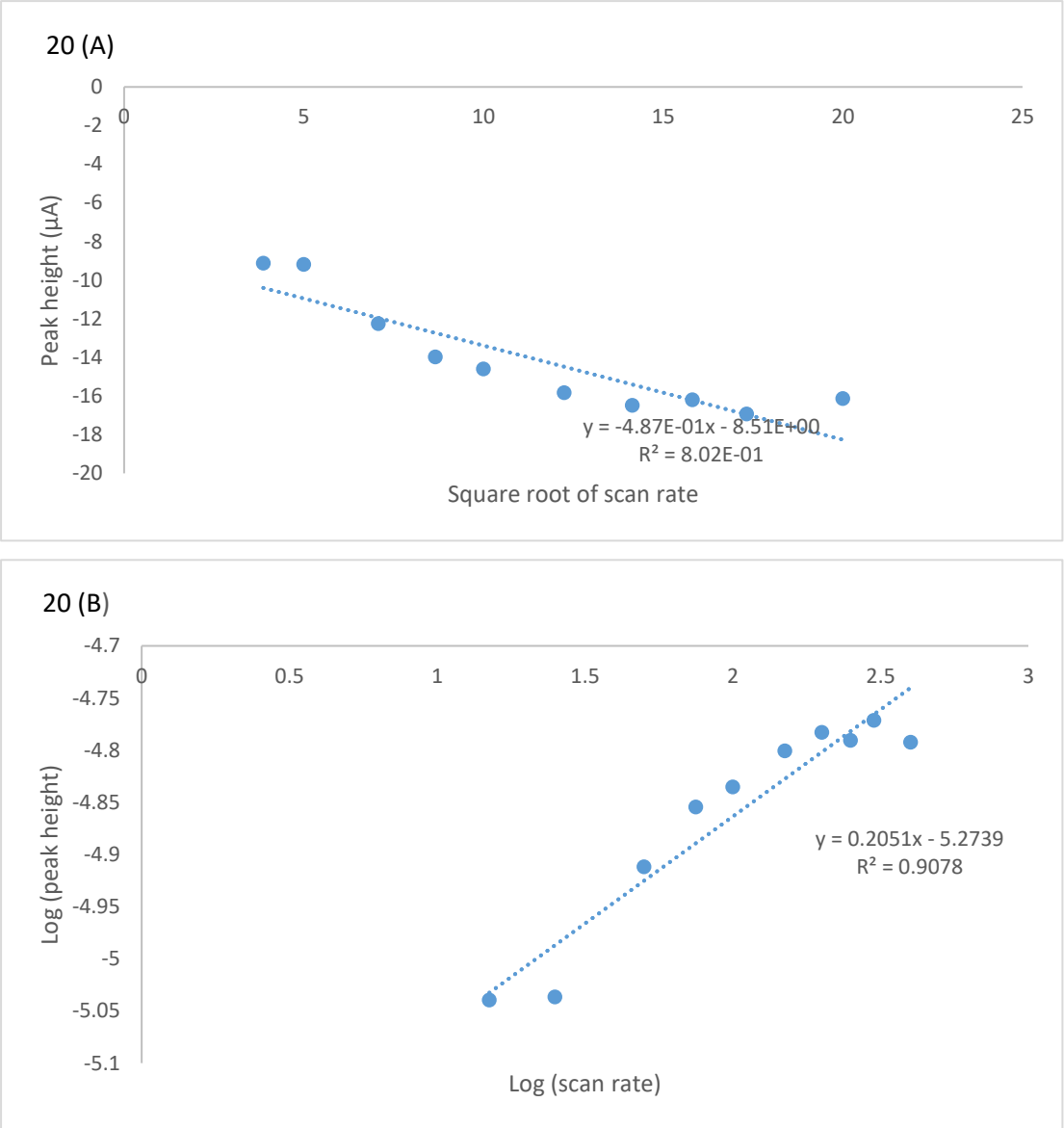


Figure 20 (A). Calibration plot showing the relationship between Peak height and Square root of scan rate of 500 µg mL-1 MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Calibration plot showing the relationship between Log (peak height) and Log (scan rate) of 500 µg mL^{-1} MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

A plot of 'peak height' against the 'square root of scan rate' was found to be linear which indicates a diffusional process, as indicated with the following regression equation: I_p (A)= -4.87 x 10^{-7} A – 8.51 x 10^{-6} A/$(V^{s-1})^{1/2}$, R^2= 0.802 (Figure 20 A). When plotting log peak height against log scan rate, a gradient close to 0.2 was found (Figure 20 B). It was expected that a gradient between 0.5 and 0.65 would be produced, indicating a diffusional process with some surface adsorption. However, as the value is lower than expected, further studies would have to be conducted to understand the process and ensure factors such as the electrode surface or model buffer solution were not affecting the results.

3.3.5. Detection of MDPHP (Street Samples)

The electrochemical responses of six different 500 µg mL^{-1} MDPHP street samples (referred to as "D15", "D16", "D19", "T1D", "T2C" and "T4B") were studied in the optimum 0.1 M acetate buffer at pH 5.5 as determined in the initial experiment using the MDPHP control sample. A set concentration and pH were used in this part of the study as the aim was to determine whether the street samples contained MDPHP and could be electrochemically reduced.

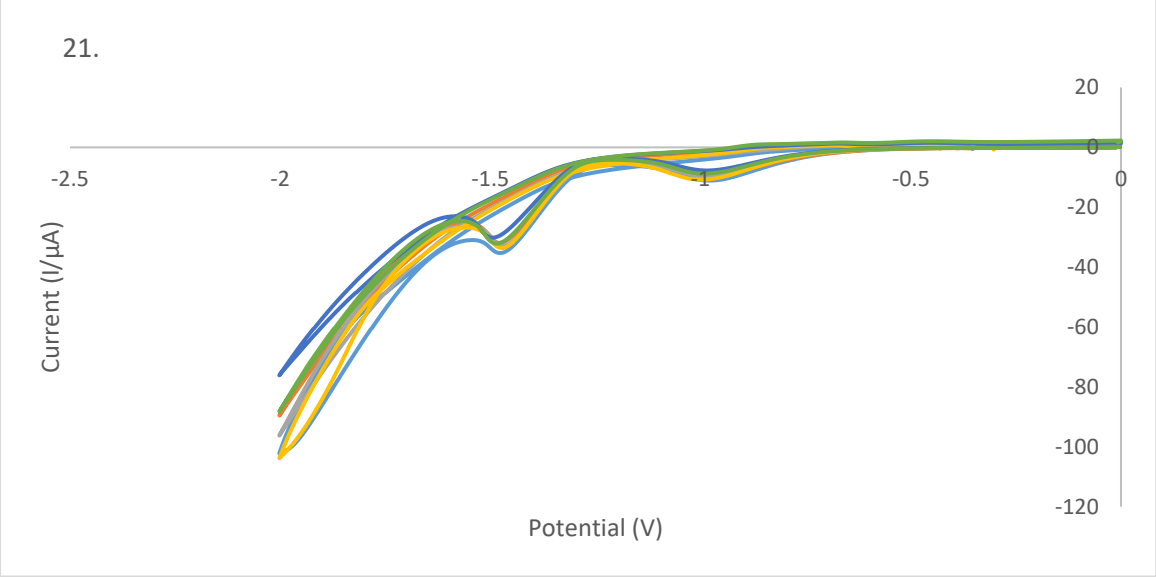


Figure 21. Cyclic voltammograms showing the electrochemical response of 500 µg mL^{-1} MDPHP street samples (D15 (blue), D16 (orange), D19 (grey), T1D (yellow), T4B (dark blue) and T2C (green)) in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

Each street sample was studied over the range of 100 – 1000 μg mL^{-1} using cyclic voltammetry within the same potential window of 0 to -2 V as used in the control sample study. Figure 21 shows a summary of cyclic voltammograms produced by 500 μg of each street sample. The cyclic voltammograms shift to a more negative potential and increase in peak height as the concentration of street sample increased, which is attributed to a higher concentration of electroactive species in the solution that are available for reduction at the surface of the electrode. Table 3 shows the calculated Limit of Detection (LOD), Limit of Quantification (LOQ) and Relative Standard Deviation (RSD) of each street sample. D15 is shown to have the lowest LOD and LOQ.

Table 3. MDPHP LOD, LOQ and RSD using cyclic voltammetry

Street Sample	Limit of Detection (LOD μg mL^{-1})	Limit of Quantification (LOQ μg mL^{-1})	Relative Standard Deviation (RSD %)
D15	0.130	0.433	2.92
D16	0.201	0.671	2.88
D19	0.192	0.641	2.47
T1D	0.223	0.745	3.18
T2C	0.272	0.907	4.57
T4B	0.222	0.739	4.11

3.3.6. MDPHP Spiked Samples- Adulterants

The selectivity of the method was tested in order to determine if commonly found adulterants in street samples affected the electrochemical response of MDPHP. Cyclic voltammograms of each street sample spiked with three common adulterants (paracetamol, caffeine and benzocaine) were recorded within the potential window previously used to detect the MDPHP control sample.

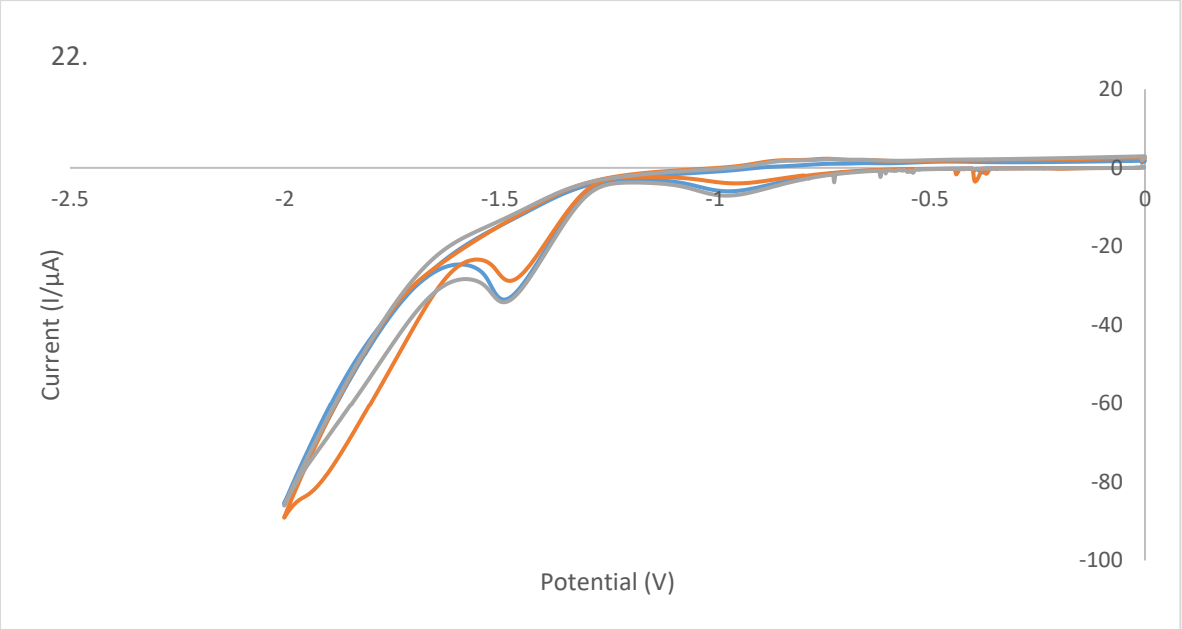


Figure 22. Cyclic voltammograms showing the electrochemical response of MDPHP T2C spiked separately with 500µg of paracetamol (blue), caffeine (grey) and benzocaine (orange) in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

The voltammograms of the MDPHP street sample (T2C) spiked with each adulterant are shown in figure 22: each adulterant shows a small oxidation peak at approximately -0.9 V, which indicates that there will be little interference with the electrochemical response of the reduction peak of MDPHP. The expected reduction peak of MDPHP is visible and the adulterants do not interfere with the peak potential or intensity.

3.3.7. Detection of MDPHP (Control) in Acetate using DPV

The electrochemical reduction of 25 – 500 µg mL^{-1} N-ethylpentylone (control) in 0.1 M acetate buffer pH 5.5 was studied using differential pulse voltammetry in attempt to improve sensitivity as performed by Elbardisy *et al* in 2019[38] . Conditions were firstly optimised and the optimum conditions were found to be: modulation amplitude: 0.15 V; modulation time 0.15 s; interval time: 0.2 s; step: -0.005 V and scan rate: 0.025 V/s.

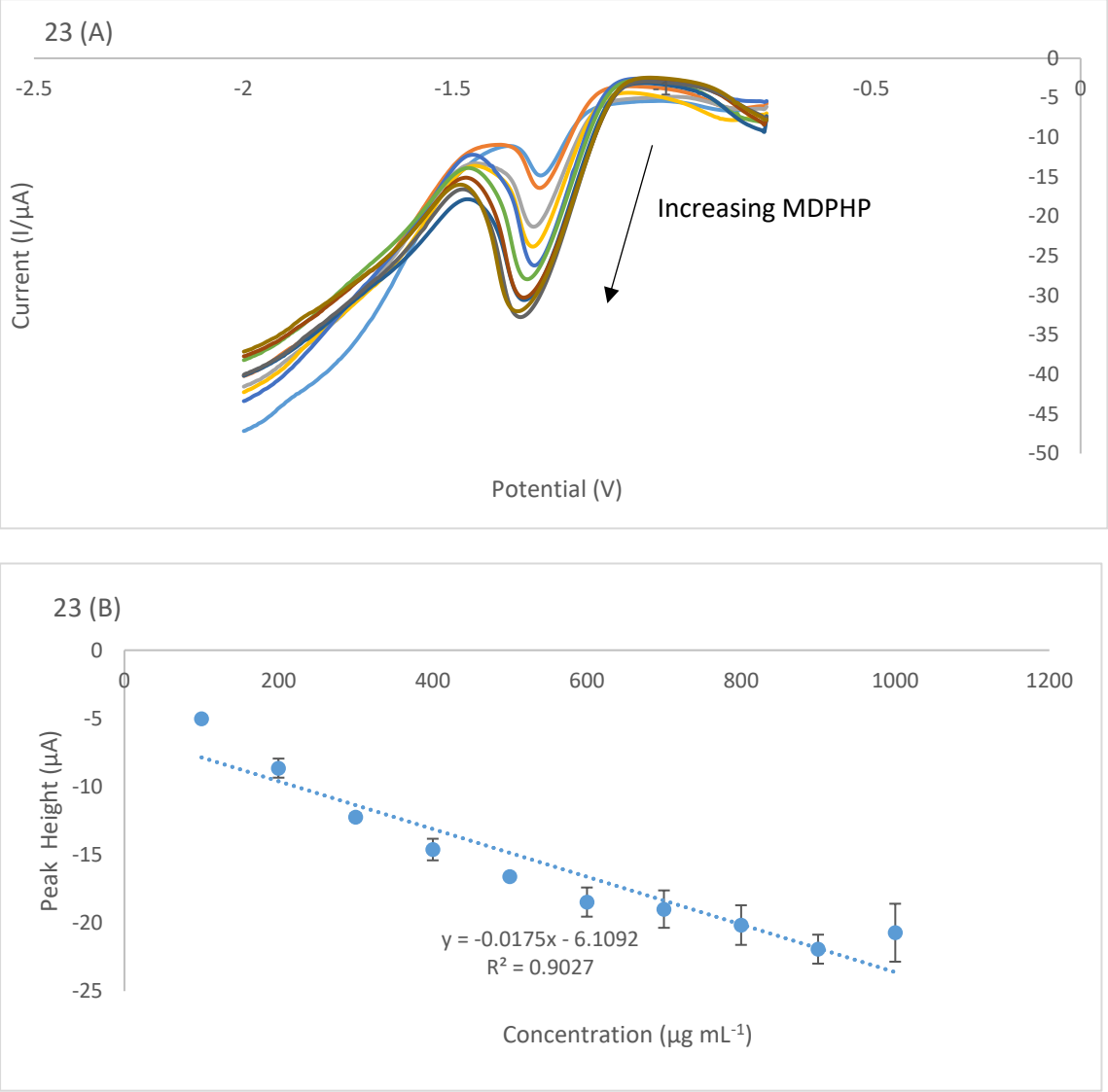


Figure 23 (A) Differential pulse voltammograms showing the electrochemical response of MDPHP in 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl). (B) Linear calibration plot of peak height against concentration of MDPHP. *N*=3.

The electrochemical response shows a reduction peak at a similar potential to where MDPHP was detected in the cyclic voltammograms. As the concentration of MDPHP is increased the peak intensity increases and the peak shape becomes more defined. Plotting the peak height (Ip/ uA) against concentration (µg mL^{-1}), in the range of 100 – 1000 µg mL^{-1}, generated a linear plot providing an R^2 value of 0.9027. The Limit of Detection was calculated to be 0.348 µg mL^{-1} and the RSD is 5.66%. As expected, the differential pulse voltammograms in figure 23 (A) show sharper and more well-resolved peaks than in comparison to the cyclic voltammograms in figure 19 (A).

3.3.8. Detection of MDPHP (control) in a Biological Sample (Diluted Human Urine)

To ascertain the relevance of the method investigated to the real life testing of biological samples, the electrochemical voltammetric response of MDPHP was tested in a sample of diluted human urine using cyclic voltammetry. The urine sample was diluted with pH 5.5 acetate buffer and spiked with MDPHP over the range of 100 – 1000 µg mL^{-1} and a calibration curve was constructed.

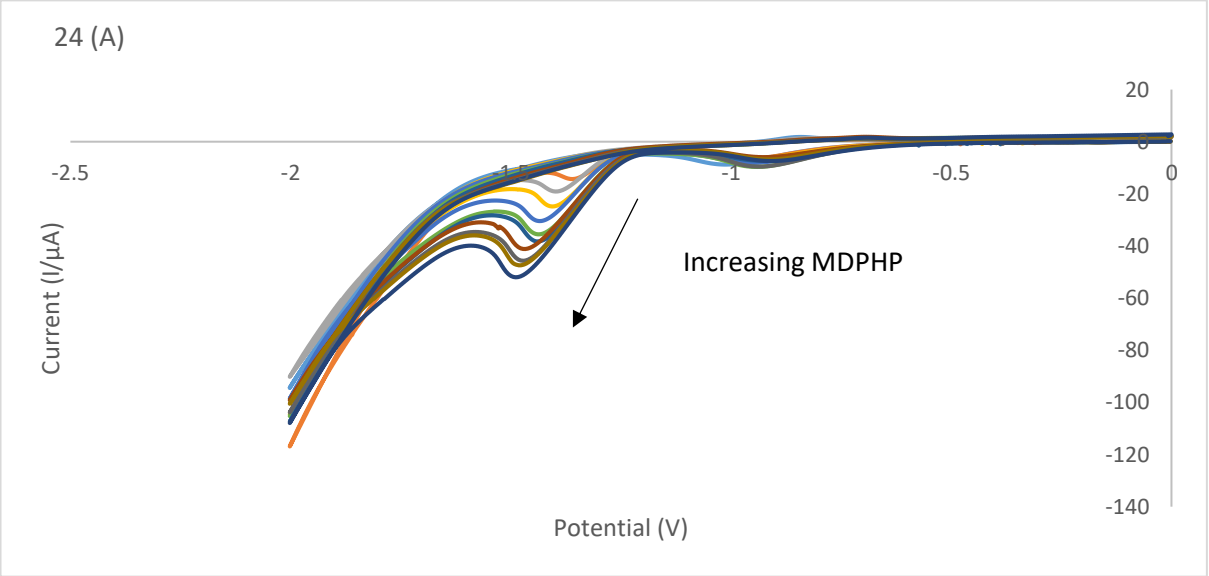


Figure 24 (A) Cyclic voltammograms showing the electrochemical response of MDPHP in human urine diluted with 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

As shown in figure 24 (A), as the concentration of MDPHP increases, the peak potential shifts towards a more negative potential and the peak intensity increases, which is due to the increase in electroactive species at the electrode surface. The blank urine solution does not contain electroactive species that will interfere with the reduction of MDPHP as the voltammogram shows a small oxidation peak, due to other species that may be in the urine sample, but does not affect the electrochemical response of MDPHP.

Plotting the peak height (Ip/µA) against concentration (µg mL^{-1}) resulted in a linear plot, which is shown in figure 24 (B). Figure 24 (B) also displays the calibration curve constructed previously of MDPHP in acetate solution,

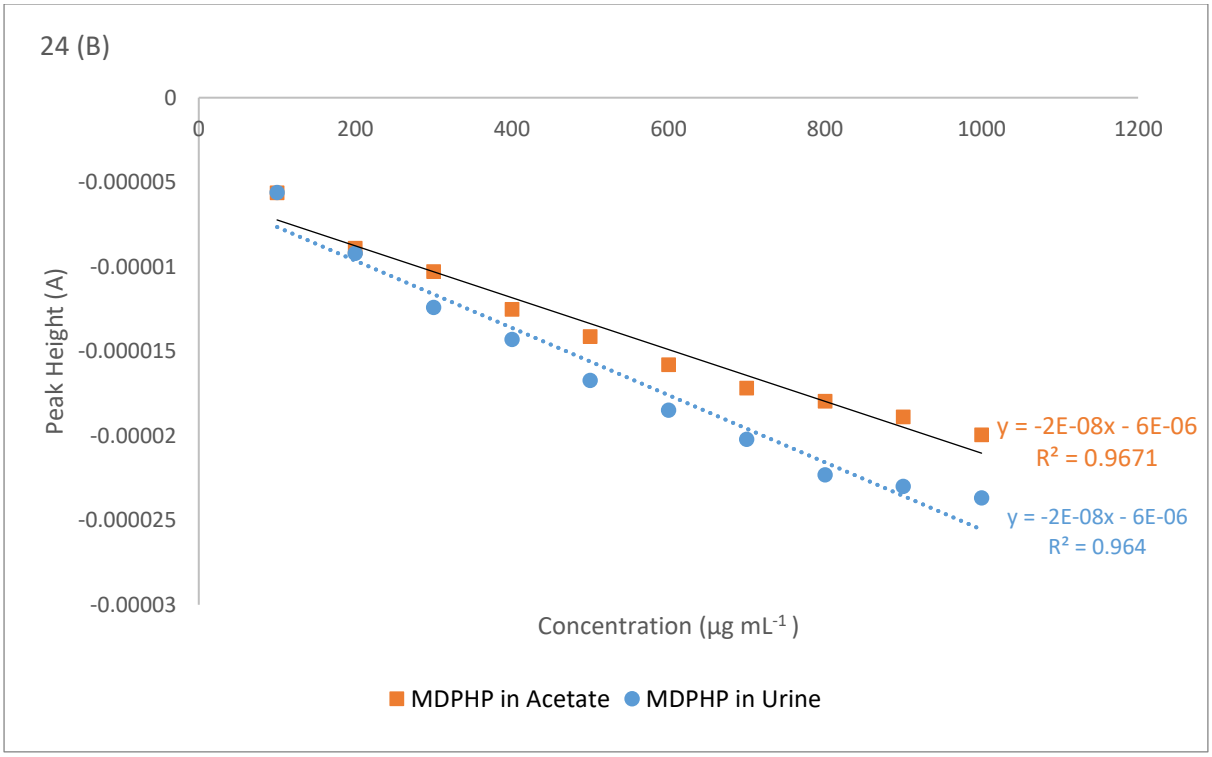


Figure 24 (B) Overlay of calibration plots showing peak height vs concentration of MDPHP (control) in acetate solution pH 5.5 and diluted human urine

The LOD corresponds to 0.205 µg mL^{-1} which is similar to the LOD found for MDPHP in the acetate buffer of 0.195 µg mL^{-1}.

3.3.9. Lowest Detection of MDPHP in a Biological Sample (Diluted Human Urine)

As the method proved successful and relevant in the detection of MDPHP in a biological sample of diluted human urine, the method was tested to determine the lowest concentration of MDPHP detectable using cyclic voltammetry. Figure 25 shows the cyclic voltammograms recorded over the range of 0 (blank) to 6 μg mL^{-1}.

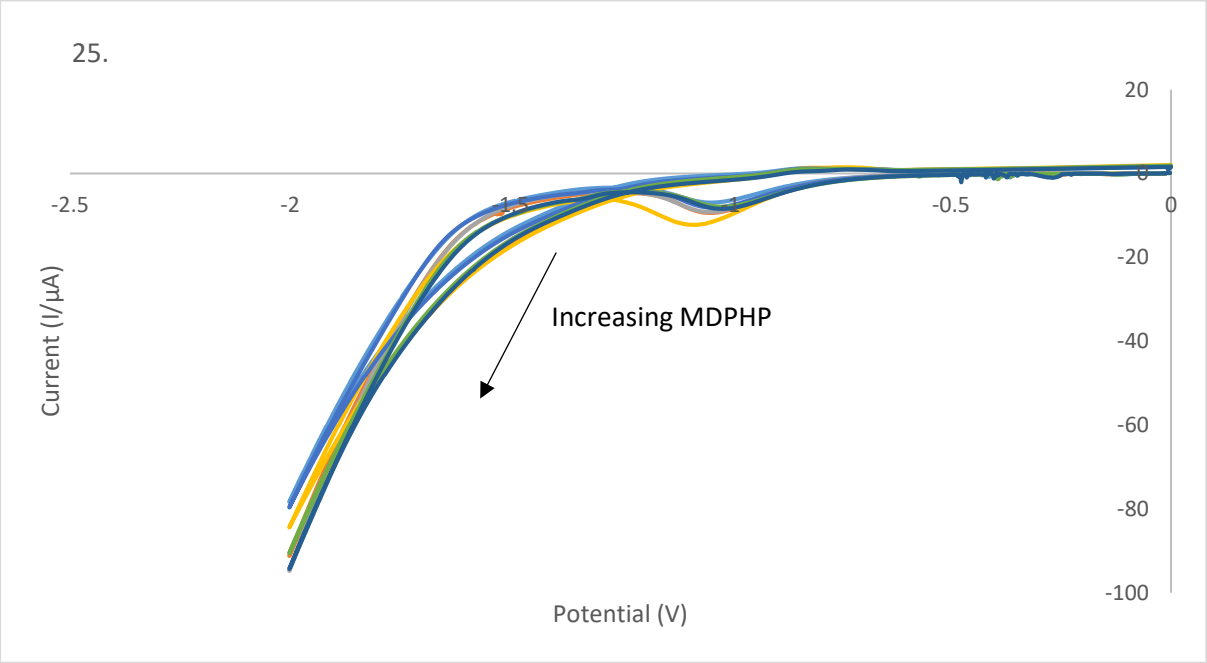


Figure 25. Cyclic voltammograms showing the electrochemical response of 0 – 6 μg mL^{-1} MDPHP in human urine diluted with 0.1 M acetate buffer at pH 5.5 using screen-printed graphite macroelectrodes (vs. Ag/AgCl).

Between 0 to 3 μg mL^{-1} there is no electrochemical response as there is no peak detected at the expected potential, the first electrochemical response is detected at 4μg mL^{-1} as there is a fluctuation at the expected potential and the peak becomes more apparent in the remaining higher concentrations. Therefore, the detection of MDPHP in a biological sample is possible at a low concentration of 4μg mL^{-1} using cyclic voltammetry.

3.3.10. Quantification of MDPHP in Street Samples

The percentage quantification of MDPHP in each street sample was studied using cyclic voltammetry and compared to data acquired from the technique of gas chromatography-mass spectrometry (GC-MS). A linear calibration plot was constructed using the control MDPHP in the range of 2000 – 2600 μg mL^{-1} with an R^2 value of 0.9995. Peak heights of the six MDPHP street samples were recorded at the concentration 0f 2500 μg mL^{-1}, the accurate mass of the samples weighed out were recorded in order to plot the data accurately. The average peak height of each street sample was recorded against the accurate concentration of each respective sample and this data was overlaid on the previously constructed MDPHP control calibration graph. Equation 3 was used to calculate the percentage quantification of MDPHP in each street sample.

$$\%\ Quantification\ = \left(\frac{Peak\ height\ of\ Street\ Sample}{Peak\ height\ of\ Control\ Sample}\right) \times 100$$

Equation 3. Used to calculate percentage quantification of MDPHP in each street sample

Results from the electrochemical method of cyclic voltammetry and gas chromatography-mass spectrometry are compared in table 4: the results between the two techniques are found to be concurrent apart from one street sample, D19, which shows a 9.3 % difference in both results. The electrochemical technique shows a significantly lower percentage at 51.2 % compared to the 60.5 % produced by GC-MS. Infrared spectroscopy (IR) was used to test the sample in question, which did not indicate the presence of any inorganic cutting agents that may have affected the result. The underestimation in quantification using the electrochemical technique may be due to surface adsorption on the electrode: the analyte may be sticking to the surface and preventing the diffusion process from occurring within the timescale of the measurement.

Table 4. MDPHP street sample quantification data comparison between GC-MS and electrochemical technique

Street Sample	GC-MS Quantification (%)	Electrochemistry Quantification (%)
D15	91.7	91.9
D16	79.0	81.8
D19	60.5	51.2
T1D	82.7	83.4
T2C	81.0	81.1
T4B	67.8	68.1

3.3.11. Summary

MDPHP is shown to be detectable using cyclic voltammetry, in acetate buffer over a range of pH's and it was determined that pH 5.5 acetate buffer was the optimum medium for the electrochemical response. Both control samples and street samples were successfully detected in linear ranges of 100 – 1000 $\mu g\ mL^{-1}$ in acetate buffer (0.1 M, pH 5.5). The corresponding limit of detections in acetate buffer (0.1 M, pH 5.5) were calculated to be 0.195 $\mu g\ mL^{-1}$ (control sample) and 0.130 $\mu g\ mL^{-1}$ (street sample). The technique of differential pulse voltammetry was also successfully utilized to detect the control sample and the linear range was found to be 100 – 1000 $\mu g\ mL^{-1}$ with a limit of detection of 0.348 $\mu g\ mL^{-1}$. In comparison, cyclic voltammetry was the preferred technique as there is a more linear relationship between peak height and concentration, and a lower LOD. The preferred technique successfully detected MDPHP in diluted human urine with a limit of detection of 0.205 $\mu g\ mL^{-1}$ and a lowest possible detection of 4$\mu g\ mL^{-1}$. The method of cyclic voltammetry was also shown to be a selective method, as control samples were spiked with the three common adulterants, paracetamol, benzocaine and caffeine and were shown not

to interfere with the expected electrochemical response of MDPHP. The quantification of MDPHP in street samples was studied by comparing data using two different techniques of cyclic voltammetry and gas chromatography-mass spectrometry (GC-MS): the results are found to be concurrent apart from one result as the electrochemical technique shows a lower percentage. This may be due to surface adsorption on the electrode as infrared spectroscopy (IR) was used to test the sample in question, and did not indicate the presence of any inorganic cutting agents.

In regards to the electrochemical process, a scan rate study was conducted, which produced a gradient value of 0.2, which was lower than the expected value and requires further work to understand the electrochemical process.

4. Conclusion

For the first time, the electrochemical detection of both N-ethylpentylone and MDPHP, were found to be possible using the electrochemical techniques of cyclic voltammetry and differential pulse voltammetry in both model buffer solutions and biological samples. The use of cyclic voltammetry offered a limit of detection of 0.046 μg mL^{-1} and 0.195 μg mL^{-1} for NEP and MDPHP control samples in a model buffer solution. Whereas differential pulse voltammetry offered a limit of detection of 0.130 μg mL^{-1} and 0.348 μg mL^{-1} for NEP and MDPHP control samples in a model buffer solution. MDPHP was successfully detected using cyclic voltammetry in a biological sample of diluted human urine with a limit of detection of 0.205 μg mL^{-1} and the method was proven to be selective as common adulterants did not interfere with the response of either NEP or MDPHP. These results are significant for the development of an in-field portable sensor as the techniques used prove to be reliable, rapid and simple. The disposable nature of the screen-printed electrodes is a great solution to the issue that previous literature focused primarily on laboratory-based techniques that cannot be used in-field. Electrochemical techniques were proven to be successful in this thesis and can be used to address the current issue that frontline workers such a healthcare and police staff do not have a quick and easy technique to determine if NEP or MDPHP is present in the system. The primary aims of this thesis were successfully achieved and can be used as a basis for future work which is outlined in the next section.

5. Future Work

Further studies into the chemical process may be undertaken in future to understand the electrochemical process in further detail, particularly in regards to MDPHP as the scan rate study did not produce the expected results. A more extensive scan rate study may be carried out to learn about the electrochemical process. The development of a portable sensor using the data collected is also a possibility.

As NEP was detected successfully in the tested matrix, this can be used as the basis for future work in regards to the detection of NEP in a biological sample, if shown to be successful this will strongly support the development of an in-the-field portable sensor.

MDPHP was successfully detected in a biological sample using cyclic voltammetry and further investigations to improve the sensitivity can be conducted by utilising differential pulse voltammetry. A comparison of the detection of MDPHP in both cyclic voltammetry and differential pulse voltammetry would be the next step in the development of an in-the-field portable sensor.

6. References

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