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Recent developments in electrochemical detection of illicit drugs in diverse matrices

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Abstract

Drug abuse is a global problem, requiring an interdisciplinary approach. Discovery, production, trafficking, and consumption of illicit drugs have been constantly growing, leading to heavy consequences for environment, human health, and society in general. Therefore, an urgent need for rapid, sensitive, portable and easy-to-operate detection methods for numerous drugs of interest in diverse matrices, from police samples, biological fluids and hair to sewage water has risen. Electrochemical sensors are promising alternatives to chromatography and spectrometry. Last decades, electrochemical sensing of illegal drugs has experienced a very significant growth, driven by improved transducers and signal amplifiers helping to improve the sensitivity and selectivity. The present review summarizes recent advances (last 10 years) in electrochemical detection of the most prevailing illicit drugs (such as cocaine, heroin, and (meth)amphetamine), their precursors and derivatives in different matrices. Various electrochemical sensors making use of different transducers with their (dis)advantages were discussed, and their sensitivity and applicability were critically compared. In those cases where natural or synthetic recognition elements were included in the sensing system to increase specificity, selected recognition elements, their immobilization, working conditions, and analytical performance were discussed. Finally, an outlook is presented with suggestions and recommendations for future developments.

Key words: illicit drugs, electrochemical sensors, recognition elements.

1. Introduction

A drug is any kind of substance that, when entering the body, causes physiological and/or psychological changes in a normal body behaviour (National Institute on Drug Abuse, 2004). Their acute and chronic effects in the human body are dependent on the type of drug, its purity, dose and way of ingestion (Novak and Kral,

2011). Drugs are classified in multiple ways, based on their origin, (chemical) structure, (pharmacological) effects, legality and social acceptance. Drugs can be either *naturally* occurring, *semi-synthetic* or fully *synthetic products* and classified as legal, illegal or prescript (Mathur and Hoskins, 2017). By-law-forbidden-drugs are illegal to own, use or sell. The risk of premature mortality and morbidity from illicit drug is dependent on its dose, frequency and route of administration (Mushayabasa and Tapedzesa, 2015). The main representatives of naturally occurring illicit drugs are indole hallucinogens, opium alkaloids such as morphine, cocaine and cannabis-derived tetrahydrocannabinol (THC). Semi-synthetic illicit drugs are formed via various chemical manipulations of the substances extracted from natural components. A well-known representative of this group is heroin, which is formed via acetylation of morphine. Illicit drugs can also be fully synthesized through different chemical processes. Amphetamine-type stimulants (ATS) are the most common drugs within this group.

Regardless of whether a drug is legal or not, its consumption can possibly lead to addiction (Compton and Volkow, 2006). Different countries across the world have made international agreements, in the form of United Nations conventions, to control production, possession, sale and use of illicit drugs. For most drugs, in parallel to international regulations, additional national legislations are in place. The history of the international drug control and establishment of the international drug control system have been explored in the United Nations Office on Drugs (UNODC) 2008 report *100 Years of drug control* (UNODC, 1972). Although many time and efforts have already been invested in drug control, (ab)use of illicit drugs is still a worldwide problem. The world drug report of 2019 states that an estimated 1 out of 18 adults, or 271 million people, between the age of 15 and 64 years, at least once consumed an illicit drug in 2017 (UNODC, 2017). Cannabis remains the most popular drug worldwide, amphetamines being placed secondly. (Prescribed) opioids are found at the third place and are far less common. Furthermore, among 29 million drug addicts, 12 million people consumed it through injection. Half of this number (~ 6 million people) lives with hepatitis C and almost a quarter (~1.6 million people) are infected with the human immunodeficiency virus (HIV) (UNODC, 2016). Therefore, the long-term consequences of drugs (ab)using cannot be underestimated. The Global Burden of Disease Study 2017 estimates that in 2017 approximately 585.000 deaths worldwide were drug-related (UNODC, 2017). Analytical methods for the detection of illicit drugs are thus indispensable. Authorities, including police, are interested to monitor sewage water, environmental and street samples for mapping drugs and their precursors and metabolites in order to obtain crime statistics and rates of consumption (Groeneveld et al., 2015). Waste water can be screened to locate drug production sites, indicate drug abuse and/or investigate its dispersity (Zuccato et al., 2008). Identifying and quantifying a type of drugs in human body fluids and hair can be used as evidence in court. Post-mortem examination of blood, hair, urine and/or brain can help to determine the cause of death (Lehrmann et al., 2009). On another side, drug tests can also be used in a preventive way. School based random student drug testing (RSDT) is only one example of overall drug prevention. The goal of these tests is to identify young adults in need and educate them about the acute and chronic drug effects, which hopefully stimulates them to stay drug-free. Although there is insufficient evidence to support or refute the efficacy of RSDT in schools and evaluation with long term follow-up will be needed, these programs are already implemented in the United States (Warner et al., 2013).
So far, the majority of analytical techniques used for the detection and identification of illicit drugs are chromatographic methods, both gas and liquid chromatography (Fernández et al., 2004; Hernández et al., 2014; Kala et al., 2008). Although these techniques are characterized by a high accuracy and specificity, their drawbacks include high cost and need for a sophisticated sample pre-treatment, complex instrumentation and trained personnel for data processing. Furthermore, chromatographic analysis is a relatively long and

expensive procedure, which cannot be performed on-site (Lurie and Of, 1997). In the past, several reviews have already been published on different portable analytic devices for the detection of illicit drugs. Pereira De Oliveira et al. (2018) investigated new trends in forensic devices for explosives, gunshot residue, liquid fuels, illicit drugs, beverages, alternative biological fluids and agrochemicals from 2013-2017, while Yanez-Sedeno et al. (2019) reviewed biosensors for poisons, drugs, toxins, explosives, and chemical and biological weapons. de Araujo et al. (2018) focused their research on portable analytical platforms, such as paper-based devices, electrophoresis chips, and electrochemical sensors, for the detection of drugs of abuse and explosives. The current review is engrossed on (dis)advantages of different electrochemical sensing approaches for the detection of illicit drugs, developed within the last 10 years. While the work of Florea et al. (2019a) mainly focuses on the development of immunosensors for forensic drugs within the last two years, and Zanfrognini et al. (2020) describe recent sensing systems for the detection of drugs of abuse based on the direct oxidation/reduction of the analyte on the electrode surface, the current review is centred around the use of different recognition elements and the importance of their immobilization strategies. In addition, electrochemical sensors without recognition elements were described in detail. Since the term "bio-sensor" only refers to a system based on natural recognition elements, the general term "sensor" will be used throughout this review.

2. (Bio)sensors for detection of illicit drugs

A (bio)sensor generally consists of three elements: a (bio)recognition element, transducer and signal display. The interaction between the analyte and the (bio)recognition element is converted by the transduction system into a readout (Masikini et al., 2015). Sensors are categorized into four main groups, depending on the applied transducer: electrochemical transducers (potentiometric, voltammetric, conductometric, etc.), optical transducers (such as surface plasmon resonance (SPR) sensors), piezoelectric transducers (such as quartz crystal microbalance (QCM) sensors), and thermometric transducers which measure the amount of heat with a sensitive thermistor to determine the analyte concentration (Ertürk and Mattiasson, 2017). Electrochemical sensors are predominant and are the focus of this review. They possess the advantages of being highly sensitive, rapid and low cost, while enabling miniaturisation and on-site use (Labib et al., 2010, 2009a). In order to develop a sensor for the detection of illicit drugs, some specifications must be accomplished. The sensor must be relatively small and portable to allow its on-site usage, have a fast response time, operate independently and be robust. For rapid analysis of human samples or for on-site monitoring of environmental samples, the sensor must have a short analysis time or provide real-time monitoring of a signal. The binding event between a target and a recognition element (if such is used) must be independent on the matrix composition and environmental changes, such as presence of organic solvents or algae, changes in pH, pressure and/or temperature. Sample pre-treatment must be minimal to make the sensor time- and economically-efficient. The analysis must be performed with a high accuracy and precision. The response must be selective for the target analyte(s) and must not be influenced by any transducer-induced noise.

In order to achieve the requirements for specificity, several parameters need to be taken into account. Interfering compounds can be present in the matrix and disrupt the measurements. Its effect is dependent on the type of interference (structure, functionalities present, concentration,...), measuring system and matrix. Several strategies can be tested to exclude the undesired compounds or to minimize its effect on the results. Firstly, optimization of the measuring procedure can be performed (range of the voltammetric scans, choice of buffer (acid/base, pH, concentration, content), type of transducer, etc.). Furthermore, optimization of the sample pretreatment or selective preconcentration steps can exclude the interference. Alternatively, the sample complexity can be reduced, e.g. there are ways to generate, in a measuring device, plasma from blood (by

applying membranes preventing passage of blood cells but allowing the liquid fraction to flow through). Secondly, the system may contain natural or artificial recognition elements for specific interaction with analyte(s). This recognition element is immobilized on an (working) electrode (transducer). Electrochemical measurements are performed using two- or three electrodes systems. Three electrodes systems contain auxiliary (AE), reference (RE) and working (WE) electrodes, whereas a two electrodes system consists of only an RE and WE (Guy and Walker, 2016). A two-electrodes system is used when either a fixed current or potential is applied between the WE and AE, and another variable is measured. In case it is aimed to carefully control and measure both potential and current passing through the cell, a three electrodes system is employed. WE's are most commonly metals, such as platinum, gold, mercury, or carbon since these materials show an excellent redox behaviour and potential window. Screen-printed electrodes (SPEs) are gaining popularity in the market of WE's. The SPE-technology uses layer-by-layer depositions of ink upon a solid substrate (Alonso-Lomillo et al., 2009; Taleat et al., 2014).

3. Types of recognition elements

In general, recognition elements can be divided into two groups, natural (and engineered natural) or artificial (synthetic), depending on their origin. Natural recognition elements such as microorganisms, antibodies (Abs), aptamers, enzymes, tissues, DNA probes, and cells have already been successfully applied in the biosensor technology (Cheun et al., 1996; Mannelli et al., 2005; Park et al., 2013; Rocchitta et al., 2016; Sharma et al., 2016; Wang et al., 2013). Artificial recognition elements include synthetic peptides and molecularly imprinted polymers (MIPs). To the best of our knowledge, no synthetic peptide for illicit drug detection has been applied yet. Therefore, this review will not further focus on this type of artificial element.

3.1 Antibodies

Antibodies (Abs), also known as immunoglobins (Ig), are large Y-shaped glycoproteins mainly produced by differentiated B cells. They are still the most popular recognition elements in diagnostics due to their excellent dissociation constant (K_d) values (nanorange, 10^{-9} mol/L) and their unique ability to selectively recognize an antigen via an antigen-binding (Fab) variable region. Tips of this region contain paratopes which bind to epitopes of an antigen. At the base of an antibody molecule, an Fc region is situated. Each antibody possesses two large heavy chains and two small light chains, held together by disulphide bridges. The Fc region holds amine functionalities while the Fab region has a terminus with carboxylic functionalities. Presence of these functional groups facilitates the Ab-immobilization for e.g. electrochemical applications. Different Ab immobilization techniques can be found in literature, each with their own (dis)advantages. During passive adsorption, a working electrode is submerged in an antibody solution and incubated for a certain time under controlled parameters such as the concentration, temperature, humidity, and pH (Parkash et al., 2014). Although this mechanism is relatively easy and rapid (no Ab-modification needed), weak bonds are formed with random orientation of the biomolecules, and affinity loss caused by their denaturation is often observed (Jung et al., 2008). The most common Ab-immobilization is a cross-linking to chemically-activated surfaces via aldehydes (e.g. glutaraldehyde), epoxides (ether bond) or carbodiimides and succinimide ester (e.g. an EDC-NHS coupling mechanism) (Jung et al., 2008; Sharma et al., 2016). Covalent immobilization assures a stable bond between Ab and the electrode surface, but does not ensure universal orientation of the immobilized Abs. Several studies showed that randomly oriented antibodies have a 2-3 fold lower capacity than the well-oriented ones (Cho et al., 2007; Danczyk et al., 2003; Kang et al., 2007). For the antibody-oriented immobilization, several mechanisms are known such as an avidin/streptavidin-biotin, Zn/Ni-histidine, Ab-

ligand interaction (Protein A, Protein G, Protein A/G, secondary antibodies), and metal affinity coordination. Although these mechanisms are often more complicated, a defined orientation of antibodies improves the overall sensitivity of a sensor (Parkash et al., 2014). One of the major drawbacks of antibodies in environmental analysis is their limited working conditions. Optimally, Ab-based sensors are applied at physiological pH (7.2-7.4) and temperature (37-38°C) or within some insignificant fluctuations. Beyond this range, degradation or denaturation occurs, resulting in a loss of the recognition/binding ability (Usami et al., 1996).

Butler et al.(2006) used passive adsorption as an immobilization technique for polyclonal antibodies to detect methamphetamine (MA) in saliva and urine, while in the study of Zhang et al.(2012) an antibody was bound to colloidal Au nanoparticles (AuNPs) to increase the sensitivity. A lower (approximately 3 times) limit of detection (LOD) was observed in the case of Zhang, although it should be noted that these studies were performed for different matrices.

3.2 Aptamers

Aptamers are single-stranded DNA or RNA oligonucleotides which show, in comparison with antibodies, a higher stability to different incubation conditions and a longer shelf life (Cai et al., 2006). They are characterized by medium recognition ability, with K_d values in the micro- to nanomolar (10^{-6}-10^{-9} mol/L) range, which places them in between antibodies and MIPs. Aptamers are produced via the *in vitro* SELEX (Systematic Evolution of Ligands by Exponential Enrichment) technology. This production process can easily be scaled up and is not dependent on the use of bacteria, cell cultures or animals. Aptamer immobilization on the surface of an electrode can be performed similar to Ab immobilization. The most common methods include physical adsorption, avidin-biotin affinity interaction, electrochemical grafting, using 4-carboxybenzenediazonium for coupling (Ocaña and del Valle, 2014), or covalent binding after electrochemical activation of the electrode via amide bonding, thiol-gold binding, or carbodiimide cross-linker chemistry. Upon the binding with a target analyte, the 3D-conformation of an aptamer switches to a locked structure leading to significant changes in electrochemical properties.

So far only a couple of aptamer-based sensors for detection of illicit drugs have been described. Baker et al.(2006), Zhang et al. (2012) and Yang et al.(2016a), and Oueslati et al.(2018) designed electrochemical, voltammetric, two impedimetric, and capacitive sensors respectively, based on aptamers for detection of cocaine. Baker et al.(2006) immobilized a methylene blue (MB)-tagged aptamer via self-assembly of the alkanethiol group while the aptamer of Zhang et al.(2012) was divided into two fragments, Cx and Cy, where both fragments were modified with thiol groups to assure their adsorption on a gold WE. Yang et al. (2016a) compared two different immobilization strategies for a cocaine sensor. In the first approach, a thiolated ssDNA was first immobilized onto the gold electrodes while in the second approach the ssDNA and dsDNA aptamers were co-immobilized. Due to less steric hindrance, a larger charge transfer resistance was found in case of the co-immobilization. Oueslati et al. (2018) immobilized aptamers via physical adsorption. Although we believe that covalent binding would be better in terms of repeatability and reproducibility, the developed sensing system shows great potential with a response time sampling-to-result of only 30 s and an LOD of 1.34 pM in neat serum. Ebrahimi et al.(2012) developed an aptasensor for MA where AuNPs were exploited to increase the load capacity of the aptamers, and hence to increase the sensitivity.

In case of natural recognition elements, the incubation time (also known as the accumulation or waiting time) is of crucial importance. This time occurs after the sample loading and allows binding between the recognition

elements and the analytes. Most developed (bio)sensing systems have an optimized incubation time where a compromise is reached between the signal response and the total analysis time. So far, not much research was devoted to this parameter. Recently, Zorea et al.(2020) published their research on the influence of the antibody density on the electrode's surface and the antigen incubation time using non-faradaic electrochemical immunosensors. At lower frequencies, a significant difference was observed in function of time for the response. This was not observed at higher frequencies.

3.3 Molecularly imprinted polymers

MIPs are synthetic polymers with specific recognition sites complementary in shape, size and functional groups to a template molecule (Sellergren, 2007; Vlatakis et al., 1993). MIPs are characterized by a high mechanical and thermal stability and demonstrate an excellent chemical resistance in a broad pH-range (Sellergren, 2007; Svenson and Nicholls, 2001; Vlatakis et al., 1993). Although MIPs have lower K_D values (microrange, 10^{-6} mol/L) in comparison with natural recognition elements, their outstanding robustness make MIPs a preferred receptor for applications where natural receptors can degrade, denature or lose their affinity for example in environmental analysis (Vasapollo et al., 2011). MIP particles can be first synthesized and then immobilized on an electrode, or their synthesis can be directly performed on the surface of an electrode via electro-polymerization, drop-coating of a pre-polymerization mixture, lithography, or *in situ* polymerization. Electropolymerization and *in situ* polymerization are found to be superior to all other polymerization techniques for (bio)sensoring due to their great adherence to a transducer surface and the simple and fast mechanisms. When the potential is high enough, coupling of monomers occurs. Therefore, the polymer film is directly formed on the electrode surface (Xia et al., 2001). When the particles are first synthesized, immobilization is performed via an extra step of covalent or electrostatic binding or via incorporation of the particles in membranes such as gelatin, agarose, and poly-tyramine. Although the incorporation in a membrane is a relatively easy process, which does not require functional groups at the surface of the MIP, it can lead to a decrease of availability of the binding sites and a higher variability between measurements. If the particles are immobilized via a linker to the electrode's surface, the covalent approach is favored over the electrostatic approach due to the higher stability of the formed bonds. Until now, the amount of immobilization protocols is still limited.

For potentiometric sensors, selective membranes were embedded by dispersing MIPs in a plasticized polyvinyl chloride (PVC) matrix or carbon paste (Smolinska-Kempisty et al., 2017; Zhuo et al., 2016). Another option is sol-gel based immobilization. Agarose gels have already been documented as a method for MIP immobilization onto different surfaces (Patel et al., 2009, 2008). Kriz et al. used this type of network to immobilize specific cocaine MIPs that detected down to 0.1 µg/mL of cocaine in serum (Kriz and Mosbach, 1995). Florea et al. (2018) investigated the use of graphene modified electrodes for detection of cocaine in street samples. Graphene was applied to increase the conductivity as it offers a high surface area with more binding sites. MIPs were directly formed on the surface by electrodeposition. Both p-aminobenzoic acid (PABA) and o-phenylene-diamine (OPD) were tested as monomers. Although OPD demonstrated a higher binding affinity to cocaine during the modeling stage, PABA proved to be the more convenient in reality. The PABA-based sensor was applied in a concentration range of 50-500 µM. To enhance the communication between the imprinted sites and the electrode and improve the homogeneous distribution of these binding sites, palladium nanoparticles were integrated in the sensing layer for further research. The oxidation peak current varied linearly with the cocaine concentration in the range of 100–500 µM, with a detection limit of 50 µM. The sensor was tested in saliva and river water (Florea et al., 2019a). Our research group chose itaconic acid as a co-monomer for the synthesis of MIPs towards N-formylamphetamine (NFA). This introduced free

carboxyl groups on the exterior surface of the MIP which can interact both electrostatically and covalently with an amine group. Therefore tyramine was selected as the linker (Graniczkowska et al., 2016).

4. Types of transducers

The transducer is a physical device that converts a measured signal to a corresponding, mostly quantifiable signal, more often digital. To select an optimal transducing approach several characteristics must be taken into account: the desired LOD or cut-off limit, the available sample volume and the sample matrix. Electrochemical transducers report changes in electrochemical characteristics of a detecting system such as a potential (potentiometry), conductivity (conductometry), capacitance, impedance or current (amperometry/voltammetry). These changes are proportional to the concentration of an analyte. The LOD and working range of the electrochemical sensors towards different illicit drugs are summarized in Table 1-3.

4.1 Voltammetric sensors

Voltammetric sensors measure the current (*i*) at a varying potential (*E*). They apply a potential that forces a change in the concentration of an electroactive species at the electrode's interface by oxidizing or reducing that species. Within this group, different subclasses can be distinguished such as cyclic voltammetry (CV), normal pulse-(NPV), differential pulse- (DPV), squarewave-(SWV) and (anodic, cathodic or adsorptive) stripping voltammetry (Mendoza et al., 2015). Barreira Rodriguez et al. (1990) were the first to investigate the voltammetric behavior of heroin (Supplementary information (SI) Figure 1) using a carbon paste electrode. The anodic waves observed from heroin correspond to the oxidation of two functional groups present on its structure. The first peak at 0.5V represents the oxidation of the tertiary amine group to the corresponding secondary amine, giving norheroin (3,6-diacetyl normorphine). Since heroin is unstable in aqueous samples at alkaline pH, it undergoes rapid deacetylation through hydrolysis forming 6-monoacetylmorphine (6-MAM). Therefore, the second peak corresponds to the oxidation of the acetylgroup to the phenolic group at pH>6. After the activation of the electrode and 15 s of waiting time, the voltammetric scan was started. This waiting time allows a target analyte to interact with the modified electrode prior to the analyses and can be seen as a pre-concentration step. The developed sensor could be used for heroin determination in seized drug samples over a wide concentration range. To this end, 15 mg of the samples were dissolved in 10 mL 0.01M $HClO_4$ and 1 mL of this solution was placed in an electrolytic cell containing 10 mL Britton Robinson buffer (pH=6). More recently, a SWV method was published using bare graphite electrodes for the detection of heroin in street samples, dissolved in phosphate buffer at pH 7 and 12. At pH 7, an irreversible oxidation peak was found at 0.81V due to the oxidation of the amine group. At lower potential (0.40V), an additional peak was found from the oxidation of the phenol group of 6-MAM, which is an active metabolite of heroin. At pH 12, the intensity of the second peak increased due to the fast hydrolysis of heroin to 6-MAM in alkaline medium. In acid medium, no peaks were observed. This dual-pH electrochemical strategy allowed fast heroin detection in street samples, even when mixed with common mixing agents. Prior to the samples analysis, a preconditioning step was included for 180 s (Florea et al., 2019a).

Garrido et al. (2004) studied the oxidative behavior of heroin in aqueous solutions studied via DPV using a glassy carbon paste electrode. Both the oxidation of the tertiary and its follow-up secondary amines, and the oxidation obtained by hydrolysis of the 3-acetyl group to the phenolic group at an alkaline pH were followed. Since the hydrolysis was strongly dependent on the pH, the studies were performed in a pH range from 2 to 10. In other research, they focused on the electrochemical profile of amphetamine and ATS. Using a glassy

carbon electrode, amphetamine (AMP), MA, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) were studied in different buffers (from pH 1.2 to 12.2) using CV, DPV, and SWV (SI Figure 2). A quantitative method was developed for MDMA in seized samples (ecstasy tablets dissolved in deionized water) and human serum. AMP could not be detected since the aliphatic primary amine oxidizes at a higher potential than allowed by the working range of glassy carbon electrodes. MA gave an anodic peak at pH>9 at 0.92V due to oxidation of the secondary amine. From pH 2, the anodic peak was observed at 1.17 V for MDA due to the electron removal from the aromatic nucleus, forming a radical cation. MDMA showed two anodic peaks, one at 1.18V (the oxidation of the aromatic nucleus, analogous to MDA) and one appearing from pH 4 at 1.31V. At pH>9, a third peak appeared at 0.86V from the oxidation of the secondary amine (Garrido et al., 2010).

Tadini et al. (2014) developed a method for the detection of MDMA by CV using a chemically modified electrode (CME) with Nafion (Nf), cucurbit[6]uril, and methanol. Cucurbit[n]urils (CB) bear a cavity with [n] carbonyl groups oriented outwards, forming electron-rich portals on both sides of the cavity which can form complexes with the target MDMA. An anodic peak was found at 1.16V from the oxidation of the aromatic nucleus, resulting in a cation radical. The formation of the delocalized cation over the aromatic ring destroys the strong host-guest solvophobic interactions, expelling the aromatic nucleus from the CB cavity and favoring an interaction of this ion with a carbonyl oxygen. It could be concluded that the CB cavity showed more affinity for the target analyte than for its oxidation product. This electrode looked very promising since it was more sensitive than the glassy carbon and platinum electrode. Cumba et al. (2016) focused on MDMA and para-methoxyamphetamine (PMA) and measured these voltammetrically via CV and DVP with different commercially available electrodes, such as glassy carbon, boron-doped diamond and screen printed graphite electrode. The SPE showed the optimal response and did not need any pre-treatment step compared to the glassy carbon electrode. Moreover, it is low-cost and was found to be highly reproducible. PMA showed a peak at +1.162V while an oxidative peak for MDMA appeared at +0.924V. Solid samples were crushed and dissolved in PBS (pH 7). PMA and MDMA were determined in the range of 0.50-4.98 µg/mL with an LOD of 0.04 µg/mL for MDMA and 0.03 µg/mL for PMA in PBS, respectively. When both analytes were present in the same sample, the LOD increased to 0.14 µg/mL with a linear range of 2.00-19.60 µg/mL (Figure 1).

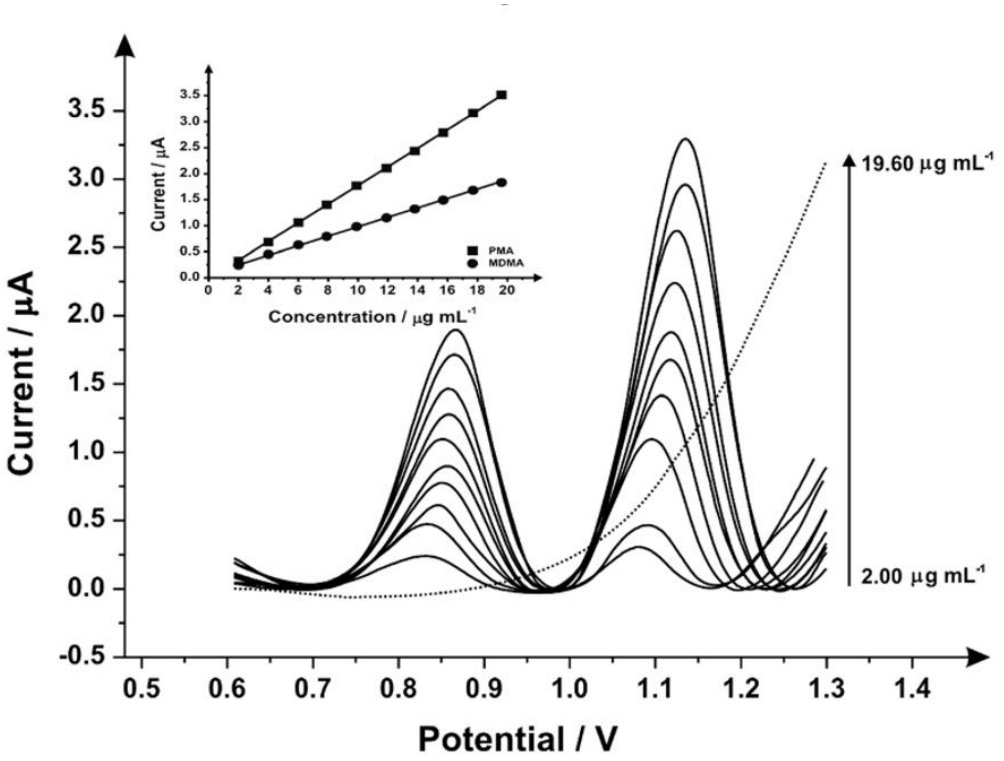

Figure 1: DPV obtained utilizing the screen printed graphite electrode by adding aliquots of MDMA/PMA (in phosphate buffer pH 7). The dotted line represents a blank. The first anodic peak is caused by the oxidation of MDMA, while the second one is derived from the oxidation of PMA (Cumba et al., 2016).

Bartlett et al. (2016) developed screen-printed carbon electrodes for the detection of MA in saliva and undiluted saliva. The mechanism was based on the oxidation of the target, mediated by N,N'-(1,4-phenylene)-dibenzenesulfonamide. This mediator reacted with MA, forming an electro-active adduct which could undergo reduction, as described more in detail by Adams and Schowalter (Adams and Schowalter, 1952). In the absence of MA, the oxidation peak was found at +0.38V together with a negligible reduction peak, coming from the mediator. When MA was present, two new peaks were observed at +0.15V and -0.046V, and the increase of the reduction peak at -0.088V was detected. Additionally, the oxidation peak from the mediator was increased. The procedure consisted of a 10 s waiting time after the sample loading and showed a response time of 55 s. Although the measuring time was significantly shorter (55s) in comparison to an existing lateral flow immunoassay, the reached LOD (400 vs 10 ng/mL for the sensor and the immunoassay, respectively) was too high for this approach to be accepted as a screening test. Therefore further research is still necessary to increase the sensitivity of the system. MA was detected by Oghli et al. (2015) in solid street and biological samples by CV and DVP using a pre-treated pencil graphite electrode (PPGE). It was shown that with an increased pre-treatment time of 600 s, an increase in the MA oxidation signal was found. Further extension of this time led to a decrease of the obtained signal. The well-defined anodic peak of MA was found at 0.74 V, stemming from the oxidation of the secondary amine in comparison to the non-pretreated pencil graphite electrode (NPGE), where only a very weak peak was observed. The same LOD (50 nM) was achieved in biological fluids (urine) by Svorc et al. (2014) using a self-assembled boron-doped diamond electrode. DVP measurements show the oxidation peak at a potential higher than +1.23V at pH 10. Human urine samples could be analyzed without any pre-treatment. Rafiee et al. (2015) used a AuNPs/multiwalled carbon nanotube (MWCNT) Nf modified SPE for the detection of the same target dissolved in alkaline solutions (0.01M NaOH). Oxidation of the secondary amine was observed at +0.45V. The analysis was started with an

optimized waiting time of 200 s after the deposition of a sample. Preliminary tests of the electrode were performed using CV, while further quantitative analyses were performed by SWV. Nevescanin et al. (2013) determined AMP and MDMA using gold electrodes by CV and SWV. The goal of this research was to investigate the voltammetric behavior of the ATS by CV and to quantitatively determine the MDMA- and AMP content in illegally produced tablets (dissolved in 0.05M $NaHCO_3$) and human urine samples by SWV/CV. Prior to the analysis, 220 s of waiting time was included.

Novak et al. (2013) developed a method based on SWV for the detection of cannabinoids in food products (plants e.g. hemp) using microparticles at a paraffin-impregnated graphite electrode. Cannabinoids are poorly soluble in water and can be oxidized in the same electrode as other phenols. Therefore, water-insoluble microparticles were incorporated with transfer electroactive compounds onto the surface of a paraffin-impregnated graphite electrode. When the electrode was submerged into an aqueous electrolyte, the attached compounds were used as reactants in the electrochemical analysis. At pH 7, a single anodic peak for Δ9-tetrahydrocannabinol (Δ9-THC, SI Figure 3), cannabinol and cannabidiol was found at 0.55 V from the oxidation of the phenol group to a phenoxy radical. Balbino et al. (2014) investigated the use of two different conventional working electrodes, a glassy carbon and a platinum disc, for the detection of Δ9-THC in seized samples by SWV. Tetrabutylammonium tetrafluoroborate (TBATFB) was chosen as supporting electrolyte and after the waiting time of 30 s, a well-defined anodic peak was found at 0.0V and -0.01V for the glassy carbon and platinum disc respectively. The glassy carbon electrode was found superior due to a lower LOD in comparison to the platinum working electrode (6.2×10^{-10} vs 2.7×10^{-8} for the glassy carbon and platinum electrode, respectively). Later Balbino et al. (2016) published a CV-method using the same type of glassy carbon electrode for the detection of Δ9-THC in suspected marijuana plants. The same accumulation time of 30 s was kept. The samples were weighed, dissolved in hexane, filtered and diluted with a Fast Blue B salt (FBB) solution. If Δ9-THC was present in the sample, it got derivatized by the Fast Blue B salt, where consequently oxidation of this intermediate occurred and an anodic peak appeared at 0.0 V. Fast Blue B salt is a commonly used reagent added to samples for colorimetric confirmation (it generates a deep red or purple chromophore) of marijuana-contaminated samples.

Oiye et al. (2009) developed a fast, non-destructive method for the detection of cocaine (SI Figure 4) in confiscated samples dissolved in 0.1 M $NaCLO_4$ in water. A platinum electrode was modified with cobalt-hexacyanoferrate (CoHCFe). When cocaine interacted with cobalt ions, it formed a complex which partially passivated the film on the surface. Once the complex was formed, the mobility of the Na^+ ions and the subsequent electron transfer for the Fe^{II}/Fe^{III} redox couple was blocked, leading to a decrease of the current peaks. Siqueira de Oliveira et al. (2013) determined cocaine by CV in confiscated samples using a modified carbon paste electrode with different methoxy-substituted N,N'-ethylene-bis(salicylideneiminato)-uranyl(VI) complexes. The uranyl oxygen of the Schiff base complex interacted with the carboxyl group of cocaine. A well-defined peak was found for cocaine at 0.62V. In the same year, Siqueira de Oliveira (2013) published the use of other uranyl Schiff base films, $[UO_2(3\text{- MeOSalen})(H_2O)]H_2O$ and $[UO_2(5\text{-MeOSalen})(H_2O)]H_2O$ for the detection of cocaine but using platinum and glassy carbon electrodes. The accumulation time was set on 5 s. Freitas et al. (2017) reported on the development of a portable and simple batch injection analysis system based on square wave voltammetry (BIA-SWV) for screening of cocaine and adulterants in seized cocaine samples using a boron-doped diamond electrode. The samples were dissolved and diluted in a supporting acid electrolyte. In this acidic medium, the amine group of cocaine is protonated and a higher energy barrier is required for oxidation to occur. Therefore, the reaction will only take place at a higher

potential (>1.9V). Measurements were performed at 2.1V, where the peak of cocaine was clearly separated from those of other common adulterants. De Jong et al. (2016) developed a wearable fingertip sensor for the on-spot detection of cocaine and its cutting agents in street samples. Carbon SPE's were placed on the glove using a conductive, flexible gelatin hydrogel as electrolyte. A peak was found at 1.04V due to the oxidation of the tertiary amine of cocaine. A conditioning step of 5 s was included. The LOD using this system was significantly lower than the LODs of the traditionally used color tests (2 μM versus 13.8 μM, respectively). Solid street samples were dissolved in 0.02 M KH_2PO_4 buffer (pH=7) with 0.1 M KCl. Due to the cocaine signal suppression in presence of levamisole, which could potentially lead to false negatives, two optimized strategies were later on published by the same group (De Jong et al., 2018; Florea et al., 2018). In the first strategy, disposable graphite electrodes were applied in a buffer with higher pH (12 instead of 7), where the oxidative peaks of both cocaine and levamisole could be distinguished, allowing simultaneous detection. However, there should be mentioned that a signal decrease was found in time for cocaine, probably due to stability issues of the target at pH 12. Cathodically pretreatment of these graphite electrodes for 360 s lowered the LOD from 5 to 3 μM (De Jong et al., 2018). The second approach used functionalized the carbon SPE with conductive polymers. Based on the computational calculations, two monomers, PABA and OPD were selected and immobilized via electro-polymerization (CV) of the carbon SPE's. OPD showed the highest binding score for cocaine, but PABA had a higher conductivity, which made this monomer a better choice for the selected application. SWV measurements, performed under the same conditions in phosphate buffer at pH 7, showed that cocaine was detected in a concentration range of 50–500 μM and in the range of 100–500 μM in the presence of 30% levamisole (Florea et al., 2018). To enhance the communication between the imprinted sites and the electrode and improve the homogeneous distribution of these binding sites, palladium nanoparticles were integrated in the sensing layer. The oxidation peak current varied linearly with a cocaine concentration in the range of 100–500 μM, with the detection limit of 50 μM. The sensor was tested in saliva and river water (Florea et al., 2019a). Baker et al. (2006) developed an aptamer-based sensor for the rapid and label free detection of cocaine at μM level in adulterated samples and biological fluids. The methylene blue (MB)-tagged aptamer self-assembled on a gold electrode via the alkanethiol group. The aptamer folds into a three-way junction in the presence of the target, resulting in an alteration of the electron transfer, and causing an increase of the observed reduction peak. The equilibrium was rapidly obtained: after 80 s, 97% of the total signal change was observed and in less than 4 min completely saturated. For further measurements, the accumulation time was set on 80 s. The sensor's variability was 3%, and even after six sequential injections and regeneration, the initial sensor loss was within 1%. AMP could not be detected since it oxidizes at a higher potential than the working window of glassy electrodes. More recently, Tavakkoli et al. (2019) published a sensitive aptasensor, also for cocaine, using CV (to study the changes in the electrochemical properties of the active area of the electrode) and SWV (to achieve a quantitative method). The 5'-disulfide-functionalized end of the aptamer was immobilized on a nanoporous gold (NPG) electrode followed by conjugation of the 3'-amino-functionalized end to 2,5-dihydroxybenzoic acid (DHBA) as a redox probe. In the presence of cocaine, the aptamer changes from an open unfolded state to a closed conformation, reducing the distance between DHBA and the electrode surface and enhancing the electron-transfer efficiency. The incubation time was optimized and set at 40 min. More recently, an electrochemical sensor for psychoactive drugs in water was developed by Masemola et al. (2020) using an exfoliated graphite (EG)-AuNPs modified copper wire. The sensor was able to detect cocaine in water with an LOD of 0.82 mg/L, under the pH of 12.25. An oxidation peak was found at +1.09V, caused by the irreversible oxidation from the tertiary amine of cocaine to an imine. The sensor was further successfully tested on methylphenidate, amphetamine and heroin as well, thus showing the application of the EG-AuNPs electrode for electrochemical detection of psychoactive drugs and their

metabolites in synthetic and real water samples. A MIP-based sensor was developed using SWV for detection of cocaine in saliva, river water and street samples. Therefore, a SPCE was modified with graphene and Pd NPs prior to electropolymerisation by CV, using PABA as monomer and cocaine as template. In presence of cocaine, an oxidation peak was found at 0.88V due to the entrapment of cocaine inside the MIPs. Street samples were dissolved in PBS (pH=7) (Florea et al., 2019a).

Merli et al. (2014) developed a method for the detection of lysergic acid diethylamide (LSD, SI Figure 5) in spiked toons and biological samples, such as hair, plasma and urine, to investigate the most optimal extraction method for each matrix, using adsorptive stripping voltammetry. Two oxidation waves were found at $E_{1/2}$=860 mV (E_p=913 mV) and $E_{1/2}$=1250 mV (E_p=1139 mV), each characterized by a complex two electron process. Measurements were performed using a pretreated glassy carbon electrode, samples were diluted in DMF/tetrabutylammonium perchlorate. A waiting time of 50 s set prior to analysis. More recently, Ribeiro et al. (2017) published a new voltammetric method to determine LSD in seized blotters using a glassy carbon electrode. An anodic peak was found at 0.88V, $KClO_4$ was used as supporting electrolyte in aqueous solutions. A preconcentration step of 60 s was included.

Oiye et al. (2017) published research on a new class of illicit drugs, called N-benzyl-substituted phenethylamines (NBOMes). As a proof of concept, a SWV method for the fast and sensitive detection of 25H-NBOMe (SI Figure 6) in blotter samples was developed using a glassy carbon disk as WE. After preconcentration for 30 s, analysis show an anodic peak at 1.35 V due to the oxidation of NBOMes. Souza et al. (2018) developed an SWV method for the detection of NBOMes and their correlates, 2,5-dimethoxyphenethylamines (2C-X group, SI Figure 6) in blotting paper (diluted in methanol and supporting electrolyte) using a boron-doped electrode. A first peak was found at +1.2V for all 25X-NBOMes and 2C-B due to the electrochemical oxidation involving two-electron transfer with the substitution of the halide group by a hydroxyl group on the aromatic ring, followed by the formation of a quinone derivative. A second peak at +1.8V, only found for 25X-NBOMes, represents the dealkylation process of the secondary amine. More recently, Belchior de Andrade and Gonzalez-Rodriguez (2019) published a new method for the selective detection of 25I-NBOH and 2C-I in blotter paper using an SPCE via differential pulse voltammetry.

4.2 Amperometric sensors

Amperometric sensors are a subclass of voltammetric sensors, where a fixed potential is applied to an electrochemical cell, and the corresponding current is measured. Next to amperometric devices, chronoamperometry is known (Gueshi et al., 1978; Haddad, 1990). Chronoamperometry measures the current as a function of time when a square wave potential is applied. This current changes according to the diffusion gradient of a target from a solution to the sensor's surface (Guy and Walker, 2016).
Fernández-Abedul and Costa-Garcia (1997) developed a flow injection amperometric sensor for the detection and quantification of cocaine samples confiscated by police authorities using a non-pre-treated carbon SPE. The single and irreversible peak, only observed at pH<6.5, referred to the electro-oxidation of the tertiary amine to the corresponding secondary amine. During the enzymatic reaction, cocaine underwent N-demethylation to norcocaine, and formaldehyde was released and checked spectrophotometrically (λ=412 nm) (Kleeberg and Klinger, 1982). Theoretically CYP450 2B4 could also react with dissolved oxygen, but this irreversible autooxidation has been reported to be very slow, and thus does not interfere with the cocaine detection (Shumyantseva et al., 2006). Using carbon-based SPEs, the purity of cocaine street samples was evaluated enzymatically (CYP450 2B4) by chronoamperometry. The enzyme was trapped in the structure of

a working electrode, and its interaction with cocaine was measured by voltammetry. The reached capability of detection was 0.2 mM in the calibration range from 0.2 to 1.2 mM (Asturias-Arribas et al., 2013). Alanso-Lomilla et al. (2009) also detected cocaine in street samples using CYP450 2B4, but the linkage of CYP450 2B4 on bare gold and carbon SPEs was performed covalently through 4-nitrobenzenediazonium tetrafluoroborate monolayers. Au NP were incorporated to increase the sensor's sensitivity. Even though CYP450 has a good affinity for cocaine, searching for the most optimum conditions of the experimental variables (pH, E_{ap}, composition of the different SPCE's and curing conditions) was needed in order to develop a selective biosensor (Kleeberg and Klinger, 1982).

Man et al. (2009) assessed the use of different sensing technologies to track illicit MA drug laboratories. Therefore, the target gases were first identified for different MA production processes, including the Birch reduction, P2P (phenyl-2-propanone), and the red phosphorous methods. Advantages offered by amperometric gas sensors include their short response time, low power consumption, the LODs in the ppm to ppb range, the large linear working ranges (>3 orders magnitude), and the possibility to detect on-site. MDA and its analogues MDMA (SI Figure 2) and 3,4-methylenedioxyethylamphetamine (MDEA, SI Figure 2) were detected in urine and saliva by Butler et al. (2006) using a screen printed immunosensor, offering a non-invasive and faster alternative for blood analysis. The polyclonal antibodies used were immobilized on carbon SPEs by passive adsorption. Both MDA and MDMA were labelled with horseradish peroxidase (HRP) to increase the sensitivity. From the results, it could be concluded that with an increasing alkyl chain (MDA<MDMA<MDEA), the phenyl- and its methylenedioxy group became more dominant, leading to a more efficient antibody binding.

More recently, a label free sensor for the detection of MA in human blood was developed by Zhang and Liu (2014) using prussian blue as a mediator. Therefore, different layers were deposited on the gold electrodes: L-cysteine, Prussian blue (PB) and a (3-mercaptopropyl) trime-thoxysilane (MPS) film. Before the Ab immobilization, the nano-Au colloid was added to increase the sensitivity. PB was incorporated for two reasons: it was used as an electron transfer mediator to enhance the performance; and second, as "artificial peroxidase", to amplify the amperometric signal of the immunosensor. An MPS film was added to prevent the PB film from leaking. Moreover, the thiol groups of MPS served as binding sites for the nano-Au immobilization. Both the LC and MPS film were thus present to enhance the stability of the immunosensor. The nano-Au particles were used to increase the amount of adsorbed antibodies. The immunochemical incubation time was studied as well, a maximum response was found after 35 min. Therefore, 35 min was set as the optimum incubation time.

Already in 1995, Kriz and Mosbash described a competitive MIP-based sensor for the detection of morphine. Measurements were performed in a concentration range of 0.1-10µg/mL after dissolving the target analyte in 20 mM citrate buffer at pH 6 containing 10 % ethanol. The intention was to apply this sensor later on to real biological fluids. The outer part of the sensor was dipped into a suspension containing the MIP in a 4% aqueous agarose solution. Subsequently, the agarose was cross-linked by epichlorhydrine and sodiumborohydride. The quantitative detection method involved a two-step process. First, morphine (SI Figure 1) bound selectively to the MIP. In the second step, an excess of electro-inactive codeine (a competitor) was added, releasing morphine, which was detected via amperometry. The oxidation of morphine was based on the electron oxidation of the 3-hydroxy functionality, followed by its ionization and dimerization of the free radical to pseudomorphine, as described by Wilson et al (1984). As in codeine, the hydrogen from the 3-hydroxy group is replaced by a methyl group, it is less susceptible to electrochemical oxidation. Moreover, at the selected

potential (+500 mV), the oxidation of codeine was negligible. Further results showed that the exposure to strong basic/acid environments did not interfere with the binding event between the MIP and the target (Kriz and Mosbach, 1995). To the best of our knowledge, no other amperometric sensors based on MIPs for the detection of illicit drugs were published.

4.3 Impedimetric sensors

Impedance describes the relationship between voltage and a current for a non-steady-state behavior. Ebrahimi et al. (2012) used an impedimetric spectroscopic aptasensor for the detection of MA with high affinity and a K_D value in the nM range. Gold electrodes were first functionalized with AuNPs to amplify the electrochemical signal before the aptamers were immobilized via passive adsorption. This research was used as a proof-of-concept for folding and unfolding of the selected aptamer in presence of AMP and MA rather than for the monitoring of real samples. Hua et al. (2010) developed an aptasensor for the detection of cocaine in serum. The aptasensor comprised thiolated DNA sequences which self-assembled on the AuNPs present on a chitosan-pretreated glassy carbon electrode. Interaction of the amine groups of chitosan with the Au NPs results in a higher immobilization surface, which should increase the sensor sensitivity. If a sample contained cocaine, a three-way junction complex was formed, which increased the charge density and steric hindrance on the surface of the electrode, thus blocking the electron transfer, which was measured. Incubation was set at 15 minutes in order to have an efficient binding between the aptamer and cocaine. Zhang et al. (2012) developed a label-free aptasensor for cocaine, but these analyses were based on the formation of a supramolecular aptamer fragments/target complex. The anti-cocaine aptamer was divided in two fragments (C_x and C_Y), and through modification of their 5' or 3' ends with a thiolated group and subsequent treatment with mercaptoethanol, one of the two fragments was immobilized on the gold surface. In presence of the target and the other fragment, the immobilized fragment self-assembled into the supramolecular aptamer fragment/target complex. This step included an incubation time of 30 min in order to achieve binding between the different species. Binding resulted in an increase of negative charge on the electrode and thus the increase of R_{et}, which was measured. Human plasma diluted in a buffer was analyzed in the range of 0.1 – 20 µM (30.34 µg/L – 6.067 mg/L), yielding similar data as in blank buffer. Yang et al. (2016a) developed a so-called DDIAS, a DNA-directed immobilization aptamer sensor, for the detection of cocaine in sewage water, which had first been filtered and preconcentrated using solid phase extraction in 0.8 M phosphate buffer with 1.0 M NaCl, 5 mM $MgCl_2$ and 1 mM ethylene tetraacetic acid (EDTA). Two different aptamer immobilization methods were investigated. In the first approach, the thiolated ssDNA was first immobilized onto the gold electrodes while in the second approach the ssDNA and dsDNA aptamers were co-immobilized (Figure 2).

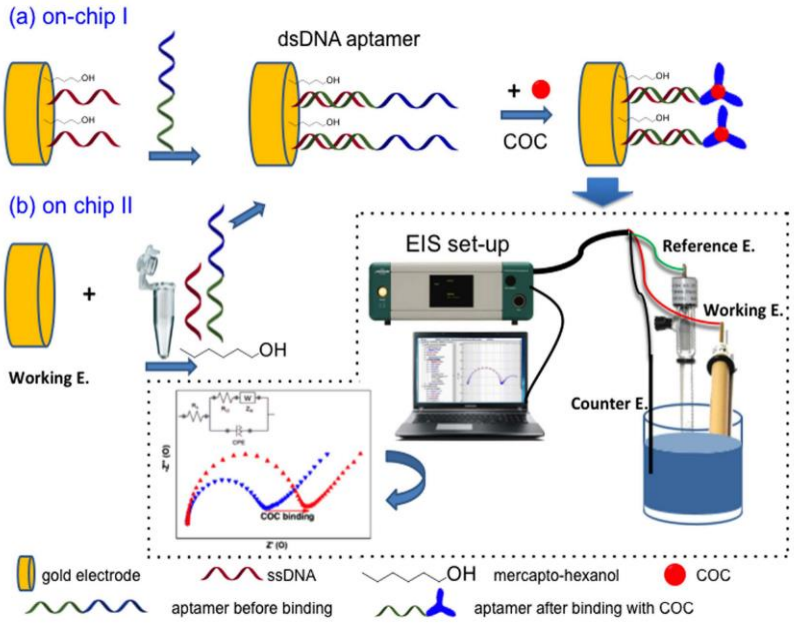

Figure 2: Schematic illustration of the DDIAS for the detection of cocaine using EIS (Yang et al., 2016a).

In order to control the surface density and reduce the non-specific binding, blocking with 6-mercapto-hexane (MCH) was used in both strategies, with an optimized ratio of ssDNA:MCH of 1:4, based on the research of Keighley et al. (2008). Also here, in the presence of cocaine, the configuration of the aptamer changed from a "two-stem loop" to a "three-way junction", which resulted in an increase in the charge transfer resistance during the analyses. Due to less steric hindrance, a larger charge transfer resistance was found in case of co-immobilization of ss- and ds-DNA compared to the first immobilization approach and was concluded to be better. Both types of aptamer-probe electrodes were incubated with the samples for 1h.

4.4 Capacitive sensors

Capacitive sensors are a sub-category of impedimetric sensors. The principle of a capacitive sensor is based on the measurement of a change in capacitance caused by a change of dielectric properties and/or thickness of a dielectrical layer when a target binds to a recognition element immobilized on an electrode surface. Despite the increasing popularity of this electrochemical sensor for (bio)medical applications (Labib et al., 2010, 2009b, 2009c; Limbut et al., 2007; Teeparuksapun et al., 2010), so far only two publications were devoted to tracing illicit drugs, more specifically one on ATS detection in sewage water and one for cocaine in blood serum. A highly sensitive sensor was developed by our group to monitor trace amounts of NFA in aqueous samples. NFA is an intermediate formed during the synthesis of AMP via the Leuckart reaction. MIPs were chosen as the recognition element and were immobilized on gold electrodes using tyramine as a linker. The obtained MIPs were characterized by a low cross-reactivity towards AMP and di(beta-phenylisopropyl). With such sensor, real-time monitoring of the binding effect is possible. Results could be drawn after 7-8 min, taken the death time from the sample transport to the flow cell into account (Graniczkowska et al., 2016). A capacitive aptasensor was developed by Oueslati et al. (2018) for the detection of cocaine in blood serum. Therefore, the specific aptamer solution was drop coated on gold electrodes and incubated for 24 h in a humidor. The developed assay showed a sample-to-result time of 30 s, which is a great result in comparison to other cocaine sensor and/or other developed aptasensors in general.

4.5 Potentiometric sensors

Using potentiometric sensors, the difference in potential is (passively) measured between the WE and RE without polarizing the electrochemical cell. The change in potential is logarithmically dependent on the concentration of an analyte (Wang et al., 2008). Ion-selective electrodes (ISE) have been used in the majority of potentiometric sensors. They generate a signal at the interface of an anion-selective membrane and a solution due to the transfer of ionic species between these two phases (Singhal, 2011). Already in 1992, Watanabe et al. developed a potentiometric sensor for the detection of MA in urine. When different ion exchangers and solvent mediators were tested, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate (NaTFPB) and tricresylphosphate (TCP) were found as the most suitable options to incorporate in the PVC matrix. While exploring the applicability of this sensor, it was found that 2-fluoro-2'-nitrodiphenyl ether (FNDPE) was a better solvent mediator in comparison to TCP when the matrix changed from urine to serum. Therefore, the influence of the solvent mediator using potentiometric sensors cannot be neglected. As a continuation of that work, a cocaine membrane selective electrode was developed by the same group using the same ion exchanger (NaTFPB) and tetrakis (2-ethylhexyl) pyromellitate (TEHPY) as solvent mediator. TEHPY was chosen as the mediator since it suppressed the response to lipophilic quaternary ammonium ions and strengthened the response towards cocaine. The selectivity of the membrane was tested in pharmaceutical mixtures, to check cross-reactivities with morphine, codeine, MA, ephedrine, and methylephedrine. The response time was set on 10 s. After that moment, 90% of the final signal was reached (Watanabe et al., 1995). At the same time, Elnemma et al. (1992) investigated PVC-based potentiometric ISE for the quantification of cocaine in illicit powders and tablets, concluding that the highest sensitivity and selectivity was obtained when using tetraphenylborate (TPB) as an ion exchanger and dibutyl sebacate or dioctylphtalate (DOP) as solvent mediators.

More recently, Smolinska-Kempisty et al. (2017) synthesized MIPs by solid-phase imprinting to monitor cocaine in serum. As a template, the cocaine metabolite benzoylecgonine was used. The MIP particles were incorporated in a PVC matrix, with addition of nitrophenyloctyl ether (NPOE) as a plasticizer and potassium tetrakis(4-chlorophenyl)borate (kTpBCl) to increase the membrane conductivity and reduce anion interference. After drying, the obtained membrane was punched and placed inside a commercial ISE. AMP was detected in water by Gallardo-Gonzalez et al. (2018) using a highly selective microsensor based on [3,3'-Co(1,2-closo-$C_2B_9H_{11})_2]^-[C_9H_{13}NH]^+$ as an ion-pair complex. After incorporation of the ion-pair complex in a PVC membrane, it was drop-casted onto gold micro-electrodes modified with a solid conductive layer of polypyrrole. The time needed for the sensor to obtain a stable signal was reported to be less than 10 s.

5. General conclusions and future outlook

As covered in this review, various electrochemical sensors for detection of the most prevailing illicit drugs, such as cocaine, heroin, and (meth)amphetamine, were described. However, the use of both artificial and natural recognition elements in these sensing systems remains rather limited. If a recognition element is included to increase the system's sensitivity and specificity, the preference has been given to aptamers and antibodies, where the latter has a leading position. This could be explained by the fact that an antibody's affinity is superior to an aptamer, while being widely commercially available and relatively easy to produce. Therefore, antibodies offer the best option for sensing in physiological media. Having a polymer backbone, MIPs are extremely robust and highly resistant to pH and temperature changes and presence of strong chemicals, being in addition the easiest, cheapest, and fastest recognition element to produce. Therefore, they

are favoured for sensing in harsh conditions, such as detection in sewage, environmental analysis, tracing of clandestine laboratories or suspicious activities, and the mapping of transportation and distribution of illicit drugs and precursors, where extremely high sensitivity is out of focus.

An appropriate technique to immobilize the selected recognition element is crucial. Independent on the type of recognition elements, the immobilization techniques must be carefully selected in order to obtain an optimal steric orientation of receptors on an electrode's surface. For natural recognition elements, a covalent binding is favoured in comparison to a passive/non-specific adsorption. However, the non-covalent avidin/streptavidin-biotin interaction is proven to have a 2-3 times higher binding capacity compared to the traditional EDC-NHS-based technique. For MIPs, covalent binding on transducers assures a bigger amount of sterically available binding sites and higher sensor stability in general.

The most commonly used electrodes are gold and (glassy or paste) carbon. Gold electrodes could be easily modified and show a large cathodic potential range, but they are susceptible to surface oxidation. Glassy carbon allows a more negative potential of the surface than gold or platinum, but it is difficult and expensive to fabricate. On the contrary, carbon paste electrodes are easy to use over a wide potential range and are inexpensive, but prone to mechanical and chemical (e.g. organic solvents) damage. We believe that CPEs will further gain even more popularity and will be used in a majority of electrochemical sensors for detection of illicit drugs. CPEs are flexible in design, with a possibility for miniaturization and automatization. A large choice of the used materials is available, such as silver, carbon ink, or nanostructure materials: Au, Pt, Pd, and other metal NPs, carbon nanotubes, and graphene-based inks).

Looking at different transducers, voltammetry, and more specifically SWV, is the most widely used for the facile and rapid quantitative sensing of illicit drugs, such as cocaine, 25-NBOMe and Δ9-THC. The choice of a supporting electrolyte, pH, and applied voltage could be optimized in order to increase the sensitivity and remove any unwanted peak overlap. Impedimetric and capacitive sensors have shown great potential for several pharmaceutical and biomedical applications, and hence could offer a good alternative in drug sensing. The same holds true for ISE (potentiometry), but a careful selection of an ion exchanger and solvent mediator is important here. So far, all research focusing on illicit drugs was concentrated around PVC matrices where (different) plasticizers have to be included. Phthalate esters, such as dioctyl phthalate (DOP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di(2-propylheptyl) phthalate (DPHP) could be mentioned as commonly used plasticizers in industry.

To conclude, a deep fundamental investigation has been already performed upon the electrochemical behaviour and sensing of illicit drugs. To the best of our knowledge, this has been limited to academic research only, and no commercial market was conquered yet. However quite a number of research projects have been aiming commercialization (Horizon 2020 projects such as microMole and NOSY). On the other hand, other techniques such as surface enhanced Raman spectroscopy (SERS) and infrared spectroscopy (IR) have improved drastically and became well-known and widely used approaches to detect illicit drugs. These techniques are still relatively expensive and on-site, fast screening is often difficult. Electrochemical sensors could offer a relatively cheap and fast sensitive alternative to chromatography for screening of a big amount of samples, detection on-line, or working in complicated matrices. They show a great potential for further growth in close future. Although up till now the focus was often on a single target analysis, it could be envisaged that multiplex detection has been becoming a key area of interest.

Table 1 summarizes the developed electrochemical sensors for the detection of opiates for each matrix with their corresponding working range and LOD. For heroin and morphine, a cut-off value of 10 (27 nM) and 2000 ng/mL (7.01 μM) in urine was respectively set by SAMHSA.

Table 2 summarizes the developed electrochemical sensors for the detection of psychodysleptica for each matrix with their corresponding working range and LOD. SAMHSA determined a urinary cut-off of 50 ng/mL (159 nM) for Δ9-THC.

Analyte	Matrix	Sensing approach	Transducer	Linear range	LOD	Ref
Δ9-THC	Plants	CV	Glassy carbon disk	1.0-3.5 μM	1.0 μM	(Balbino et al., 2016)
	Food (plants)	SWV	Microparticles at a paraffin-impregnated graphite electrode	n.m.	n.m.	(Novak et al., 2013)
	Seized samples	SWV	Glassy carbon* and platinum disc**	2.0-21.0 nM*, 8.0-40.0 nM**	0.62 nM*, 27 nM**	(Balbino et al., 2014)
LSD	Spiked toons, hair, plasma and urine	(adsorptive stripping) V	GCE	0.003-0.278 nM	0.004 nM	(Merli et al., 2014)
	Seized blotters	CV	GCE	1.86-12.5 μM	0.987 μM	(Ribeiro et al., 2017)
25H-NBOMe	Blotter paper	SWV	Glassy carbon electrode	4.25 μM-49.6 μM	1.28 μM	(Oiye et al., 2017)
25X- NBOMe* and 2C-X**			Boron doped electrode	1-555 μM	0.1μM* and 0.3 μM**	(Souza et al., 2018)
25I-NBOH*, 25I- BOMe** and 2C-I***		DVP	SPCE	0.01-0.08 mg/mL	0.011*, 0.004**, and 0.012***mg/mL	(Belchior de Andrade and Gonzalez-Rodriguez, 2019)

Table 3 summarizes the developed electrochemical sensors for the detection of stimulants for each matrix with their corresponding working range and LOD. SAMHSA determined a urinary cut-off for AMP, MA and MDMA of 500 ng/mL (3.7 μM, 3.36 μM and 2.58 μM respectively), and for cocaine of 100 ng/mL (329.6 nM).

Analyte	Matrix	Sensing approach	Transducer	Linear range	LOD	Ref
AMP	Aqueous solutions	POT	Gold with [3,3'-Co(1,2-closo-$C_2B_9H_{11})_2]^-$ $[C_9H_{13}NH]^+$ as ion- pair complex in PVC matrix	10 μM-1 mM	12 μM	(Gallardo-Gonzalez et al., 2018)
AMP[1], MDMA[2]	Solid samples* and urine**	CV* and SWV**	Au electrode	n.m.	n.m.	(Nevescanin et al., 2013)
	Solid samples	DPV	CPE	n.m.	110.9-258.9 μM[1*], 0.1 mM 38.4-229.2 μM[2*], 0.1 mM 30.9-91.6 μM[2**]	(Barreira Rodriguez et al., 1990)
MDMA	Street samples dissolved at pH 7* and 12**	SWV	SPCE	10-500 μM* and 4.2 μM – 48 μM	10 μM 3.5 μM	(Florea et al., 2019b)
	Standards	CV	CME with Nf/CB[6]/ methanol	10-1000 μM		(Tadini et al., 2014)
MA	Standards	EIS	Apt based sensor with Au NPs	n.m. (POC)	n.m.	(Ebrahimi et al., 2012)
Morphine	Biological fluids	Amp	MIPs immobilized on agarose cross-linked electrode	0.35-3.5 μM	0.175 μM	(Kriz and Mosbach, 1995)
	Air	(Gas)Amp	Fingerprint of target gasses	n.m.	n.m.	(Man et al., 2009)
	Blood	Amp	Ab/nano-Au/MPS/PB/LC Au electrodes	10 nM-5.0 μM	7.5 nM	(Zhang and Liu, 2014)
	Solid samples	Amp	Ab sensor using PB as peroxidase	1.0 nM-2.0 μM	0.21 nM	(Zhang and Liu, 2014)
		SW-stripping V	Au-colloid/MWCNT modified SPE	3-50 μM	6 nM	(Rafiee et al., 2015)
		VOL	Pretreated pencil graphite electrode	n.m.	50 nM	(Oghli et al., 2015)
	Saliva	CV	SPCE. Oxidation is mediated by *N,N'*-(1,4-phenylene)-dibenzene-sulfonamide	n.m.	2.68 μM	(Bartlett et al., 2016)
	Urine	DVP	Self-assembled boron-doped diamond electrode	0.07-80 μM	50 nM	(Svorc et al., 2014)
	Urine* and blood serum**	POT	NaTFPB in PVC matrix	10 μM-0.01M	10 μM*, 20 μM**	(Watanabe et al., 1993)

MA, MDA, MDMA	Seized samples* and serum**	V	GCE	8-45 µM*, 12-45 µM**	3.7 µM*, 2.4 µM**	(Garrido et al., 2010)
MDA, MDEA, MDMA	Saliva* and urine**	Amp	SPCE immunosensor	0.61-400 ng/mL	0.36 ng/mL*, 0.042 ng/mL**	(Butler et al., 2006)
NFA	Sewage water	Capacitive	MIPs immobilized on gold electrodes, using tyramine as a linker	50-1000 µM	10 µM	(Graniczkowska et al., 2016)
Cocaine	Standards	CV	Carbon paste electrode modified with $[UO_2(X\text{-}MeOSalen)(H_2O)]\ H_2O$	0.1-1.3 µM	0.326 µM	(Tessari et al., 2010)
		SWV	Au/NPG/aptamer (DHBA)	0.05-1 and 1-35 µM	21 nM	(Tavakkoli et al., 2019)
	Serum	Capacitive	Apt on Au electrode	14.5 fM-1.45 pM	1.34pM	(Oueslati et al., 2018)
	Seized samples	Amp	Home-made CPE	0.2 µM-10µM	0.2 µM	(Fernández-Abedul and Costa-García, 1996)
		(chrono-) Amp	SPCE. Oxidation of cocaine mediated by CYP450 2B4.	0.2-1.2 mM	0.2 mM	(Asturias-Arribas et al., 2013)
		(Flow injection) Amp	non-pretreated SPCE	0.2 µM-10µM	0.2 µM	(Fernández-Abedul and Costa-García, 1996)
		V	Pt electrode with CoHCFe	0.24-1.5 mM	0.14 mM	(Oiye et al., 2009)
			CPE modified with *N,N'*-ethylene-bis(salcylidene-iminato)uranyl(VI)-complexes	1.0-1.3 µM	0.326 µM	(Siqueira de Oliveira et al., 2013)
			Pt* and GC** electrode modified with uranyl Schiff base films	0.54-9.10 µM	0.07µM* and 0.15µM**	(De Oliveira et al., 2013)
	Street solid samples	(chrono-) Amp	CYP450 2B4/ diazonium/ AuNP/ SP(C/G)E	0.10-0.83 mM	23.05 nM	(Asturias-Arribas et al., 2011)
		POT	TPB in PVC matrix	0.1M-10µM (Nerstian response)	1mM	(Elnemma et al., 1992)
			NaTFPB in PVC matrix	0.1M-1µM (Nerstian response)	0.4 µM	(Watanabe et al., 1995)
		SWV	SPCE	n.m.	2 µM	(De Jong et al., 2016)
			SPCE	10-2500 µM	3 µM	(De Jong et al., 2018)
			MIPs/GPH/SPCE	50-500 µM	50 µM	(Florea et al., 2018)
		(BIA)SWV	Boron-doped diamond electrode	19.78-98.89 µM	0.89 pM	(Freitas et al., 2017)
	Sewage water	EIS	DNA directed Apt sensor	10 nM-5 µM	10 nM	(Yang et al., 2016a)
	River water and saliva	SWV	MIPs/PdNP/GPH/SPCE	100-500 µM	50 µM	(Florea et al., 2019a)
	Serum	EIS	Apt on AuNP CH-GCE	1.0 µM- 1.5 µM	0.3 µM	(Hua et al., 2010)
			Apt fragment/target complex formation on Au	0.1-20 µM	100 nM	(Zhang et al., 2012)
		POT	MIPs integrated in PVC matrix	1 nM-1mM (Nerstian response)	n.m.	(Smolinska-Kempisty et al., 2017)
	Serum, saliva, and adulterated samples	(AC)V	Apt immobilized on Au	n.m.	10 µM	(Baker et al., 2006)
Cocaine (+ AMP, heroin, and methyl-	Water	DPV	EG-AuNPs copper wire	0.125-3 mg/L	0.82 mg/L	(Masemola et al., 2020)

phenidate)

Abbreviations: Δ9-THC, Δ9-tetrahydrocannabinol; Ab, antibody; Au, gold; Amp, amperometry; AMP, amphetamine; Apt, aptamer; BIA, batch injection analysis; CH, chitosan; CoHCFe, cobalt-hexacyanoferrate; CPE, carbon paste electrode; CV, cyclic voltammetry; DHBA, 2,5-dihydroxybenzoic acid; DNA, deoxyribonucleic acid; DVP, differential pulse voltammetry; EG, exfoliated graphite; EIS, electrochemical impedance spectroscopy; GCE, glassy carbon electrode; GPH, graphene; LC, L- cysteine; LOD, limit of detection; LSD, lysergic acid diethylamide; MA, methamphetamine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxyethy- lamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MIPs, molecularly imprinted polymers; MPS, (3-mercaptorpropyl) trime-thoxysilane; NaTFPB, sodium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate; NBOMes, N-benzyl-substituted phenethylamines; n.m., not mentioned; NFA, N-formyl amphetamine; NP, nano- particles; NPG, nanoporous gold; PB, Prussian Blue; Pd, Palladium; POC, proof of concept; POT, potentiometry; PVC, polyvinylchloride; SP(C/G)E, screen printed (carbon/gold) electrode; (SW)V,(square wave) voltammetry; TPB, tetraphenylborate.

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