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Poltorak, Lukasz; Sudhölter, Ernst J.R.; de Puit, Marcel

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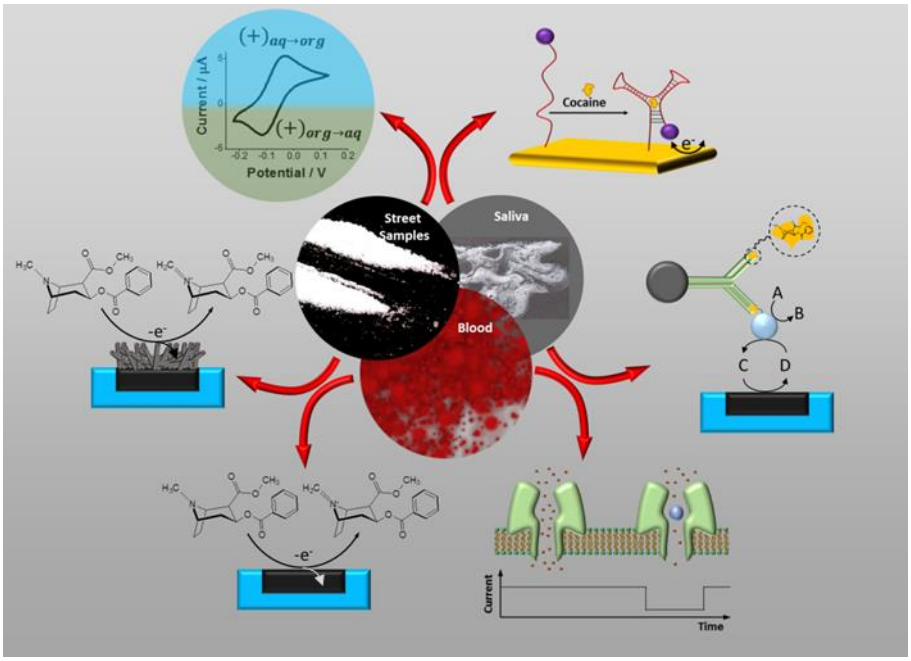
Graphical Abstract



Electrochemical cocaine (bio)sensing. From solid electrodes to soft junctions.

Lukasz Poltorak[1,2*], Ernst J. R. Sudhölter[1], Marcel de Puit[1,3]

1) Delft University of Technology, Department of Chemical Engineering, Van der Maasweg 9, 2629 HZ Delft, The Netherlands
2) Department of Inorganic and Analytical Chemistry, Faculty of Chemistry, University of Lodz, Tamka 12, 91-403 Lodz, Poland
3) Netherlands Forensic Institute, Laan van Ypenburg 6, 2497 GB, The Hague, The Netherlands

Corresponding author: l.poltorak@tudelft.nl

Abstract

In this review, we describe the importance and possible electrochemical screening methods for the illicit drug – cocaine. It covers the detection at bare and modified solid electrodes, soft electrified junctions and nanopore sensing. Emphasis is given on interfacial modification techniques and electroanalytical parameters for cocaine detection in different environments, covering the detection from both, model and real samples.

Keywords: Aptasensing, electrified liquid-liquid interface, nanopore sensing, illicit drugs, voltammetry, cocaine oxidation, carbon electrodes, surface modification.

1.1. Introduction

Cocaine is one of the most commonly used illicit drugs of abuse. It is strongly addictive, and hence, many cocaine users are subjected to the chronic intake. Not only direct (e.g. hallucinations, sudden cardiac death and stroke) and indirect (e.g. Hepatic C and HIV infections) health effects are recognized as societal problems. Cocaine production, trafficking, and distribution are highly related to the crime rate [1]. Consequently, cocaine is considered a very dangerous drug being illegal and highly regulated worldwide. The need for a method for cocaine sensing is therefore high. Currently used methodologies can be divided into colorimetric tests – albeit cheap suffer from a lack of reliability – and more sophisticated analytical techniques based on chromatography and mass spectrometry. The latter, although very accurate, requires trained personnel, dedicated lab space and still constitutes a financial barrier for the equipment itself in some developing countries. We are convinced that the gap between primitive colorimetric tests and sophisticated analytical instrumentation can be filled with electrochemical sensors. Surprisingly, electrochemical cocaine detection is a relatively new trend, and more than 90% of the reports, devoted to this topic, were published over the last 12 years. For complementary view, we also encourage to read limited, but existing, review papers that deal with illicit drugs detection (not necessary focused on electroanalysis) [2–5]. As we will show, a number of elegant electrochemistry based solutions – allowing for the selective cocaine determination from real samples – are available. Given that the electrochemical detection of cocaine is cheap, fast and in this particular case can be very specific, it may lead to a commercial success.

In this review, we will discuss different electrochemical cocaine detection methodologies including (i) direct, (ii) aptamer-based, (iii) nanopore, (iv) immuno sensing and (v) detection at soft junctions. We will emphasise on the working principle of the sensing device, modification strategies (when applied), application of the sensors for real samples and finally the overview of the electroanalytical parameters (see Table A1 in appendix A).

1.2. Direct electrochemical cocaine determination

At carbon electrodes, the cocaine tertiary amine function undergoes an irreversible oxidation to an imine. As shown in Figure 1A (see reaction 1) this is one proton, two electron process giving characteristic irreversible voltamteric singal (cylic voltammgoram recorded at a carbon paste electrode is avaialbe in Figure 1B). Reaction 1 can be followed by recombination of the cocaine iminium ion to norcocaine (Figure 1, reaction 2). Due to the limited potential window, only the non-protonated form of cocaine can be oxidized at typical carbon electrodes. Electrochemical oxidation of cocaine cation (Figure 1, reaction 3) was achieved only with boron doped diamond electrodes providing a wider potential window [6]. Surprisingly, very little attention was devoted to the

electrochemical study of cocaine metabolites (reaction 4 or 5) such as ecgonine methyl ester or benzoylecgonine, which are main cocaine biomarkers when urine is subjected to analysis.

The overall process of cocaine electrochemical oxidation is pH dependent and occurs at pH > 6.5. From the change of the anodic peak location, usually found between 0.9 V to 1.1 V vs Ag/AgCl reference electrode, the pKa of cocaine was estimated to be around 8.5, given also by the maximum oxidation current at the corresponding pH [7]. Jong *et al.* proposed an elegant electrochemical sensing platform, where direct oxidation of cocaine occurred at a screen-printed carbon electrode. The electrode was placed on a fingertip of a nitrile glove, which allowed the determination of cocaine in street samples, containing a wide range of different cutting agents [8]. With a concentration as low as a few µM obtained as the detection limit, the proposed method is fully in line with forensic needs. The same group proposed a number of different solutions to separate cocaine and interferings species overlapping signals just by switching the pH, adjusting the number of voltammetric scans [9] or after surface modification with conductive polymers [10]. An improvement in terms of the lower limit of detection (LOD) was reported when the anodic detection of the cocaine at carbon-based electrodes was combined with flow injection analysis [11].

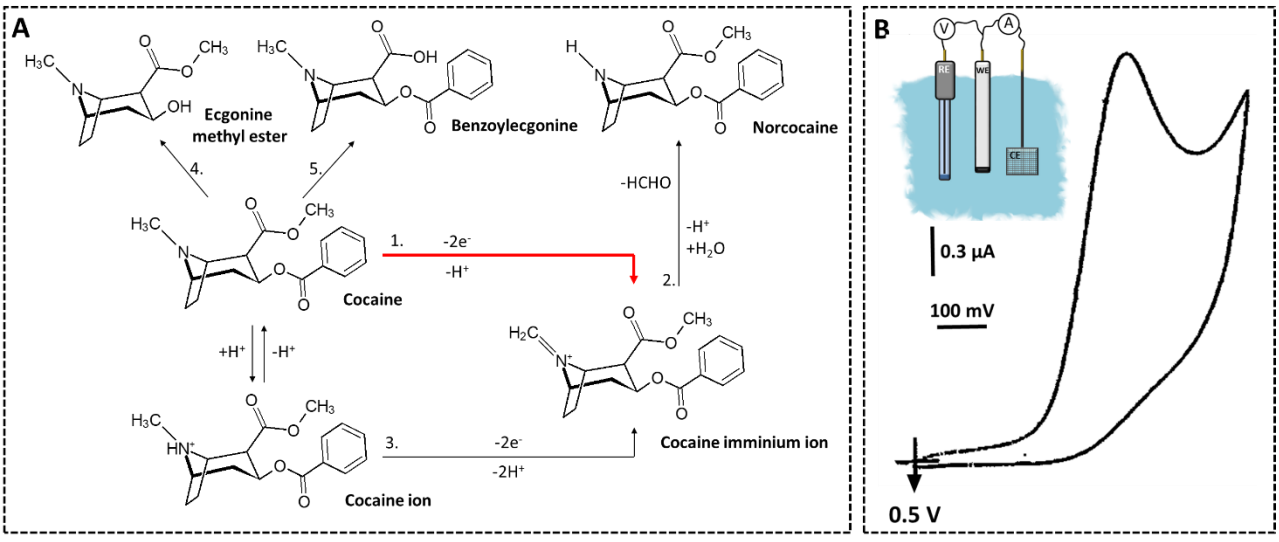


Figure 1. A - Cocaine reaction pathways. B – Cyclic voltammogram recorded for the 20 µM cocaine solutions (pH = 9, 50 mVs^{-1}) at a carbon paste electrode in a common three electrode configuration (see insert). The cyclic voltammogram was taken from ref [12].

Electrocatalytic cocaine oxidation was observed at the glassy carbon electrode (GCE) modified with multiwalled carbon nanotubes (CNT) and β-cyclodextrin, both embedded in a (conductive) polyaniline film. It was proposed that cocaine can form an inclusion complex with β-cyclodextrin,

resulting in a higher surface concentration of cocaine, whereas CNTs increase the available electroactive surface area [13]. A few reports deal with the detection of cocaine at electrodes modified with a Schiff base (imine with a nitrogen atom substituted by aryl or alkyl functionality) – Uranyl ion complex. As shown in Table A1, the investigated variables in cocaine sensing are: (i) surface modification [14,15] (ii) altered chemical structure of the Schiff base [15]; (iii) electrode material or (iv) preconditioning time, all affecting the electroanalytical output allowing for cocaine determination in μM – mM concentration range. The specificity of the sensor, although investigated, was limited only to a few other illicit drug molecules including morphine, tenamfetamine, lidnocaine and procaine. A Pt electrode, modified with a cobalt hexacyanoferrate film, was proposed for cocaine determination in acetonitrile, a polar aprotic solvent. The transducing mechanism was based on hexacyanoferrate oxidation with reduction peak dropping upon the addition of the cocaine species [16].

In our view, the biggest advantage of the direct electrochemical sensing lies in its simplicity since the analytical signal pertain to the cocaine amine function oxidation reaction. For the cocaine street samples, the bare carbon electrodes provided the desired selectivity (against a number of cutting agents) with a lower detection limit found at a few μM (meeting forensic standards set for presumptive sensing). These simple solutions are low-cost and have long shelf time of the electrode which may aspire for the commercial success. The problems can arise when the analysis is performed in more demanding environments e.g. urine, saliva or blood, as other interfering agents may also be oxidised at potential values similar to cocaine. As a matter of fact the gap in literature is present and more experimental effort is needed to comprehensively cover the topic of direct electrochemical cocaine and its metabolites sensing especially from body fluids.

1.3. Aptasensing

Aptamers are synthetic nucleic acids with a high specific structure ligand binding properties, such as the cocaine specific aptamer developed by Stojanovic *et al* [17] which undoubtedly triggered the development of the cocaine sensors. In Figure 2 four main cocaine detection scenarios are shown, utilizing an aptamer-based methodology. Figure 2A shows the general approach where the aptamer is modified with a bridging functional group on one side, providing an anchor to the transducing support, and a redox tag on the second end, contributing directly to the cocaine signaling. In the seminal works, Plaxco *et al.* [18] immobilized methylene blue (allowing the redox signalling) functionalized aptamers on a gold surface via an alkanethiol group. Cocaine-specific binding induces folding of the aptamer into a characteristic three-way junction complex which results in a closer approach of the methylene blue to the electrode surface, and hence, amplifies the voltammetric

(pulse or alternating current techniques) signal. This approach was integrated with a microfluidic system, showing an elegant method for multiple e.g. undiluted serum samples analysis [19] or a Au microelectrode array used for in vivo cocaine monitoring in rat spinal fluid [20]. A redox tag permanently situated close to the electrode surface gives high background signal even prior to cocaine binding. To partially overcome this problem Zuo *et al.* [21] split the aptamer into two fragments: the first part attached to the Au electrode surface and the second part consist of a methylene blue terminated strand present in the bulk phase. In such a configuration, significant sensitivity increase was observed as compared with the method where the redox tag is immobilized at the electrode surface. The before mentioned methylene blue can be for instance replaced with ferrocene moieties – giving an alternative redox probe [22], methylene blue – anthraquinone dual reporter serving as signaling and internal calibration probe respectively [23], Pt NPs used as electrocatalytic center for H_2O_2 reduction or amplification of the photoelectrochemical currents in a configuration with semiconducting CdS NPs situated at the aptamer terminus [24]. Cocaine sensing was also achieved with ruthenium derivatives functionalized aptamers with electrochemically triggered chemiluminescence monitoring [25], leading to an impressive 10 pM as lower limit of detection [26].

A different type of an aptamer-based cocaine sensing platform involves cocaine-induced aptamer strand conformational changes leading to an increased resistance of the charge transfer reaction of a redox probe present in the bulk phase (see Figure 2B for simplified scheme). Zhang *et al.* performed a simple, one step, Au electrode surface modification with a half of a cocaine-specific aptameric strand via an alkanethiol group. The remaining half of that strand was added, together with the analyte, to the bulk solution. Formation of a cocaine-aptamer complex at the electrode surface leads to the increase of the charge transfer resistance of the $Fe(CN)_6^{3-/4-}$ couple as monitored by electrochemical impedance spectroscopy (EIS) [27]. Other examples describe an elaborate sensing interface composed of multi-layered systems. In a series of publications, Shahdist-fard and Roushani, modified GCE with a surface enhancing “electron highways” (e.g. ionic liquid-chitosan-CNTs nanocomposite, [28], [29], [30] CdTe QDs [31] or Au NPs-cysteamine [32]) which were further decorated with metal NPs (Au, Ag or Pt) terminated aptamer. Depending on the specific design, the detection was found to be valid in pM – μM range, with an impressive LOD of 0.5 pM for GCE/Au NPs-cystamine/HS-aptemer-Au NPs platform [32]. A conceptually similar design was developed by Yilmaz *et al.*[33] who electropolymerized 3,4-ethylenedioxythiophene-2,1,3-benzothiadiazole-poly-L-phenylalanine at the GCE surface and terminate it with a cocaine-specific aptamer. Hashemi *et al.*[34] synthesized a nanocomposite holding magnetic and conductive properties. The structure consisted of a 3D-magnetic reduced graphene oxide, polyaniline and Au NPs, which were finished with the cocaine

binding aptamer. After exposure of such nanocomposite material to a cocaine containing solution and its subsequent pre-condensation at the carbon-based electrode surface (in the magnetic field) detection was achieved. Using EIS as the electrochemical tool to measure the increase of the resistance of the $Fe(CN)_6^{3-/4-}$ redox couple the detection limits were found at 29 pM and nearly 100 nM as the lower and upper cuts off, respectively.

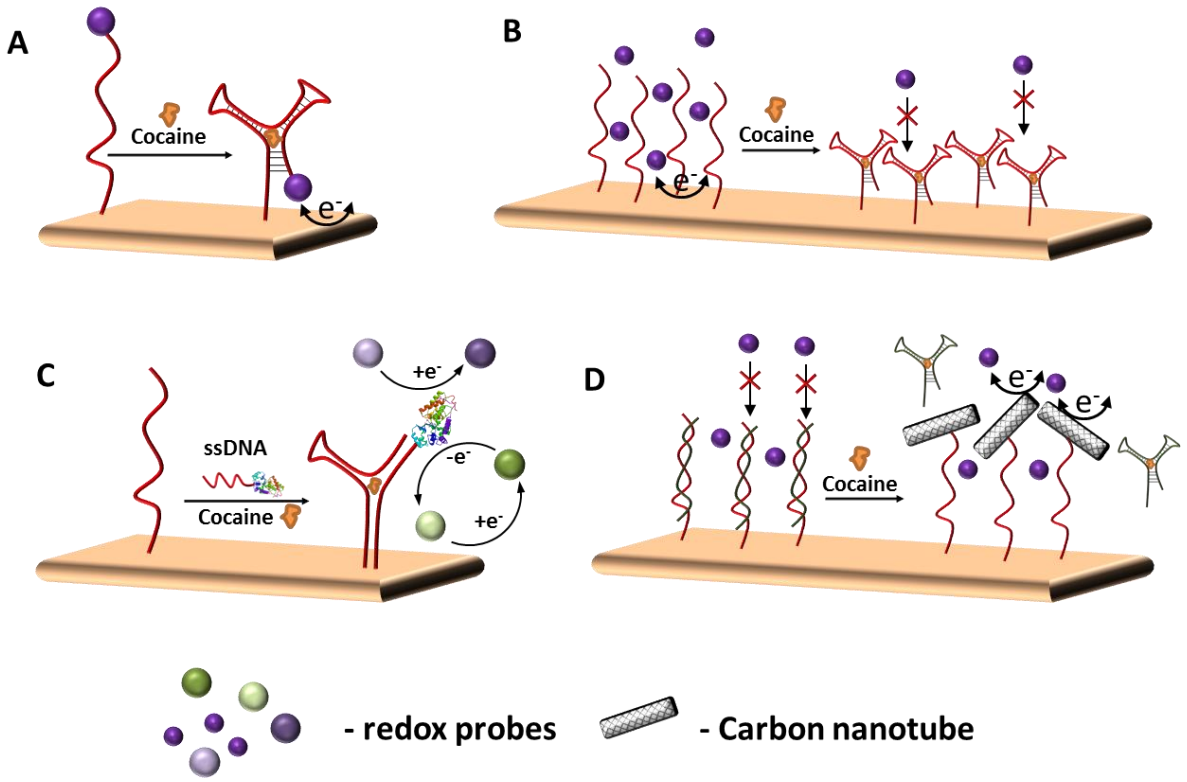


Figure 2. Cocaine aptasensing strategies. A – Oxidation/reduction of the label probe attached to the aptamer end that is brought close to the electrode surface upon cocaine binding. B - Increased charge transfer resistance of the redox probe caused by the cocaine–aptamer complex formation, C – redox recycling mechanism achieved with the help of DNA-zyme or enzymes brought close to the electrode surface due to specific aptamer – cocaine interaction and D – the electrocatalytic effect of nanomaterials bounded to aptamer film after addition of cocaine. Schemes are not to scale.

Cocaine binding to the surface-bound aptamers has been shown to reduce the conductivity of the underlining multilayer composed from ferrocene functionalized polyethylimine/Au NPs [35]. Taghdisi *et al*. constructed a platform where a cocaine-aptamer complex formation has led to a significant increase in the redox probe oxidation currents as recorded with differential pulse voltammetry [36]. In this method, as shown in Figure 2D, the Au surface was modified with a double helix with one strand covalently attached to the electrode and the second complementary strand being a cocaine-specific aptamer. When cocaine is added it interacts with the non-covalently attached aptamer resulting in a double helix de-hybridization. Formed complex leaves the surface and is replaced by

single-wall CNTs which adsorbs to ssDNA remaining at the surface. The latter efficiently increases the electroactive surface area, and hence, contributes to higher oxidation currents of the redox probe present in the bulk phase.

Incorporation of enzymes or DNA-zyme, as shown schematically in Figure 2C, into an aptamer containing a transducing layer gives another alternative for cocaine detection. The presence of biocatalytic centers, allows for the redox probe recycling which contributes to the sensor sensitivity. An elegant configuration was reported by Jiang *et al*. who decorated carbon screen-printed electrodes with a cocaine-specific aptamer terminated with alkaline phosphatase. Signaling is related to the following reactions: (i) the enzyme hydrolyzes non-electroactive p-aminophenylphosphate to p-aminophenol that is (ii) subsequently oxidized electrochemically to a p-quinone imine. (iii) The nicotinamide adenine dinucleotide (NADH) is present and reduces the p-quinone imine back to p-aminophenol, and hence the redox cycle is formed. The linear range of cocaine detection spans from 1 nM to 0.5 μM, as measured at pH = 9, due to the high enzyme stability and activity [37]. A similar detection mechanism was reported for Au electrodes modified with Horse Radish Peroxidase (HRP) terminated cocaine-specific aptamer layer [38]. Similarly, DNA-zyme's (catalytically active nucleic acid structure mimicking the properties of an enzyme) mimicking HRP activity were also used to construct electrochemical cocaine sensors [39]. Enzymes can be also used to affect the surface architectures. For instance, Klenow fragment polymerase was used to introduce ferrocene functionalized oligonucleotides between ssDNA present at the electrode surface and cocaine bounded tripartite complex [40]. In another example, a conductive magnetic graphene platform was impregnated with a cocaine-specific aptamer terminated with the redox probe – thionine. Upon cocaine binding, the aptamer desorbs from the surface, and hence deprives it from the signaling tag. Cocaine regeneration from cocaine – aptamer complex was achieved with DNase I (nuclease cleavage). This allowed for further aptamer desorption from the magnetic graphene platform improving voltammetric sensitivities [41].

Clear advantage of the cocaine electrochemical aptasensing is its high specificity originating from the cocaine – aptamer interactions. Many examples show that nM or even pM limits of detection can be obtained. Moreover, depending from the sensor design the detectable concentration ranges cover pM and/or nM and/or μM intervals and are suitable for sensing in more demanding environments e.g. body fluids and trace analysis rather than investigation of street samples. Although elegant, in a view of practical utility, these examples suffer from overcomplicated surface engineering relaying on a number of mutually cooperating components. This, together with the necessity to maintain appropriate storage conditions and protection against fouling may limit commercial applications.

1.4. Immuno- and enzymatic sensors

The toolbox of electrodes modified with a cocaine-specific affinity layer include antibodies, enzymes and other bio-molecules that upon interaction with cocaine or its metabolites gives electrochemically detectable species. Cocaine was recognized as one of the preferential substrates of a Cytochrome C which participate in its metabolic oxidation followed by hydrolysis to norcocaine. Asturias-Arribas *et al.* took advantage of this property and covalently modified screen-printed carbon electrodes with Cytochrome P450 2B4 and obtained a cocaine amperometric sensor operating in the nM concentration range [42]. In another study, the working electrode was printed from a blend of Cytochrome P450 2B4 and carbon ink giving a cocaine sensor operating in the 0.2 – 1.2 mM range [43]. For both methods, the validity of sensors was confirmed with street samples. An alternative approach was proposed by Yilmaz *et al*. who first modified the GCE surface with a polypeptide film terminated with benzoylecgonine antibody immobilized via glutaraldehyde cross linking. Cocaine or its metabolite, benzoylecgonine, binding to the antibody resulted in an increased charge transfer resistance and a drop of the oxidation peak current of the ferrocyanide, being proportional to the analyte concentration [44]. Some studies make use of a cascade of reactions. Cocaine triggered, amperometric detection of a p-benzoquinone, before oxidized by HRP, was proposed by Vidal *et al.* as is shown in Figure 3A [45] Their approach consisted of the following steps: (i) preparation of magnetic nanoparticles (NPs) functionalized with the polyclonal anti-cocaine antibody; (ii) competitive adsorption between cocaine and cocaine conjugated to HRP to functionalized NPs; (iii) followed by magnetic assisted collection and re-suspension at carbon electrodes. The observed signal amplitude was proportional to the amount of the HRP immobilized at the electrode surface, and was found to be high for low cocaine concentrations.

Cocaine lower detection limits at the pM levels was achieved using polyclonal anti-cocaine antibody-alkaline phosphatase conjugate as shown in Figure 3B. In this smart, and multistep approach, cocaine first binds to the conjugate. Next, the analyte-containing solution flows through the customized adsorbent which retains free and passes up cocaine bounded conjugate. Addition of tyrosine and glucose dehydrogenase to the working solution triggers a set of coupled reactions: (i) alkaline phosphatase controlled dephosphorylation of phenyl phosphate; tyrosinase controlled (ii) phenol hydration and (iii) catechol to o-quinone oxidation; (iv) glucose dehydrogenase-catalyzed the reduction of o-quinone back to catechol. Among many electroactive species formed, it was O_2 reduced by tyrosinase, that was monitored with a Clark electrode and was directly related to the present cocaine concentration [46]. The system with an impressive limit of detection equal to around 3 pM of cocaine concentration was reported and described by Abdelshafi *et al*. [47]. In their work, the authors developed a microfluidic electrochemical ELISA device where a monoclonal IP3G2

antibody was grafted to magnetic nano-beads and served for cocaine preconcentration whereas HRP was employed as an enzyme converting catechol to electrochemically detectable *o*-benzoquinone.

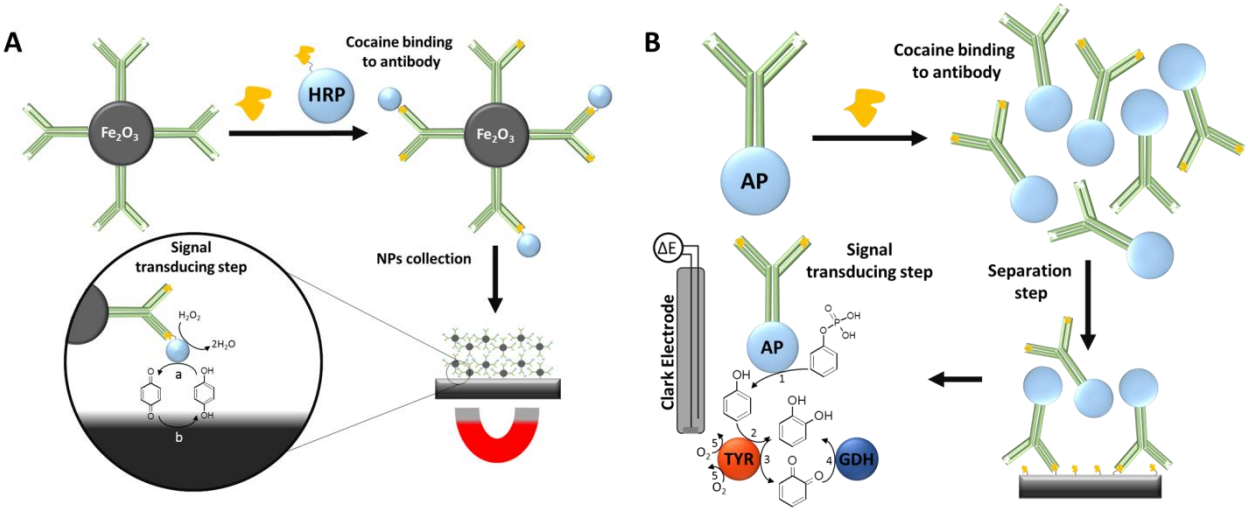


Figure 3. A – Magnetic NPs based immune-sensing utilizing polyclonal anti-cocaine antibody and Horseradish Peroxidase (HRP); (a) is the hydroquinone oxidation step; (b) is the p-benzoquinone electrochemical reduction step. B – Immuno-sensing utilizing alkaline phosphatase and polyclonal anti-cocaine antibody conjugate. 1 – stands for phenyl phosphate dephosphorylation, 2 is the phenol hydration step, 3 is the catechol oxidation by Tyrosinase (TYR), 4 stands for o-benzoquinone reduction by Glucose Dehydrogenase (GDH) and 5 is the O_2 reduction reactions at TYR that can be monitored with oxygen selective electrode.

Similarly to aptasensing, cocaine (or its metabolites) – antibody interaction assures a high detection specificity. Proposed enzymo- and immunosensing methodologies are designed in a way allowing for cocaine sensing down to and within pM/nM levels. Common drawbacks that can be mention here include (i) the complexity of the multi-component systems requiring harmonized functioning; (ii) time-dependent signal response; (iii) sensitivity of a sensing platform to pH variation and denaturation conditions.

1.5. Cocaine detection at soft junctions

1.5.1. Electrified liquid-liquid interfaces and ion selective electrodes

In electrochemistry soft interfaces can be narrowed down to the electrified liquid-liquid interface, polymeric membranes and self-assembling molecular ensembles (e.g. lipid bilayers). Detection at the electrified liquid-liquid interface, known also as the interface between two immiscible electrolyte solutions (ITIES), is not restricted to redox reactions alone, but can also arise from interfacial ion transfer reactions. There are two main requirements allowing for the molecular detection at ITIES: (i) the existence of a permanently charged or ionisable functionalities within the molecular structure of interest and (ii) phase partitioning needs to occur within the available potential window. ITIES was used to determine the lipophilic properties of some illicit drugs, including opioids and amphetamines

[48]. Samec *et al.* were the first who found that the protonated cocaine can undergo a simple ion transfer across the water and o-nitrophenyl-n-octyl ether (NPOE) interface (see Fig. 4A for schematic) [49]. In the recent report by Poltorak *et al.*, ITIES was used as the screening device for cocaine detection in the presence of a number of cutting agents used to adulterate street samples [50]. In such a configuration, as shown in Fig. 4B, symmetric ion transfer voltammogram obtained during protonated cocaine interfacial transfer indicated the reversibility of the process. As the detection is governed by molecular partitioning over the water and 1,2-dichloroethena interface (which is affected by the chemical structure), good selectivity was obtained even at the pristine interface. With a low limit of detection of a few µM and broad detection range up to the mM level, the validity of this approach was confirmed on real street samples.

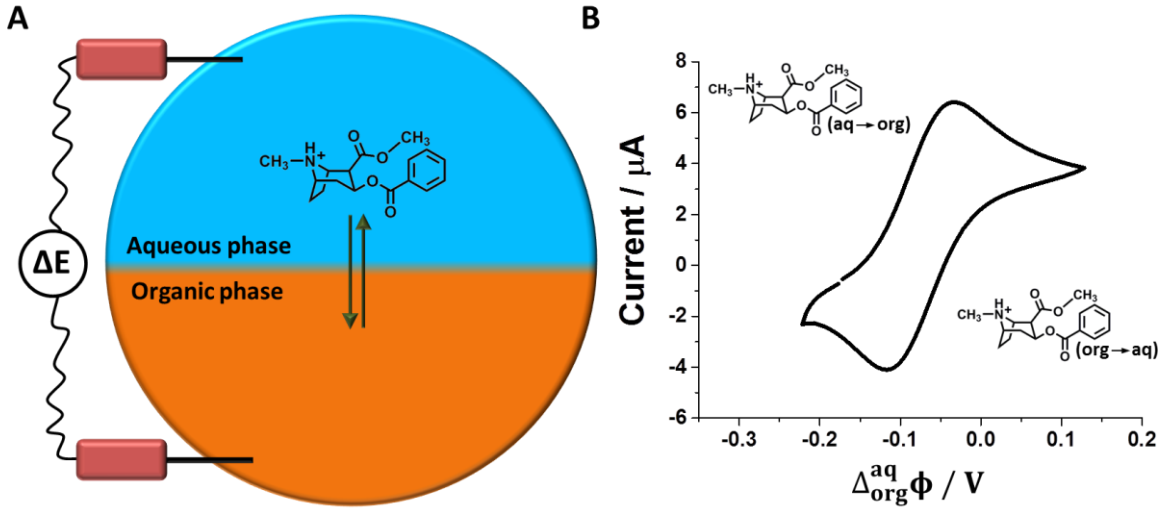


Figure 4. A – cocaine cation transfer across the polarized liquid-liquid interface. B – Ion transfer voltammogram recorded for cocaine transferring across the water – 1,2-dichloroethane interface. In parenthesis, the direction of ion transfer is given: positive current correspond to cocaine transfer from the aqueous to the organic phase, where a negative current corresponds to cocaine transfer from the organic to the aqueous phase.

Cocaine can also be detected with a properly adjusted membrane composition of an amine-selective electrode. Elnemma and Hamada were the first who reported the fabrication of cocaine-selective electrodes with a polyvinylchloride membranes utilizing di-n-octylphthalate or di-n-butyl sebacate as plasticizer and cocaine tetraphenylborate as an ion exchanger. Ion selective electrodes with such a composition displayed (i) Nernstian response over four decades of cocaine concentration (ii) in a wide pH range 3 – 7 [51]. Further improvement was proposed by Watanabe *et al.* who used the much more hydrophobic sodium tetrakis[3.5-bis(trifluoromethyl)phenyl]borate as the cation-exchanger and tetrakis(2-ethylhexyl) pyromellitate as the plasticizer. Utilization of membrane

components with higher lipophilicity improved the membrane selectivity, further lowered the limit of detection and finally allowed for measurements in an extended pH range from 1 to 8 [52]. An interesting approach was proposed by Smolinska-Kempisty *et al*. where the PVC membrane of an ion selective electrode was doped with the cocaine molecularly imprinted nanoparticles. The optimized example (covering nanoparticles formulation and their loading in the membrane) allowed for the cocaine detection in an impressive range from 1 nM up to 1 mM even in a real matrix, such as blood serum [53].

1.5.2. Nanopore sensing

Chemical detection employing nanopores rely on ionic current measurements passing through a pore with an extremely small pore volume. These ionic currents are measured in time and are constant for a fixed potential difference applied across the pore. The presence of an analyte, with a size just smaller than the pore size, increases the measured electrical resistance, if the analyte is translocated through the pores. This is recorded as a current drop with a very characteristic current – time pulse-wise response: (i) the pulse duration informs about the analyte translocation time; (ii) the pulse intensity is proportional to the analyte volume and finally (iii) the pulse frequency allows determination of the analyte concentration [54]. Nano-pore techniques employing α-Hemolysin were developed to date (see Figure 5) utilizing aptamers and specific host-guest interactions. Cocaine-induced aptamer conformation changes allow for the sensing by two different scenarios (i) α-Hemolysin pore blockage by the folded aptameric structure with the capture time proportional to the cocaine concentration [55]; and (ii) ssDNA translocation, initially hybridized with the aptameric chain, being released upon cocaine binding [56],[57]. For the latter, the translocation pulse frequency was directly related to the cocaine concentration. A different approach was proposed by Abelow *et al.* who instead of using a biological pore prepared glass nanopipette (utilizing glass capillary pulling technique) with a radius equal to 20 or 65 nm [58]. Stepwise surface modification of the nanopipette orifice with aptameric chains significantly lower amount of the translocating $Fc(CH_2OH)_2$ that was monitored by voltammetry at Pt electrodes located inside the nanopipette. The addition of cocaine to the investigated solution, triggered the aptamer conformation changes into the folded structure. As a consequence, the pore opened and the $Fc(CH_2OH)_2$ could enter again, leading to a signal regeneration. As the threshold concentration of the cocaine allowing for an efficient pore opening is only slightly lower from the saturation concentration, the detectable concentration range was rather narrow and lies within the μM regime. Cocaine sensing with an artificial nanopore was also recently reported by Wang *et al*.[59] In their approach the nanopore (having around 30 nm in diameter on the narrow side) was chemically etched in a polyethylene terephthalate thin sheet. The internal walls of the pore were modified with a section of a cocaine specific aptameric. Diffusion of cocaine and the

second section of a cocaine specific aptamer resulted in a bulky complex formation and allowed the control of the pore resistance. The authors were able to detect cocaine in a range from 1 nM up to 1 µM by following the ionic currents crossing the nanopore orifice.

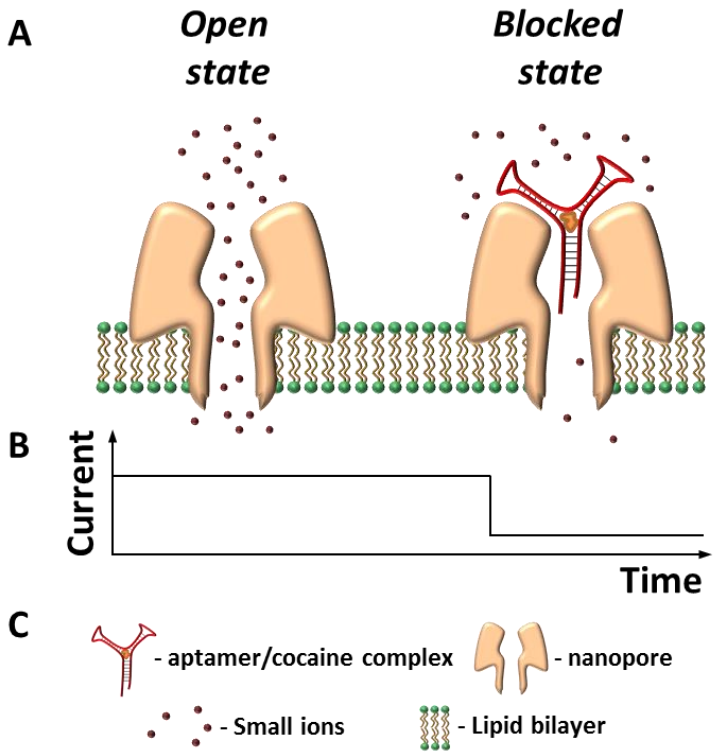


Figure 5. A – Schematic showing the general idea behind cocaine detection with (in that case) biological nano-pore. B – Shows the ionic current flowing across the nano-pore in the open state and the closed state. C – legend describing all components of the nano-pore sensing platform.

As compared with all other cocaine electrochemical sensing strategies, the detection at soft interfaces arises from the interfacial cocaine ion transfer (across the liquid – liquid interface, polymeric membrane – liquid interface or through a lipid bilayer embedded pore). In case of the ITIES and ion selective electrodes, the selectivity is governed by molecular lipophilicity, and hence, does not rely on oxidation/reduction reactions. Moreover, the cocaine can be detected in a wide pH range from 1 – 8, whereas the lower and upper detection limit oscillates from nM up to mM levels respectively. Disadvantages may include (i) the toxicity of used organic solvents for ITIES; (ii) surface fouling and electrochemical instability especially caused by surfactants; and (iii) (in some cases) problems with selectivity when detection is performed in demanding and complex environments e.g. body fluids.

1.6. Conclusions

Clearly, all the effort related to the electrochemical detection of cocaine has led to very exciting

Method	LOD[1]	Dynamic detection range[2]	Relative selectivity	Type of real samples	Tested on real sample	Real sample preparation

output in the form of a number of different sensing strategies ranging from direct charge transfer reactions to elaborated interphases with a high affinity for cocaine. Electrochemical sensors can be used to selectively detect cocaine in complex environments such as street samples and body fluids. The comparison between colorimetric tests and described here, electrochemical sensing scenarios is available in Table 1 (for more details we recommend to see Table A1 available in appendix A). Given by its simplicity, rapid analysis time and low associated costs, these methods have high commercialization potential, and hence, can be successfully incorporated into still presumptive, but significantly more accurate methods in comparison to the existing colorimetric illicit drugs screening tests. Further improvement is however anticipated. To our surprise, the beneficial effect of the electrified interface miniaturization on the electroanalytical output was not very often recognised. This includes arrays of nano- or micro- electrodes and nanogap sensors allowing the improvement in terms of LODs (small capacitive contribution and redox recycling) and sensitivities (working under non-diffusion limited conditions). Statistical validation of the developed sensing strategies on real samples is obviously needed. Also, more attention is required for electrochemical detection of cocaine metabolites from real samples such as urine. As the access to cocaine (and other illicit drugs) and related knowledge (e.g. street samples composition, new types of drug molecules, forensic analytical guidelines) is not directly available, the mutual cooperation between academic and forensic institutions have to be strengthened. We are convinced that by close cooperation, we are able to bring electrochemical cocaine sensors closer to our societal needs.

Table 1. Comparison of different electrochemical cocaine screening methods with a colorimetric test.

Colorimetric tests	µM	µM - mM	Moderate	Street samples	Commercial tests	Street samples – dissolution in buffer or supporting electrolyte; Body fluids – direct analysis (in some cases dilution in buffer was required).
Direct electrochemical detection	µM	µM - mM	High	Street samples	Yes	
Apta-sensing	pM	pM - µM	Very high	Body fluids	Spiked body fluids	
Immuno-sensing	pM	pM - µM	Very high	Body fluids	Spiked body fluids	
Enzymatic-sensing	nM	nM - µM	Very high	Body fluids	Spiked body fluids	
Ion selective electrodes	µM	µM - mM	High	Street samples	Yes	
Electrified liquid – liquid interface	µM	µM - mM	High	Street samples	Yes	
Nanopore sensing	nM	nM - µM	Very high	Body fluids	Spiked body fluids	

[1]At least one example within the group shows LOD within reported order of magnitude.
[2]Underlined dynamic detection range is reported for a few methods.

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