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Highlights:

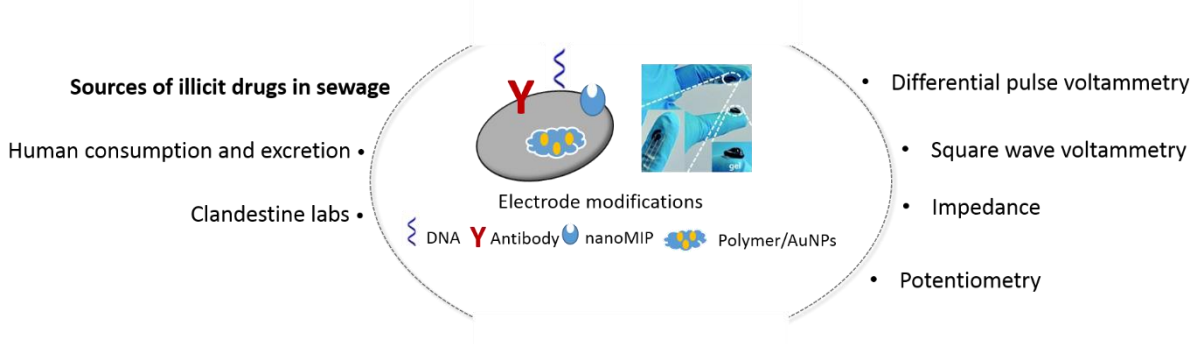
- Forensic drug market is continuously developing worldwide.
- Forensic drugs are classified as the latest group of 'emerging pollutants'.
- Electrochemical strategies for illicit drug detection in street samples, biological fluids and wastewaters are being developed.
- There is limited research available on the electrochemical detection of forensic drugs in environmental samples emphasizing the need for developing novel electrochemical strategies for their determination in water.

Abstract:

Illicit drugs consumption and trafficking is spread worldwide and remains an increasing challenge for local authorities. Forensic drugs and their metabolites are released into wastewaters due to human excretion after illegal consumption of drugs and occasionally due to disposal of clandestine laboratory wastes into sewage systems, being recently classified as the latest group of 'emerging pollutants'. Hence, it is essential to have efficient and accurate methods to detect these type of compounds in seized street samples, biological fluids and wastewaters in order to reduce and prevent trafficking and consumption and negative effects on aquatic systems. Electrochemical strategies offer a fast, portable, low-cost and accurate alternative to chromatographic and spectrometric methods, for the analysis of forensic drugs and metabolites in different matrices. Recent electrochemical strategies applied to the detection of illicit drugs in wastewaters, biological fluids and street samples are presented in this review, together with the impact of drug consumption on the environment.

Keywords: illicit drugs; electrochemistry; wastewater analysis; street samples; biological fluids; forensic science.

Graphical abstract:



Introduction

The use of illicit drugs is a persistent and deteriorating concern in our society. In the last decade, a significant increase in illicit drug consumption was reported, both for Europe and globally, with the estimated amount of drug users rising with 23% from 208 million to 255 million users worldwide [1,2].

Globally, cannabis is the most used drug, reaching 183 million people for consumption each year, followed by opioids and amphetamines with around 36 million users each. "Ecstacy", opiates and cocaine form the next three drug classes with around 20 million users each worldwide [1]. Cocaine is, however, the second most used drug in Europe [2].

The global abuse of illicit drugs holds multiple severe consequences for our society, mainly for health and health treatment costs, economic damage, increasing criminality, but also for the environment via contamination of wastewaters, surface waters and soil [2,3]. Therefore, it is essential that illicit drugs can be detected in an efficient way to reduce and prevent trafficking and consumption, in seized street samples and biological fluids, and also in wastewater. Expanding to the detection of metabolites of illicit drugs (present in urine) might also help in estimating the total drug consumption in specific areas by analyzing wastewater streams (wastewater-based drug epidemiology), which is a main focus of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [4-6].

The present review focuses on the impact of illicit drugs as emerging pollutants on the environment and the advancements recently made in the field of electrochemical sensing of drugs of abuse. Electrochemical strategies applied to their direct analysis in wastewaters are presented, as well as detection methods to identify drugs in seized street powders and biological samples. Drugs identification in street and biological samples combats drug trafficking and consumption and thus has an indirect environmental impact.

Impact of illicit drugs consumption on the environment

The direct impact of illicit drugs on the environment rather than the wastewater-based drug epidemiology is a less studied phenomenon, however, illicit drugs and their metabolites are continuously released into wastewaters due to human excretion after illegal consumption of drugs and occasionally due to disposal of clandestine laboratory wastes into sewage systems [7]. Because the effects of these drugs on aquatic systems were relatively unknown until recent years, illicit drugs were only recently classified as the latest group of 'emerging pollutants' [8,9]. Several studies on different locations indicate that the drugs and metabolites are often only partially removed by wastewater treatment plants (WWTPs) and sewage treatment plants (STPs), leading to significant exposure of the compounds to surface waters and soils [8,10,11]. Because of the limited effect of wastewater treatment, illicit drugs and their metabolites are released and detected in the aquatic environment (i.e. rivers, groundwater, lakes, channels) [3,12]. The concentration of a few illicit drugs in natural water systems occur at a similar degree to different emerging contaminants like diclofenac, causing their inclusion into the list of priority substances of the European Union Water Framework Directive [13]. The presence in surface waters immediately raises the concern of the presence of illicit drugs in ground- and drinking water. Some studies showed a presence of cocaine, 3,4-methylenedioxymethamphetamine (MDMA), methadone and benzoylecgonine in these systems, exposing humans and other organisms to these substances [14,15].

The exotoxicity of illicit drugs receives less attention compared to legal pharmaceuticals, but the possible negative effects on biota, aquatic organisms and the ecosystem might be at a similar level to pharmaceuticals [16]. It was proven that amphetamine sulphate showed a high toxicity to rainbow trout hepatocytes [17], while cocaine showed to cause defects because of mutation in distinct genes,

leading to a disturbed dopaminergic signaling in the brain of mutagenized zebrafish [18]. Moderate concentrations of cocaine in water also caused primary DNA damage in Zebra mussels [19], while the presence of morphine led to reduced phagocytic and intracellular esterase activity, lipid peroxidation and cell adherence in freshwater mussels [20]. It is clear that the extent of the effects of illicit drugs depend on the drug type and the tested organism.

The United Nations Office on Drugs and Crime has for each class of drug a report on the "recommended methods for the identification and analysis of *drug*" [21-29], including color tests, anion tests, microcrystal tests and immunoassays for screening of drugs, and more sophisticated methods for confirmatory analysis e.g. ultraviolet spectrophotometry (UV), gas chromatography coupled with mass spectrometry (GC-MS) and flame ionization detection (GC-FID), high performance liquid chromatography (HPLC), fourier transform infrared spectroscopy (FTIR). Concerning optimized methods for analysis in wastewaters, different methods have been developed, with GC-MS, LC-MS, SPE-LC-MS(-MS) the most important ones for the detection of illicit drugs in environmental settings, both for on-line and off-line settings for SPE [4, 30].

Electrochemical strategies to detect drugs of abuse in real samples

Electrochemical methods are particularly amenable to forensic analysis due to their great performance in turbid, complex matrices. Electrochemical platforms are not affected by optically absorbing and fluorescent molecules, as in the case of spectroscopy, and can be readily functionalized with nanomaterials, allowing to reach low detection limits needed when analyzing environmental or clinical samples. Moreover, electrochemical methods may avoid the lengthy pretreatment and purification steps that are usually required when analyzing real samples. Another advantage is the possibility to integrate electrochemical sensors into a flow-injection setting, which is perfect for the real-time on-line monitoring of wastewater streams. Sensing devices incorporating such methods are becoming increasingly attractive due to their low cost, ease-of use and portability for point-of-use applications [31].

There is only limited literature available on the detection of illicit drugs in environmental samples emphasizing the need for developing novel electrochemical strategies for determining, monitoring and possibly decontaminating wastewater, surface water, drinking water of these emerging pollutants. Yang et al [32] developed the first aptasensor for electrochemical detection of cocaine in wastewaters and explored its potential to evaluate trends of cocaine use. The group used a DNA-directed immobilization method of an aptamer specific for cocaine onto gold chips. Two approaches were employed and compared in terms of immobilization efficiency (Figure 1). In the first strategy ssDNA was firstly immobilized onto gold chips, while the second approach used the co-immobilization of ssDNA and dsDNA aptamer. In both cases mercaptohexanol was added to reduce non-specific binding, which is important when analyzing complex matrices. Cocaine detection was achieved following a simple principle: upon cocaine binding the aptamer changes its configuration forming a two junction loop, which leads to an increase in the charge transfer resistance (R_{ct}) in impedimetric measurements. The second immobilization strategy proved to be more efficient inducing a bigger ΔR_{ct} value due to less steric hindrance effects. The sensor exhibits a LOD of 0.01 µM with a dynamic range from 10 nM to 50 µM. The practical applicability of the sensor for cocaine detection in water samples was assessed, however a pre-treatment step had to be introduced. Cocaine was analyzed in spiked tap water and wastewater samples after the latter was subjected to filtration to remove large molecules. An original aspect of the work was the assessment of the sensor for estimation of drug consumption at community level. The sensor was used to analyze wastewater collected daily during one week monitoring, after a pre-concentration step using solid phase extraction to reach LOD, and it showed remarkable results, comparable with mass spectrometry, indicating the usefulness of electrochemistry for wastewater based epidemiology studies.

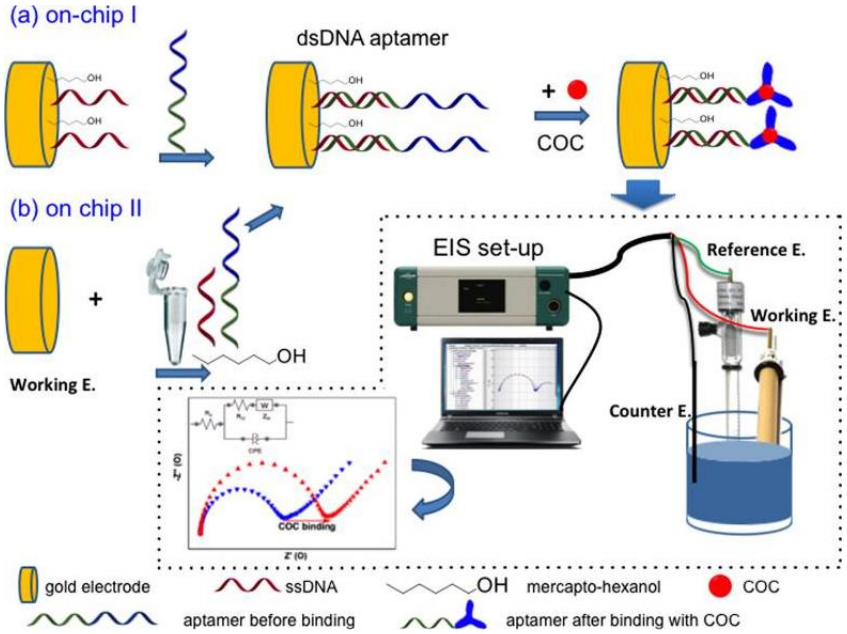


Figure 1. Electrochemical strategy based on DNA aptamers for cocaine detection in wastewaters [32].

Amin et al [33] reported a sensor for the detection of tramadol, an opiate painkiller emerging as drug of abuse, in wastewaters. The modification of glassy carbon electrodes with Nafion® and gold nanoparticles (AuNPs) protected with with cetyltrimethylammonium bromide (CTAB) proved to improve the electron transfer as shown by impedimetric measurements. The square wave anodic stripping voltammetry response of the sensor was linear over the concentration ranges of 0.5-1 $\mu g \cdot mL^{-1}$ and 2-12 $\mu g \cdot mL^{-1}$ with a low detection limit of 0.3 $ng \cdot mL^{-1}$, suitable for analysis of wastewaters. The influence of likely interferences present in water samples, e.g. urea, glucose, citric acid, Na^+, K^+, Cl^-, on the performance of the sensor was further evaluated. The sensor maintained its response with a RSD of 5% or below when the interfering compounds were present in 1-5 folds with respect to tramadol, however in 5-20 fold concentration range a decrease of 20% of tramadol signal was observed, which could raise challenges in case of real samples analysis. Hospital water samples were analyzed with the developed sensor, following a filtration through an acetate membrane filter syringe. In addition to filtration the water samples had to be deaerated, as dissolved oxygen interferes with tramadol detection, posing some difficulties in the case of on-site detection. Tramadol was successfully quantified in three out of four analyzed samples (in one sample the matrix effect being too strong), however the results were not validated with a well-established method.

While the literature available on electrochemical detection of forensic drugs in environmental samples in recent years is scarce, the detection of drugs of abuse in seized street samples and in biological samples such as saliva and urine has attracted increasing interest. Detecting and thus preventing drug use and abuse has an impact on the prevention of environmental contamination as well. Drugs can enter wastewaters as such or in the form of their metabolites via human excretion, e.g. urine, after illegal consumption or via accidental or intentional disposal from clandestine drug laboratories. A source of environmental pollution by forensic drugs is represented also by their illegal bury in soil, disposal into sinks and toilets reaching sewerage systems or into public solid waste facilities. Moreover illicit drugs have the potential to enter the ambient air in powder form during consumption or handling and as smoke during consumption [3]. Implementing measures to reduce illicit drug trafficking, fabrication and use would reduce the negative effects of these compounds, showing the significant importance of these studies on the environmental impact as well. Furthermore, the strategies discussed below might also be further altered and expanded to an environmental setting.

In this light, several works focused on the rapid detection of drugs in street samples avoiding the false negatives and false positives given by color tests [34]. Our group developed a wearable fingertip electrochemical sensor for on-the-spot identification of cocaine and its cutting agents in street samples [35]. The study elucidated, for the first time, the presence of cutting agents in street samples in an on-site test. The extensive screening by square wave voltammetry in phosphate buffer solution (PBS) pH 7 revealed valuable information on which adulterants may pose challenges for on-the-spot cocaine detection leading to false positive or false negative results. To reduce false negatives the study was further focused on the case of levamisole as an adulterant suppressing the peak of cocaine [36]. Two strategies were proposed to avoid this suppressing effect and reveal the signal of cocaine on unmodified carbon screen printed electrodes i.e. adjusting the pH of the detection solution to pH 12 and applying a cathodic pretreatment, allowing a fast and simple detection of cocaine in street samples adulterated with levamisole. Another strategy involved the modification of screen printed electrodes with affinity polymers for cocaine, selected by computational modelling [37]. Another portable method for cocaine quantification and adulterants screen was proposed by Richter's group [38], by coupling a batch-injection system with square wave voltammetry. Using boron doped diamond electrodes the group demonstrated for the first time the possibility for electrochemical analysis of cocaine in acid media. With minimum sample preparation the rapid screening of cocaine together with common adulterants was possible. Unmodified graphite screen printed electrodes were also employed by Cumba et al [39] for the simultaneous detection of para-methoxyamphetamine (PMA), commonly known as Dr. Death, and 3,4-methylenedioxy-methamphetamine (MDMA) in ecstasy pills at pH 7. Few other electrochemical strategies have been reported in the past two years for the determination of drugs of abuse in street samples, which involve their direct voltammetric analysis on unmodified carbon-based electrodes, e.g. Δ9-THC [40], synthetic drugs such as ethylone [41], benzylpiperazine [42] or 25H-N-benzyl-substituted phenethylamines [43].
Although simple, the limits of detection in all above presented strategies (unmodified electrodes) were in the µM range, thus, if the detection principles were to be further exploited for water samples analysis, a sample preconcentration step or further electrode modification (to increase sensitivity) would be necessary.

Dronova et al [44] achieved low LODs, in the nM range, for the direct voltammetric detection of synthetic cannabinoids, without any further electrode modification. The low LODs were suitable for applications in both street samples and saliva. Three types of unmodified working electrodes were compared, showing that Pt and (anodically pretreated) boron doped diamond electrodes are the most suitable for DPV analysis of indole and indazole-synthetic cannabionoids in acetonitrile exhibiting the lowest oxidation potentials and highest sensitivity, respectively, while GC electrodes showed the lowest sensitivity towards all analytes. This also points to the importance of testing and comparing different electrode materials when developing strategies for electrochemical detection of forensic drugs.

To achieve sensitive and selective detection of drugs of abuse in biological samples a modification of electrode surfaces with affinity elements i.e. aptamers, antibodies or molecularly imprinted polymers is usually required, to increase both selectivity and sensitivity (pre-concentration close to electrode surface). Cocaine has received quite some attention in forensic electrochemistry in recent years. Several aptasensors have been reported for its detection in urine, saliva or serum samples, most of them being based on indirect detection using a redox probe by means of capacitive, impedimetric or voltammetric measurements [45-48]. The sensors achieved low LODs in the nM range, or even fM level. However a downside of employing aptamers in these cases is that they are synthesized by SELEX to exhibit high specificity for the target i.e. cocaine and therefore do not bind cocaine metabolites i.e. benzoylecgonine, which are also present in biological samples. This might lead to an inaccurate evaluation of drug consumption. Immunosensors employ antibodies, which, having lower selectivity, can also bind structurally related compounds. Sengel et al [49] used a benzoylecgonine antibody immobilized on poly-L-phenylalanine covered GC electrode, which was able to bind both cocaine and benzoylecgonine. Yet, the sensor reached a LOD of 0.41 µM which is not sufficient to

determine cocaine and benzoylecgonine in biological samples. With a similar approach (antibody@polymer, indirect detection by DPV) the same group developed an immunosensor for the detection of synthetic cannabinoid JWH-073, reaching in this case nM level detection, probably due to higher affinity of the antibody for its target. A general concern of these biological affinity elements is their stability in extreme conditions (temperature, acidity, ionic strength, etc.), which might pose a problem in often varying unknown matrices, like wastewater. This might be a concern to use these approaches in an environmental setting.

Other type of selective recognition elements emerging in recent years, which are more stable in extreme conditions compared to biological recognition elements, is represented by nanoMIPs that can be tailor-designed to bind both illicit drugs and their metabolite. Using benzoylecgonine as template for solid-phase imprinting of nanoMIPs Piletsky's group [50] developed a potentiometric sensor, capable to bind both cocaine and its metabolites, as shown by SPR measurements e.g. low dissociation constants for benzoylecgonine 0.17 nM, norcocaine 2.45 nM, cocaethylene 2.45 nM and anhydroecgonine methyl ester was 1.79 nM. The sensor exhibits a broad linear range (1 nM to 1µM) suitable for biological samples analysis and was tested for cocaine detection in spiked serum samples showing a negligible effect of the matrix on the sensor response. Moreover the sensor can be regenerated by adding a short washing step with PBS. Errachid's group developed a potentiometric sensor for the detection of amphetamine [51] based on a ion-pair complex (metallocarborane coupled with amphetamine protonated cation) which was incorporated in a PVC sensitive membrane and casted onto polypyrrole-modified electrodes. A quick response of the sensor was obtained within the range 10^{-5} M to 10^{-3} M achieving a LOD of 12 µM.

Conclusions

This review summarizes the advancements made in recent years for the electrochemical detection of forensic drugs. Recently classified as emerging pollutants, illicit drugs and metabolites are only partially removed by treatments plants, thus exposing humans and other organisms to their negative effects. The literature available on the detection of illicit drugs in environmental samples is scarce emphasizing the need for developing novel electrochemical strategies for determining, monitoring and possibly decontaminating wastewater, surface water, drinking water of these pollutants. The few strategies presented in the literature require modifications with aptamers or polymer composites, and a pretreatment of the samples i.e. filtration and/or preconcentration by solid phase extraction to reach the desired detection limits. A publication even assessed the usefulness of electrochemical sensors to estimate drugs consumption at community levels, by weekly monitoring rugs in wastewaters, showing that electrochemical methods can be indeed amenable to such applications. There is more research available on the detection of illicit drugs in street samples and biological fluids as strategies to combat drug trafficking and consumptions. Some procedures involve the use of unmodified electrodes to screen unknown street samples towards a particular drug in presence of adulterants, however most methods require electrode modifications with e.g. aptamers, antibodies or nanoMIPs to reach low detection limits and high selectivity. Wearable sensors i.e. gloves, also attracted interest from the research community as a convenient way to perform on-site analysis. Nonetheless, despite their advantages electrochemical methods for illicit drugs detection still have a long way to become fully applicable. In particular for waste water analysis, preconcentration methods or flow injection analysis should be considered, as well as new electrode materials to improve sensitivity and selectivity. The use of electrochemistry for illicit drug decontamination could be further exploited.

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