Nanomaterial-based aptamer sensors for analysis of illicit drugs and evaluation of drugs consumption for wastewater-based epidemiology

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ABSTRACT

The abuse of illicit drugs usually associated with dramatic crimes may cause significant problems for the whole society. Wastewater-based epidemiology (WBE) has been demonstrated to be a novel and cost-effective way to evaluate the abuse of illicit drugs at the community level, and has been used as a routine method for monitoring and played a significant role for combating the crimes in some countries, e.g. China. The method can also provide temporal and spatial variation of drugs of abuse. The detection methods mainly remain on the conventional liquid chromatography coupled with mass spectrometry, which is extremely sensitive and selective, however needs advanced facility and well-trained personals, thus limit it in the lab. As an alternative, sensors have emerged to be a powerful analytical tool for a wide spectrum of analytes, in particular aptamer sensors (aptasensors) have attracted increasing attention and could act as an efficient tool in this field due to the excellent characteristics of selectivity, sensitivity, low cost, miniaturization, easy-to-use, and automation. In this review, we will briefly introduce the context, specific assessment process and applications of WBE and the recent progress of illicit drug aptasensors, in particular focusing on optical and electrochemical sensors. We then highlight several recent aptasensors for illicit drugs in new technology integration and discuss the feasibility of these aptasensor for WBE. We will summarize the challenges and propose our insights and opportunity on aptasensor for WBE to evaluate community-wide drug use trends and public health.

1. Introduction

The abuse of illicit drug is becoming increasingly serious worldwide [1—3]. For drug addicts around the world in 2017, approximately 271 million people (5.5%) aged between 15 and 64 have used illicit drugs at least once, which represented one in every 18 people [4]. A variety of illicit drugs are available on the drug market, including traditional plant-based substances (heroin, cannabis, and cocaine) and novel synthetic drugs [4]. In recent years, hundreds of new psychoactive substances have been synthesized. The massive abuse of illicit drugs is a global concern that poses a great risk and can cause many health problems, including death [1—3]. For example, in North America, the abuse of synthetic opioids such as fentanyl caused a dramatic increase in opioid overdose deaths in 2017 [4]. Therefore, to control the seriousness and harmfulness of drug abuse, it is increasingly important to effectively evaluate and supervise drug abuse.

To evaluate the abuse of drugs, wastewater-based epidemiology (WBE), which is emerging as a novel tool, has been employed to estimate drug consumption and capture drug traffickers by measuring drugs or metabolites in wastewater at defined sewage treatment plants [3,5—7]. Compared to the classical survey approach [6,8], WBE has some specific advantages, such as rapidness and nearly real-time monitoring which has been applied to assess illicit drug abuse in many different countries and areas [7,9,10]. A classical analytical method mainly relies on high-performance liquid chromatography-mass spectrometry, which holds the merits of excellent stability and high sensitivity and selectivity [11,12]. However, this method requires sample pre-treatment before analysis, for example, using solid-phase

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extraction to purify raw wastewater and reconstitute it in an organic solvent for analysis. Thus, the necessary but complicated sample pre-treatment, along with the manipulation of the instrument, requires professional staff to perform the assay and process data in a central lab. Therefore, the development of a rapid, simple and inexpensive analytical method to determine drugs in wastewater, preferably on-site, for the evaluation of illicit drug abuse is urgent.

Compared to conventional methods, biosensors hold promise and have become an alternative for overcoming the limitations of the traditional methods. As small analytical devices, biosensors are composed of biological receptors that generate an optical, electrochemical or other signals in the presence of a target. Biosensors with the ability to be miniaturized have the advantage of target analysis with minimal on-site samples in different complex matrices, such as body fluids and environmental samples [13–15]. Among the various biosensors, when the biological receptor is an aptamer, the biosensor is denoted as an aptamer sensor (aptasensor). An aptamer is an artificial single-stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA) that can specifically bind to certain targets due to its high stability, high affinity, and high specificity; additionally, it has a directional nucleic acid amplification ability and a low production cost [16,17]. Furthermore, chemical/biological labelling and modifying of aptamers with reporter molecules can promote the performance of the detection system while retaining its affinity for the specific target [17]. Moreover, an aptamer can be constructed to withstand repeated cycles of denaturation and renaturation, which leads to the feasibility of regenerating the immobilized biocomponent function for reuse [18]. Recently, aptasensors have been widely applied to monitor many different targets in different matrices, such as heavy metals [19–22], small molecules [23,24], targeted nucleic acid sequences [25], peptides [26], enzymes [27], proteins [26,28], biomarkers [13,14] and even bacteria [29,30]. The detection of a variety of illicit drugs in different matrices with aptasensors has been reported.

In this review, we first demonstrate the process of WBE that can be used in the evaluation of illicit drug consumption. Then, we focus on recent advances in illicit drug aptasensors. Depending on their read-out methods, we classify illicit drug aptasensors into optical (colorimetry, fluorescence, and SERS) and electrochemical analytical methods. Third, we highlight the latest development of new technology integration in the use of illicit drug aptasensors, such as DNA technology (DNA amplification, DNA hydrogel, and DNA sequencing technology) and device integration (microfluidic systems, paper-based platforms, and portable device integration). Finally, we discuss the possibility of illicit drug aptasensors in WBE.

2. Wastewater-based epidemiology

It is difficult to monitor and control the abuse of drugs because drug-taking behaviour is characterized by concealment and complexity. WBE has the clear potential to be implemented as a powerful tool by combining existing methods for drug abuse evaluation. Wastewater contains different kinds of chemical substances that have been excreted or consumed within the given area that is served by certain wastewater treatment plants (Tab S1) [31]. The feasibility of non-invasive and near real-time monitoring of drug use by monitoring such residues in wastewater has been demonstrated [32]. Wastewater analysis has a few merits compared with other approaches, as it is unaffected by the biases associated with self-reported results and can well identify the real types of drugs consumed, which is particularly important because drug addicts usually do not know the actual mixture of chemicals they use [33–35]. This approach can also supply timely information

on temporal and spatial profiles of drug abuse over a short period of time [36–38]. It has been used for the evaluation of lifestyle such as food and chemical consumption [31] and recently has been proposed for tracing the carrier for early warning of COVID-19 [39].

WBE has several successive steps that enable experts to quantify certain illicit drugs or their metabolic residues in untreated wastewater and then normalized to estimate the total consumption of the corresponding drugs by the population served by that sewage treatment plant [8]. First, the appropriate untreated wastewater sample is collected and analysed to quantify the target drugs. Then, drug consumption is achieved by back calculating the daily wastewater load of the target residue; this is achieved by multiplying the measured target residue concentration by the daily flow rate of wastewater. In a third step, researchers can correct the value obtained in the second step by a specific correction factor and then obtain the total consumption of the target drug. The correction factor needs to consider both the molecular mass ratio of the parent drug to its metabolite and the average excretion rate of a given drug residue. Finally, to conveniently compare the daily consumption of different cities, the total consumption value will be averaged by the whole population, which is usually expressed in daily doses per thousand people.

Currently, WBE is widely employed in many different countries and regions to evaluate the abuse of a variety of illicit drugs [10,32,40], including the UK [37], France [41], Belgium [42], Norway [43], Australia [31,44,45], USA [46], and China [47]. The wide application of WBE showed its ability to evaluate the use of cannabis, methamphetamine, amphetamine, ketamine, cocaine and other widely abused drugs [10,40]. Moreover, the geographical differences of drug abuse in different areas assessed by WBE are consistent with the results obtained by other established methods [36]. It has been demonstrated that wastewater analysis can monitor local and temporal patterns of drug use, showing its potential to provide complementary information with standard technology. It is worth mentioning that our research group is the earliest one in China to have been evaluating of illicit drug use using WBE. In the past few years, our group has performed many works on monitoring of illicit drugs and made a series of contribution for WBE [11,47–49]. One of the most important works is that we used this technique to help police track down and arrest drug manufacturers [3].

The commonly used advanced analytical technique for WBE is high-performance liquid chromatography-mass spectrometry because it can identify and quantify the illicit drug targets at very low concentrations in a particular complex sample (raw wastewater). A mass spectrometry is the most widely used technique for illicit drug quantification in WBE because it can quantify the illicit drugs or their metabolic residues in wastewater utilizing an internal standard, such as a deuterated analogue of the target [50]. High-resolution mass spectrometry as a powerful tool has also many applications for WBE, including quantification of drugs at ultralow concentrations, screening of a large number of non-target compounds, identification of new metabolites and degradation/transformation chemicals, investigation of illicit drugs and elucidation of unknowns in wastewater [51]. Although the mass spectrometry is a gold-standard technique for the quantification of target drugs in wastewater due to its excellent stability, selectivity and sensitivity, novel methods with promoted performances will be beneficial to further extend the application of WBE. However, mass spectrometry involves troublesome sample collection and purification, expensive testing, and necessary operation by professional staff become a burden for the evaluation process, especially for the testing in the field. Therefore, there is an urgent need to develop a rapid method (for example, biosensors) to provide real-time monitoring of wastewater profiles [52].

3. Aptasensors for the detection of illicit drugs

Aptasensors have emerged as an innovative and powerful technique and they have been widely employed in biomedical diagnosis, drug screening, food safety, forensic analysis, and environmental monitoring [26,53,54]. Increasing efforts have been made to construct aptasensors for illicit drug analysis, due to its low-cost and excellent stability [55]. The signals from the binding between the aptamer and illicit drug include a colorimetric signal [56], an electrochemical signal [37,57], a fluorescence signal [58] and a surface-enhanced Raman spectroscopy/scattering (SERS) signal [59]. For example, we have recently developed a colorimetric aptasensor using DNAzyme for methamphetamine determination [60]. In this part, to provide a comprehensive summary of illicit drug aptamer sensors, the highlights and prominent examples are discussed, mainly focusing on the optical and electrochemical aptasensors.

3.1. Optical aptasensors

Many optical aptasensors have been developed to detect illicit drugs so far. In the literature, the following illicit drug aptasensors have been classified into colorimetric, fluorescence, SERS, luminescence, and other optical platforms (listed in Table 1). In this section, we mainly discuss colorimetric, fluorescence, and SERS aptasensors, which are the most widely used optical sensing platforms in conjunction with aptamers.

3.1.1. Colorimetric aptasensors

Colorimetric sensors have been given more attention in relation to illicit drug detection because of its merits, such as simplicity, cost-effectiveness, amenability and even visualization by the naked eye. For example, Stojanovic and Landry first reported a colorimetric cocaine aptasensor with a limit of detection of 2 μM in 2002 [72]. Other colorimetric aptasensors for illicit detection that have been reported in recent years are also listed in Table 1.

Colorimetric aptasensors are visual and usually involve nanomaterials, typically gold nanoparticles (AuNPs), which are red when dispersed in solution. In most sensors, the electropositive amino groups of the aptamer noncovalently bind to negatively charged AuNPs, which can stabilize the AuNPs under high salt concentrations. When introduced to an illicit drug, the specific aptamer preferentially binds to the illicit drug instead of with the AuNPs. The bare AuNPs are susceptible to aggregation and lead to an intensity change and a blue shift. The peak intensity or the extent of the blue shift correlates to the concentration of the illicit drug, which can be quantified by an absorbance ratio. Based on this

strategy, many scientists have successfully constructed a colorimetric sensor for visual methamphetamine detection [61,63]. However, the detection of a low drug concentration cannot be realized due to the limitation of aggregation-induced sedimentation [73]. What is worse is that these AuNP-aptasensors based on salt-induced aggregation have difficulty resisting interference from environmental matrices.

To acquire high sensitivity and stability, a novel structure of a gold nanomaterial coated by Ag core-shell nanoparticles (Au@Ag) was synthesized, and a non-aggregation analytical strategy was applied in illicit drug detection in our work [56]. Fig. 1 shows the detection strategy of the aptasensor in which this analytical technique is composed of a certain aptamer, a reporter probe, and a capture probe. The reporter probe was Au@Ag synthesized by seed growth and then modified with reporter probe DNA. The capture probe was made with magnetic beads functionalized with capture probe DNA; both the reporter probe DNA and capture probe DNA were complementary to the specific aptamer fragment. Therefore, the aptamer could bind to the capture probe and reporter probe to form a sandwich structure through a hybridization reaction. The sandwich complex could be removed from the suspension by an external magnetic field, which would reduce the absorbance intensity of Au@Ag [74]. In contrast, the sandwich complex could not be formed in the presence of certain illicit drugs because the aptamer would bind preferably with the target drug due to an increased affinity. Thus, the concentration of Au@Ag remaining in the supernatant was proportional to the concentrations of target drugs. The colour of the supernatant changed from light yellow to deep yellow as the concentration of the target drug increased, which could be measured and even visualized by the naked eye. This simple design avoided the complex spectrum of the classical colorimetric method mentioned above, which was dependent on induced aggregation. Due to the stability of dispersed Au@Ag, the colour of the solution was always yellow without a blue shift and could only be reduced in intensity rather than with the complex spectra of aggregates.

Apart from a nanomaterial-based colour aptasensor for illicit drug detection, DNAzyme has been used as an example of an allosteric aptamer that has been applied in biosensor construction [75]. In these platforms, DNAzyme could act as either signal read-outs or recognition elements. The G-quadruplex structure was composed of repetitive G-rich sections, which could form a G-quadruplex/hemin complex under the existence of hemin that exhibited peroxidase behaviour similar to horseradish peroxidase. Herein, horseradish peroxidase mimicking DNAzymes could be used to fabricate colorimetric aptasensors. In fact, many novel colorimetric illicit drug aptasensors were constructed by using a

Table 1
Available optical aptasensors for the detection of illicit drugs.

| Detection signal	Target	Linear range	LOD	Assay time	Ref.
Colorimetry	Methamphetamine	5–400 μM	5 mM	<30 min.	[61]
Colorimetry	Methamphetamine	1.0–200 nM	0.5 nM	60 min	[62]
Colorimetry	Methamphetamine	2–10 μM	0.82 μM	15 min	[63]
Colorimetry	Methamphetamine	0.5–200 nM	0.1 nM	60 min	[56]
Colorimetry	Cocaine	1.0–150 nM	0.5 nM	60 min	[56]
Colorimetry	Cocaine	10–150 nM	3.3 nM	60 min	[62]
Colorimetry	Cocaine	5–1000 μM	10 μM	60 min	[64]
Colorimetry	Methamphetamine	8–500 nM	0.5 nM	70 min	[60]
Colorimetry	Codeine	1–20 μM	0.9 μM	<5 min	[65]
Fluorescence	Cocaine	1–500 nM	0.1 nM	<30 min	[66]
Fluorescence	Cocaine	0.5–80 nM	84 nM	25 min	[67]
Fluorescence	Cocaine	0.5–20 nM	209 pM	70 min	[68]
Fluorescence	Cocaine	0–8 μM	200 nM	20 s	[69]
SERS	Methylamphetamine	0.5–40 ppb	0.16 ppb	30 min	[59]
SERS	Cocaine	0.01–10 μM	10 nM	60 min	[70]
Chemiluminescence	Cocaine	5.0–60 μM	1.43 μM	70 min	[71]
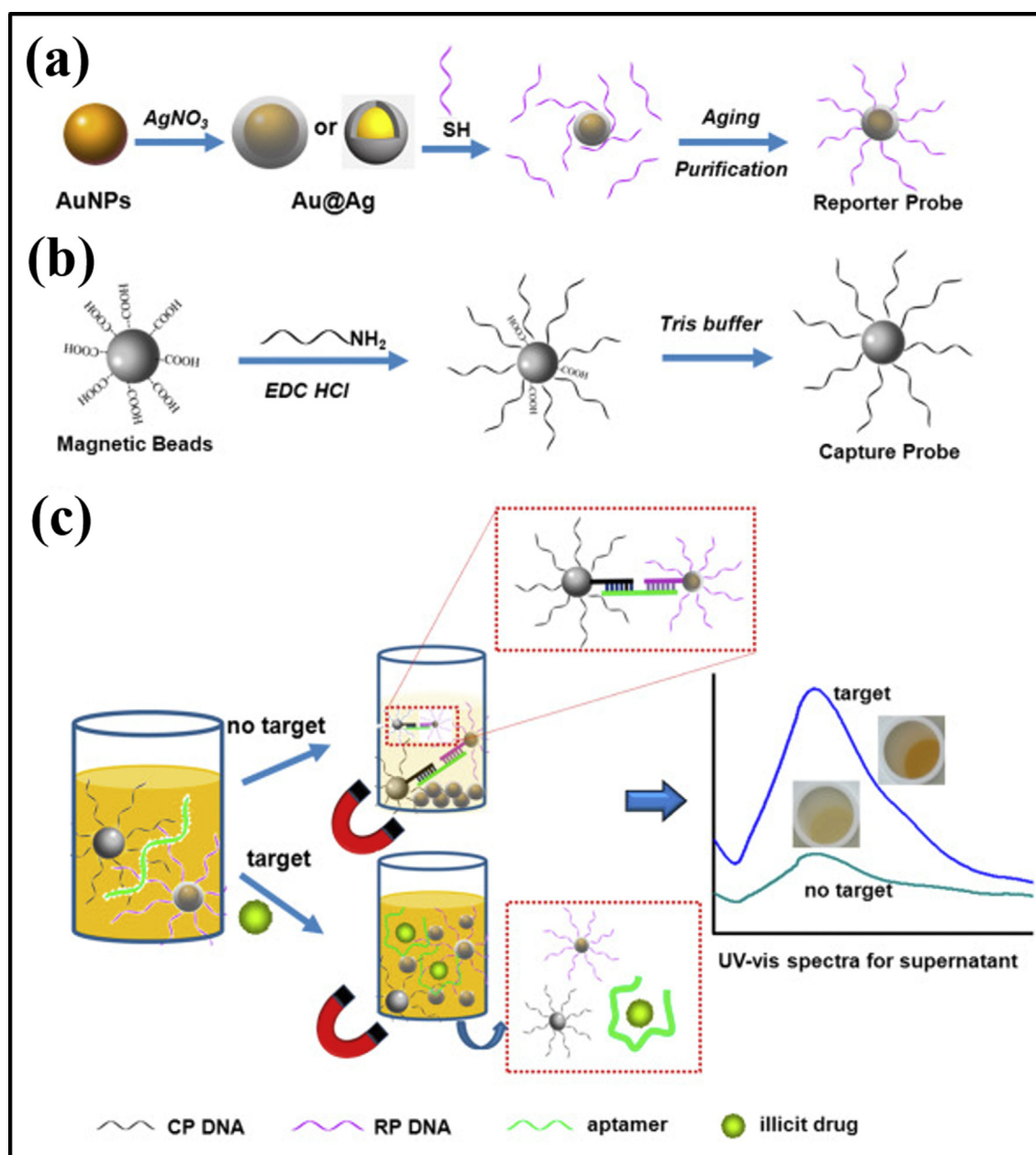
Fig. 1. Colorimetric aptasensor based on non-aggregated Au@Ag for illicit drug detection [56].

combined aptamer and peroxidase-mimicking DNAzyme [19,60,64,75]. We also designed a simple and unlabelled aptasensor for the detection of methamphetamine through a G-quadruplex-hemin DNAzyme [60].

3.1.2. Fluorescent aptasensors

Similar to a colorimetric aptasensor, a fluorescent aptasensor (listed in Table 1) has also been successfully employed in illicit drug analysis. Usually, fluorescence aptasensors require a fluorophore and a fluorescent quencher. The former is usually bound to one terminus of an aptamer, which mainly includes fluorescent dyes, gold/silver nanoclusters (Au/AgNCs), and quantum dots (QDs); the quencher is usually bound to the other terminus and mainly included a fluorescence quenching organic fluorescent molecule (dabcy and BHQ), and nanomaterial (Au/AgNPs and graphene oxide (GO)). The initial aptamer conformation could make the fluorophore and quencher close to each other, leading to low or even no fluorescence. After adding an illicit drug, the conformational change of the aptamer could separate the fluorophore and quencher, leading to high fluorescence intensity (turned on) [76]. Alternatively, the quencher could modify a complementary nucleic acid sequence that gets unbound from the aptamer upon illicit drug binding. When the fluorophore and quencher were spatially apart, the intensity of fluorescence was high. Once the aptamer bound to the target, the conformational change of the aptamer caused the fluorophore and quencher to be close to each other, leading to fluorescence loss (turned off). The fluorescence intensity could be relative to the concentration of the illicit drug target depending on whether the fluorophore and quencher contained nanomaterials or not; this is further discussed in four categories.

The first category was that the fluorescent and quenching agents did not contain nanomaterials. For example, Roncancio et al. [69]

constructed a simple fluorescence aptasensor for one-step cocaine detection with a minimal amount of sample. They discovered that cocaine aptamer I could also bind the fluorescent molecule 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) and thereby quench its fluorescence. They changed aptamer I and engineered a novel aptamer II that exhibited a high affinity for both ligands, which reduced the background signal, thus gaining an increased target signal. Using this aptamer, they successfully constructed a novel detection method that was dependent on the cocaine-mediated displacement of ATMND from aptamer II due to the competitive binding mechanism (as shown in Fig. 2A). This competitive binding method overcame the limitation of target sensitivity compared with that of the traditional target-induced conformational change one. Notably, although neither the fluorescent nor quenching agents used nanomaterials, nanomaterials could be used as auxiliary materials in these analytical strategies. For example, Abnous et al. used silica nanoparticles as auxiliary materials and designed a cocaine detection tool based on an aptamer [67].

The second category was that the fluorescent agents used nanomaterials, while the quenchers did not. The common fluorescent nanomaterials were Au/AgNCs and QDs [80,81], which were widely applied to the construction of illicit drug aptasensors [82]. Raichlin et al. developed a fluorescence sensing platform for cocaine detection based on the electron-transfer quenching of nucleic acid-functionalized CdSe/ZnS QDs with doxorubicin [77]. As shown in Fig. 2B, the formation of nucleic acid duplexes on the QDs and the assembly of aptamer-cocaine complexes on the QDs could intercalate doxorubicin into the duplex domains of the resulting recognition complexes, which quenched the fluorescence of the QDs.

The third category was that the fluorescence quenchers used nanomaterials, while the fluorescent agents did not. As a fluorescence quencher, nanomaterials, especially two-dimensional (2D)

nanomaterials (such as graphene oxide) and noble metal nanomaterials (Au/AgNPs), have been widely used in biosensors. In some cases, aptamers modified by fluorophores in an unbound state could be adsorbed on the surface of nanomaterials, such as two-dimensional nanomaterials (graphene oxide and molybdenum disulfide) that behaved as a quencher [20,66]. For example, graphene oxide, a typical 2D nanomaterial, has been used in the detection field based on quenching the fluorescence of aptamers. However, there are still some problems. Physical adsorption methods prevent the sensitivity of detection from being further improved, and false signals affect the results. Zhang et al. not only solved the problem but also reported a fluorescent cocaine aptasensor based on the use of GO [66]. They used an aptamer with poly-cytosine that could be adsorbed on the surface of GO to detect cocaine because poly-cytosine DNA could be strongly adsorbed on many common nanomaterials. Moreover, cocaine could be adsorbed on the surface of bare GO to limit further improvements on the sensitivity. Tween 20-protected GO was therefore used to prevent the cocaine from nonspecific binding because Tween 20, as a nonionic surfactant, may strongly interact with GO through its hydrocarbon lipophilic group. Apart from the 2D nanomaterial, other nanomaterials, such as Au/Ag nanomaterials (seen in Fig. 2C), have also been applied in illicit drug detection [78].

The combination of various nanomaterials was beneficial for improving the analytical performance of the determination methods, such as sensitivity. Emrani et al. combined silicon nanoparticles and AuNPs for cocaine determination [68]. The aptasensor acquired the merits of AuNPs such as unique optical properties and large surface area while the silicon nanoparticles acted as amplifiers of fluorescence intensity, and increased the affinity of the aptamer towards its target relative to its complementary strand; moreover, the hairpin structure of the complementary strand of the aptamer brought the fluorophore close to the surface of the silicon nanoparticles. In the absence of cocaine, a fluorophore that is close

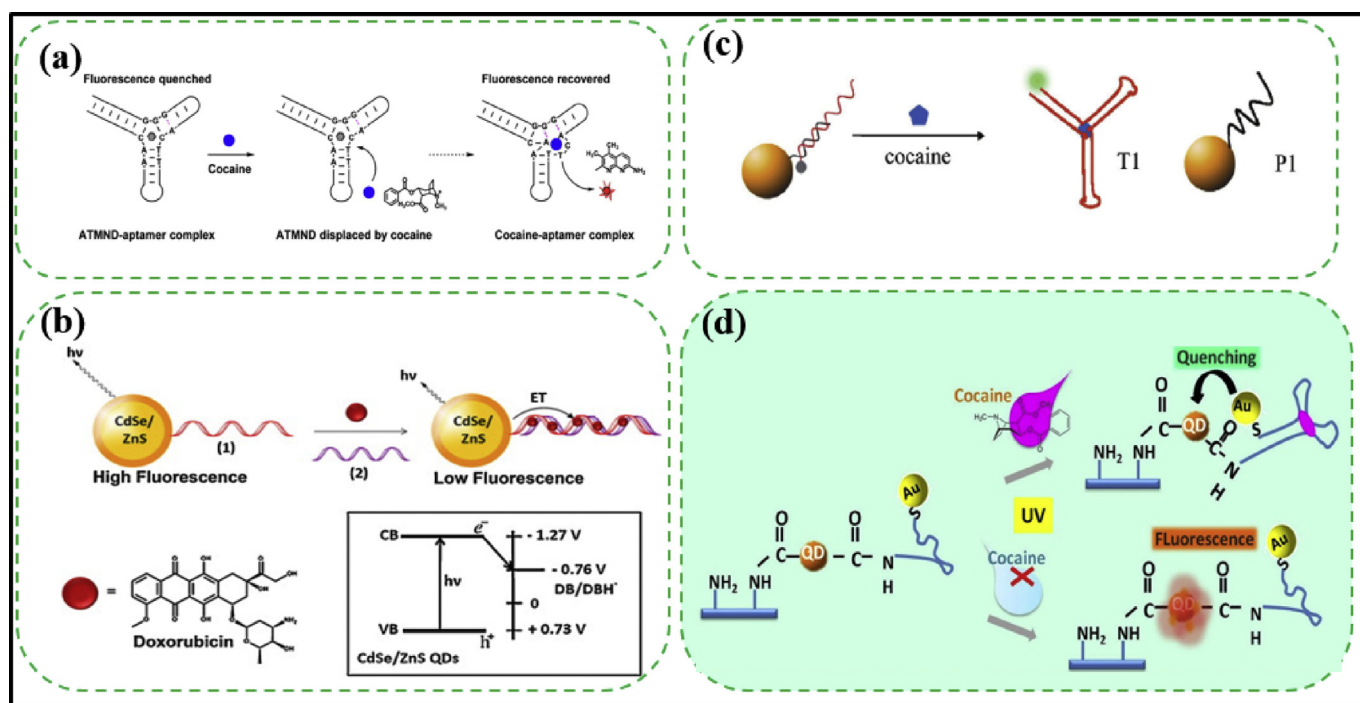**Fig. 2.** (a) Label-free aptamer-fluorophore assembly for rapid and specific detection of cocaine [69]; (b) an optical detection of cocaine based on electron-transfer quenching of DNA-functionalized QDs by doxorubicin [77]; (c) cocaine detection in blood serum using an aptasensor on AuNPs and progressive dilution [78]; and (d) an aptamer folding-based sensory platform decorated with nanoparticles for simple cocaine detection [79].

to the surface of AuNPs, results in a weak fluorescence emission. In this particular aptasensor, due to the hairpin structure that forms when in the presence of cocaine, the fluorophore was brought close to the surface of the silicon nanoparticles, causing a strong emission of fluorescence. In another example, Yan et al. reported the construction of an aptasensor by a combination of different nanomaterials (AuNPs and GO) for cocaine detection [83].

The fourth category was when both the fluorescent and quenching agents are nanomaterials. For example, Guler et al. constructed a combined platform by using QDs and AuNPs as well as a functional aptamer for a new psychoactive drug detector (Fig. 2D) [79]. For the fabrication of illicit drug aptasensors, QDs were first modified onto poly-L-lysine-coated microwell surfaces. Then, the AuNP-aptamer was bound to the QDs. The introduction of cocaine or benzoylecgonine led to a conformational change of the aptamer, which brought the AuNPs and QDs close. Hence, the extent of QD fluorescence quenching was observed when the target drug was added.

3.1.3. SERS aptasensors

SERS is an ultrasensitive analytical strategy mainly dependent on a large electromagnetic enhancement induced by a localized surface plasmon resonance of nanoscale noble metal surfaces. SERS also possesses other incomparable merits of high-resolution spectroscopic bands, low photo-bleaching and a wide range of excitation wavelengths. Currently, SERS has a wide range of applications in biomedical, environmental and forensic analyses towards small molecules, pathogens, cells and even whole living animals [84]. Recently, AuNP-based SERS sensors have attracted much more attention for the rapid detection of a variety of targets, including illicit drugs [85]. To promote analytical performance, novel shapes or structures of AuNPs have been synthesized [70].

Oroval et al. synthesized a novel shape of AuNP nanotriangles (AuNTs) and used it in SERS detection of cocaine [70]. They showed a design comprising of mesoporous silica nanoparticles loaded with crystal violet as a SERS reporter with high Raman activity and capped with an aptamer that selectively coordinated with cocaine. In this case, the cocaine resulted in the release of crystal violet, and the delivered crystal violet was detected by SERS upon cocaine adsorption on the AuNTs, which displayed an efficient electromagnetic field enhancement and high colloidal stability. We also designed a structure of Au@Ag core-shell nanoparticles for better sensitivity and developed a new biosensor based on Au@Ag for the detection of methamphetamine with SERS [59].

3.2. Electrochemical aptasensors

Electrochemical analytical techniques, as an essential branch of modern analytical chemistry, play a key role in illicit drug analysis. Electrochemical aptasensors hold the advantage of simplicity, rapid detection, high sensitivity, low cost and compatibility with use in different settings and therefore are also widely applied in illicit drug determination [57,86]. In this part, we briefly overview electrochemical aptasensors for illicit drug analysis (Table 2).

To improve the analytical performance, electrodes were usually decorated with suitable materials in the development of electrochemical aptasensors. In the last few years, various modification protocols have been explored, ranging from biological receptors such as DNA or proteins to synthetic metal ionophores. Recent reports have suggested that nanoparticle-modified electrodes can be very effective in electrochemical sensors. Therefore, we discuss this in relation to electrodes modified by different materials, mainly including nanobiomolecule-modified electrodes, noble metal nanoparticle-modified electrodes, carbon nanoparticle-modified electrodes, and some other nanoparticle-modified electrodes.

3.2.1. Nano biomolecule-modified electrodes

The most common electrochemical aptasensor was to directly modify the corresponding aptamer or DNA on the electrode. For example, Abnous et al. designed an ultrasensitive electrochemical aptasensor for a cocaine assay dependent on the H-shape structure of the aptamer and its complementary strand [95]. This aptasensor inherited merits of the gold electrode, such as a large surface area and high electrochemical conductivity, as well as excellent selectivity and sensitivity of the aptamer towards its target. Furthermore, the conjugate of the aptamer and complementary strands of the aptamer acted as a gate for the access of the redox probe to the electrode surface. In the presence of cocaine, the aptamer bound to cocaine, which left the surface of the electrode, and the gate of the H-shaped structure was opened, leading to a strong electrochemical signal.

Sometimes, to obtain better selectivity and sensitivity, new biomolecules were designed and modified on the electrode. Bozokalfa et al. reported an aptasensor for cocaine by combining the merits of an aptamer and a polythiophene bearing polyalanine homopeptide side chains (PT-Pala)-modified electrode [96]. To construct the sensing platform, PT-Pala was electrochemically modified on the electrode surface, and then the aptamer was attached to the polymer by covalent conjugation chemistry (Fig. 3A). This sensing platform had the ability to detect cocaine and benzoylecgonine.

3.2.2. Noble metal nanoparticle-modified electrodes

An electrode surface can be modified with nanomaterials, such as AuNPs, which seems to add complexity to the synthesis but improves the analytical performance of the aptasensors and provides easy functionalization. Asghary et al. developed a self-powered aptasensor for the determination of ketamine [94]. AuNPs were modified on the surface of a graphite electrode (AuNPs/graphite electrode), which endowed the electrode with a larger surface area for modification with thiolated single-stranded DNA (ssDNA). This could become a biocathode in a microbial fuel cell system (seen in Fig. 3B). This self-powered sensor was dependent on the microbial fuel cell system and acted as an efficient tool for ketamine analysis in a complex matrix. Under optimal experimental conditions, the difference between the power densities of the ssDNA-modified electrode with and without accumulated ketamine served as the determination signal with a limit of detection of 0.54 nM.

In contrast to AuNPs, Su et al. [101] used gold nanoclusters (AuNCs) as a modification and developed another electrochemical sensor for cocaine. Two-dimensional zirconium-based metal-organic framework nanosheets embedded with AuNCs (AuNCs@MOF) were synthesized by a one-pot method under moderate conditions. The optimized AuNCs@MOF nanosheets not only had good electrochemical activity, high specific surface area, and physicochemical stability but also held a strong bio-affinity towards biomolecules. Therefore, an adequate aptamer could be decorated onto the substrate modified by AuNCs@MOF nanosheets, further forming a biosensing platform that successfully monitored cocaine based on the specific binding between the aptamer and cocaine. This novel method has a great opportunity for simple and convenient cocaine analysis due to its simple operation and its merits of excellent stability, repeatability, and selectivity.

Another noble metal nanomaterial, platinum nanoparticles, was usually applied to modify the electrode for aptasensor construction [92]. Roushani and Shahdost-fard designed a voltammetric and impedimetric determination method for cocaine by using a platinum nanoparticle-modified glassy carbon electrode and rutin as a redox probe. As shown in Fig. 3C, the electrode was cocaine-responsive by decorating with platinum nanoparticles and

Table 2
Available electrochemical aptasensors for the detection of illicit drugs.

Detection method	Target	Linear range	LOD	Assay time	Ref.
ACEO	Cocaine	14.5 fM-14.5 pM	7.8 fM	30 s	[87]
DPV	Codeine	7.3 pM-7.3 nM	37 pM	45 min	[88]
DPV	Codeine	7.3 pM-7.3 nM	5.7 pM	45 min	[89]
CV	Cocaine	100−1000 nM	33 nM	60 min	[90]
DPV	Cocaine	1−8 nM	100 pM	45 min	[91]
DPV	Cocaine	1 nM-11 μM	100 pM	45 min	[92]
DPV	Cocaine	50−450 pM; 1−6 μM	5 pM	45 min	[93]
EIS	Ketamine	0−600 nM	0.54 nM	5 min	[94]
DPV	Cocaine	0.3−15 nM	0.228 nM	30 min	[95]
DPV	BE	0.5−50 μM	1.5 nM	60 min	[96]
DPV	Cocaine	2.5−10 nM	1.5 nM	60 min	[96]
DPV	Codeine	10 pM-100 nM	3 pM	30 min	[97]
DPV	Codeine	1 pM-100 nM	0.3 pM	80 min	[98]
EIS	Methamphetamine	–	–	–	[99]
DPV	opium alkaloid (codeine)	0.01−900 nM	3.2 pM	60 min	[100]

ACEO: Alternating current electroosmosis; CV: Cyclic voltammetry.
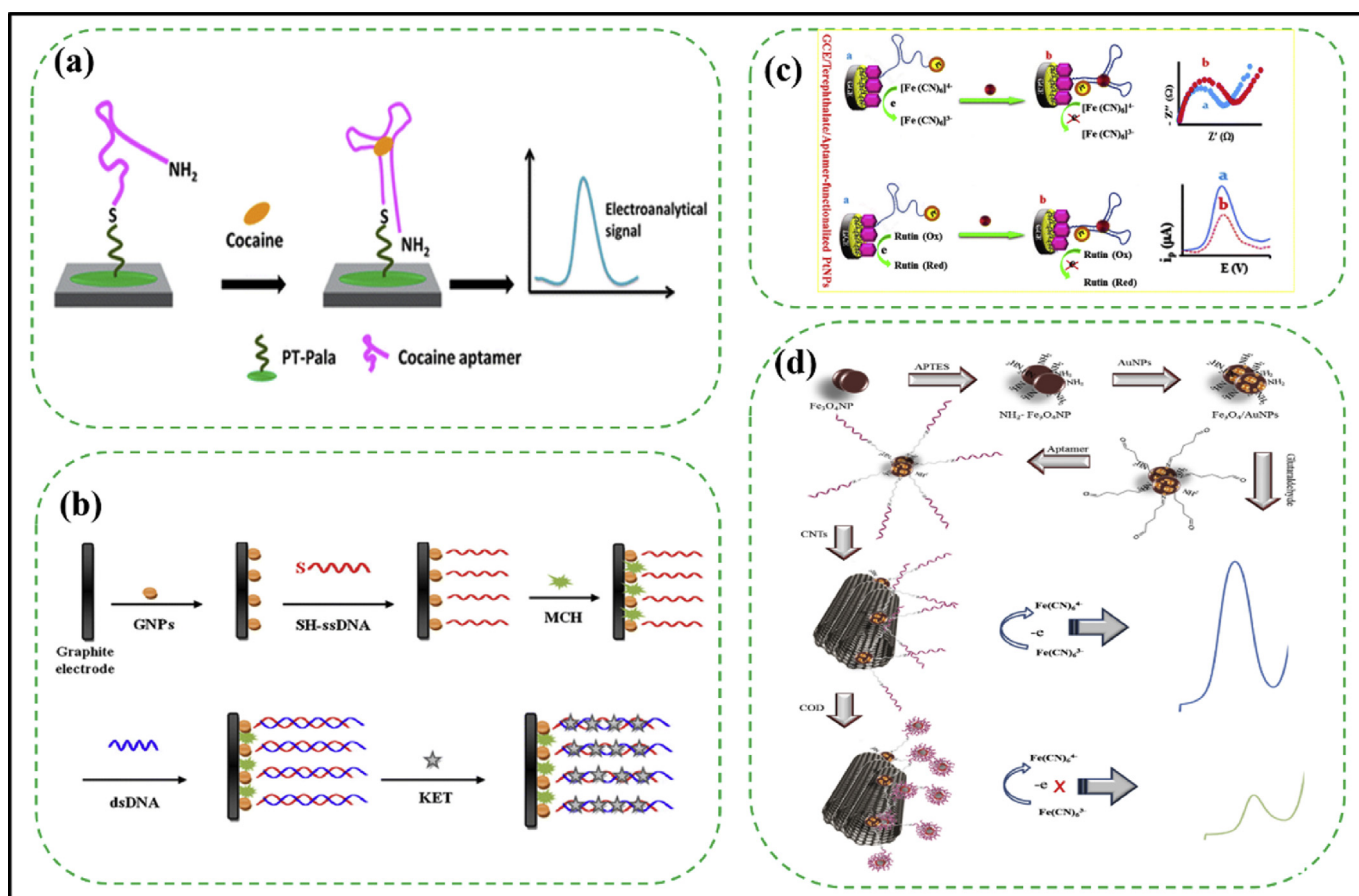EIS: Electrochemical impedance spectroscopy; BE: Benzoylecgonine.

Fig. 3. (a) Electrochemical aptasensor for a cocaine-based PT-Pala modified electrode [96]; (b) self-powered aptasensor for determination of ketamine as an anaesthesia drug in clinical serum samples [94]; (c) an aptasensor for voltammetric and impedimetric determination of cocaine based on a glassy carbon electrode modified with platinum nanoparticles and using rutin as a redox probe [92]; and (d) electrochemical aptasensor for opium alkaloid detection [100].

aptamers. The platinum nanoparticles on the electrode could accelerate the electron transfer kinetics of the rutin reduction and enhance sensitivity.

3.2.3. Carbon nanomaterial-modified electrodes

Graphene oxide (GO), a typical carbon nanomaterial, was also often used as a modified electrode material and applied in an illicit drug electrochemical aptasensor [102]. Hashemi et al. fabricated an unlabelled cocaine aptasensor that used a screen-printed carbon electrode modified by three-dimensional magnetic reduced graphene oxide (3D-MRGO)/polyaniline (PA) AuNP nanocomposite [103]. To fulfil this goal, a thiolated aptamer was bound on the surface of the nanocomposite. The signal principle of this detection method was based on the increase in the $[Fe(CN)_6]^{3-/4-}$ charge transfer resistance as an electrochemical probe under the existence of the target. A novel electrochemical cell was fabricated to collect

3D-MRGO/PA/AuNP/aptamer on the surface of the working electrode easily, which held the merits of reducing the volume of electrolyte and probe solution, obtaining repeatable responses, reusability of the screen-printed carbon electrode and user-friendly method. It was also applied to analyse cocaine in serum and urine samples to test the practical application ability of the aptasensor, and satisfactory results were obtained.

The other widely-used electrode-modified carbon nanomaterial is a carbon nanotube (CNT)-modified electrode. Using the same strategy to increase the $[Fe(CN)_6]^{3-/4-}$ charge transfer resistance as an electrochemical probe with different carbon nanomaterial-multiwall carbon nanotube-modified electrodes in the presence of illicit drug, Roushani and Shahdostfard developed a cocaine aptasensor based on conformational changes of the aptamer-modified AuNPs on an MWCNT/IL/Chit nanocomposite as the sensing system [91]. Using CNTs as a modification material, Azadbakht et al. proposed an electrochemical method for opioid analysis, specifically codeine [100]. Fig. 3D shows that their platform fabrication started by modifying NH_2-functionalized Fe_3O_4 with AuNPs (Fe_3O_4/AuNPs). CNTs were then placed on a glassy carbon electrode and modified with the Fe_3O_4/AuNPs as a signal amplifier. The proposed nanosensor integrated the advantages of the deposited Fe_3O_4/AuNPs and CNTs and the covalent attachment of the detection probe in this system. In the presence of codeine, codeine was specifically bound by the aptamer and then captured at the surface of the sensing interface, which could be used in codeine determination.

3.2.4. Other nanoparticle-modified electrodes

A variety of nanomaterials have been applied in electrode modification for electrochemical illicit drug aptasensors. For example, Wei et al. reported a ZnS nanoparticle-label-based electrochemical codeine aptasensor [88]. Taking another example, quantum dot-modified electrodes have also been reported for drug detection. Shahdost-fard and Roushani fabricated an electroanalytical tool dependent on a conformation change of the aptamer, which was covalently bound on a glassy carbon electrode covered with cadmium telluride QDs and worked as a unique modifier for cocaine detection [93].

Electrochemical sensors for illicit drugs has attracted increasing attentions. A variety of electroanalytical illicit drug aptasensors have been designed using advanced micro/nanomaterials. These highly sensitive and selective sensing platforms offer new opportunities that are simple, quick and cost-effective. Additionally, due to the synergies observed when combined with different nanomaterials, the development of an appropriate integrated analytical platform will provide novel illicit drug determination methods that are useful in both WBE and other forensic analysis fields.

4. Emerging nanotechnology for illicit drug detection

In constructing illicit drug biosensors with increased performance, some new biotechnologies have also been utilized, such as DNA technology and device integration technology, in addition to using new materials.

4.1. DNA technology

DNA biosensors provide abundant nucleic acids as materials for constructing highly ordered nucleic acid nanostructures for forming nucleic acid hydrogels and developing nucleic acid amplification technology, which has great potential for biosensing. In this section, we briefly introduce DNA technology used in the construction of illicit drug aptasensors, mainly including isothermal amplification of nucleic acid technology, nucleic acid hydrogels, and DNA sequencing technology.

4.1.1. DNA amplification

Isothermal amplification of nucleic acids is a rapid and efficient amplification technology at a constant temperature under simple conditions, such as a water bath. Since the early 1990s, many more isothermal nucleic acid amplification technologies utilizing different amplification principles have been reported [104]. Most of these techniques possess excellent sensitivity for nucleic acid testing, and some have been successfully commercialized and applied in different fields [105]. In recent years, isothermal amplification tests have been expanded to determine a wide range of targets, such as ions, small molecules, proteins, and cells [104]. Most of these targets trigger amplification reactions by using aptamers [106]. Nucleic acid amplification technology has also been used in illicit drug analysis (listed in Table 3). This chapter summarizes the isothermal amplification test used in illicit drug analysis, mainly including the strand displacement amplification (SDA) technique and rolling circle amplification (RCA) technique.

One of the most widely used isothermal amplification technologies in illicit drug aptasensors is the strand displacement amplification (SDA) technique, which combines the ability of a restriction endonuclease to nick an unmodified strand of its target DNA and the action of an exonuclease-deficient DNA polymerase to extend the 3′ end at the nick and displace the downstream DNA strand. The displaced strand serves as a template for an antisense reaction and vice versa, resulting in exponential amplification of the target. Li et al. developed a novel SDA technique for cocaine determination based on SERS [109]. Fig. 4A shows that Raman molecule-modified bio-barcode DNA and AuNPs are used to prepare Raman probes. Magnetic beads acted as the carrier of the amplification template and signal output products for circumventing the problem of high background noise that was induced by excess bio-barcode DNA. Under the existence of cocaine, two unlabelled proximity probes hybridized with each other and subsequently opened a hairpin connector probe to perform the SDA reaction, including the target recycling amplification and strand-displacement amplification. There are many available cocaine aptasensors that depend on SDA [107,108,110−112]. This assay was also successfully performed in real samples, such as human serum, with satisfying results, which confirmed the feasibility of this amplification detection method.

Some experts have tried to make modifications to SDA. Wang et al. reported an autonomous replication of nucleic acids by polymerization/nicking enzyme/DNAzyme cascades for the amplification determination of cocaine [113]. This analytical platform depended on the tailoring of a DNA template on which recognizing the target DNA or forming the aptamer-substrate complex triggered autonomous isothermal replication/nicking processes and the displacement of a Mg^{2+}-dependent DNAzyme that could catalyse the generation of a fluorophore-labelled nucleic acid as a readout signal for the analysis (seen in Fig. 4B). The target-triggered isothermal autonomous replication/nicking process on the template could form the Mg^{2+}-dependent DNAzyme tethered to a free strand consisting of the target sequence. This activated additional template units for the nucleic acid self-replication process, which could then determine the target nucleic acid sequence. According to the above concept, this amplification detection method for cocaine was based on aptamers. The modification of the cocaine-detection template by the addition of a nucleic acid sequence enabled the autonomous secondary coupled activation of the polymerization/nicking machinery and DNAzyme generation path, which led to an improved analysis of cocaine.

The other widely used DNA amplification technology in illicit drug aptasensors is rolling circle amplification (RCA) [114,115]. In its

Table 3
Summary of isothermal amplification methods based on cocaine aptasensor.

| Methods	Detection signal	Linear range	LOD	Assay time	Ref.
SDA	Fluorescence	5 μM-1 mM	5 μM	60 min	[107]
SDA	Fluorescence	2 nM-200 μM	2 nM	60 min	[108]
SDA	SERS	0.5−500 nM	0.1 nM	120 min	[109]
SDA	fluorescence	1 μM-100 mM	1 μM	30 min	[110]
SDA	Fluorescence	200 nM-200 μM	0.2 μM	16 min	[111]
SDA	Fluorescence	0.2−100 μM	190 nM	60 min	[112]
SDA-Cascade Amplification	Fluorescence	10 nM-5 mM	10 nM	−	[113]
RCA	Fluorescence	1.0−50 nM	0.48 nM	−	[114]
RCA	Electrochemical	2−500 nM	1.3 nM	−	[115]
Nuclease-based amplification	Fluorescence	2−50000 nM	2 nM	−	[116]
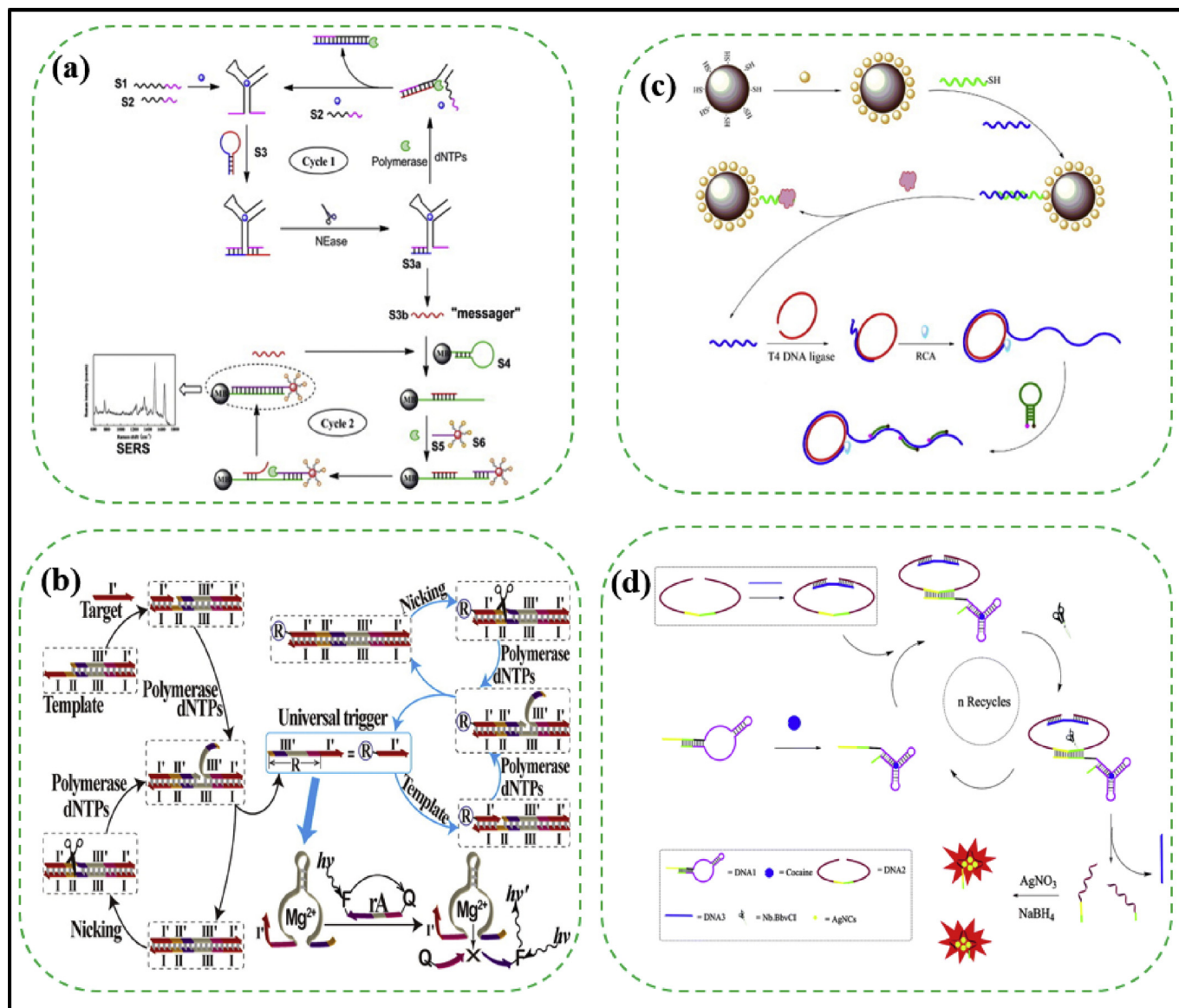
Fig. 4. (a) DNA amplification for illicit drug aptasensors. (a) SERS-dependent SDA for a cocaine aptasensor [109]; (b) SDA-cascade amplification autonomous replication of nucleic acids by polymerization/nicking enzyme/DNAzyme cascades for the amplified detection of cocaine [113]; (c) cocaine detection via RCA of short DNA strands separated by magnetic beads [114]; and (d) label-free, nuclease-based amplification and ultrasensitive fluorescence detection of cocaine [116].

original formulation, an RCA reaction involves many rounds of isothermal enzymatic synthesis in which Φ29 DNA polymerase extends a circle-hybridized primer by continuously progressing around a circular DNA probe of several dozen nucleotides to replicate its sequence over and over again. Ma et al. developed a novel determination by the combination of an aptamer and RCA for cocaine detection [114]. As shown in Fig. 4C, an aptamer was coated on AuNP-modified magnetic beads hybridized with a short DNA

sequence. When cocaine was added, the short DNA sequence was displaced from the aptamer due to the specific binding between the cocaine and aptamer. Next, the short DNA sequence was separated by the magnetic beads and used as the original RCA primer. The RCA amplicons could be detected by the generation of a fluorescence signal upon the molecular beacons hybridizing with the amplicons. Apart from SDA and RCA, there have been a few other DNA amplification technologies that have been reported for illicit drug aptasensors. An example is the use of DNA-templated AgNCs as indicators and a nicking endonuclease-assisted signal amplification method; Fig. 4D shows an unlabelled fluorescence determination of cocaine [116].

4.1.2. DNA hydrogel

Increased attention has been paid to DNA hydrogels for the construction of biosensors because they have a wide range of triggers, such as pH, ionic strength, temperature, and the presence of an electric field. They have also been used in illicit drug analysis by combining with an aptamer. Zhi et al. developed a visual detection platform for cocaine that was dependent on an aptamer cross-linked hydrogel [117]. Fig. 5A illustrates the mechanism of the colorimetric strategy. Two pieces of DNA, strand A and strand B, which were complementary to an adjacent area of the aptamer, were grafted onto linear polyacrylamide polymers for the formation of polymer strands A and B (PS-A and PS-B, respectively). When mixed in equal amounts, the polymers grafted with strand A and strand B were transparent in liquid form. When the aptamer linker was added, the aptamer initiated a hybridization of strands A and B with the aptamer sequence, thus cross-linking the linear polyacrylamide polymers. As the hybridization proceeded, the cross-linking ratio of the polyacrylamide increased, which increased the viscosity of the polymer solution. The polymer finally transformed into a gel. In the presence of cocaine, the aptamer would bind with it, which caused the dissolution of the gel because of a reduction in the cross-linking density by more favourable aptamer-target binding. If an enzyme was introduced before the addition of the aptamer, the enzyme would be trapped inside the three-dimensional network of the hydrogel (represented as pink symbols in Fig. 5A). When cocaine was added to break the gel, the enzyme was released and took part in its catalytic role for signal amplification. A cascade event was thus set in motion, whereby target binding triggered an enzymatic reaction, which could change the colour of the substrate, providing a visual cue.

Using this specific target-responsive DNA hydrogel, Ling et al. [118] combined a glucoamylase-trapped aptamer-cross-linked hydrogel with a glucometer for portable detection of cocaine. As shown in Fig. 5B, in the presence of cocaine, the hydrogel was dissolved and the glucoamylase was released, which catalysed the hydrolysis of amylose to produce more glucose for a quantitative readout using a glucometer. As shown in Fig. 5C, Mao et al. [119] also utilized a DNA crosslinked hydrogel as a target-responsive unit and gold nanorods as a multicolour signal readout circuit for visual detection of cocaine. The colour variation of the gold nanorod solution was correlated with the cocaine concentration. These simple platforms will have a wide range of applications for visual determination of different illicit drugs because the aptamer cross-linked hydrogel can be targeted to any ligand depending on the corresponding aptamer.

4.1.3. DNA sequencing technology

In recent years, nanopore sequencing as a novel DNA sequencing technology has attracted much more attention. This novel DNA sequencing technology has been applied in illicit drug analysis. Rauf et al. [120] fabricated an unlabelled nanopore aptasensor based on a target-induced strand release for cocaine analysis in real

samples. Wang et al. [121] also reported a cocaine sensor by using a combination of a single nanochannel and aptamer. These nanopore and single nanochannel-based aptasensors can specifically recognize cocaine with good sensitivity and excellent selectivity. A cost-efficient and simple to prepare sensor for rapid for and unlabelled detection in real-time were the motivating merits for a more widespread application in illicit drug analysis. Therefore, nanopore sequencing studies have provided a new method for the development of satisfactory aptasensors towards various drugs.

4.2. Engineering aptasensors for portable assay

In addition to increased efforts being devoted to illicit drug aptasensor construction, many attempts have been devoted to integrating existing analytical systems or miniaturized commercial equipment with illicit drug aptasensors into field-portable devices. Many miniaturized or portable devices, such as microfluidic chips, paper devices, and commercial devices, have been employed in the field of illicit drug aptasensors by device integration and are available for on-site detection. We discuss several engineering illicit drug aptasensors by the integration of various components.

4.2.1. Microfluidic chips

Microfluidic devices have attracted more interest from analytical scientists due to their characteristics of functional integration, high portability, short assay time, and minimal sample/reagent consumption [125]. Additionally, miniaturization minimizes the risk of sample contamination and enhances the analytical performance in comparison with those of conventional analytical tools. Therefore, microfluidic chips have been widely applied in illicit drug aptasensors [126,127]. Du et al. designed a convenient microfluidic platform by integrating an electrochemical aptasensor, which was an Au−Ag dual-metal array three-electrode system on-chip for cocaine analysis [122]. Fig. 6A demonstrates that microfluidic channels covered the glass chip and different targets were transported to the Au electrodes integrated at different positions of the chip. These electrodes were premodified by certain aptamers to capture the corresponding target. The characteristic of this microfluidic platform was that multiple targets could be recognized and detected depending on the readout signal on a certain electrode by only one electrochemical probe. $[Ru(NH_3)_6]^{3+}$, as an electrochemical probe that produced a chronocoulometric signal in this method, could quantitatively bind to surface-confined nucleic acids with electrostatic interactions; additionally, AuNPs were applied for sensitivity improvement by amplifying the detection signal. This Au−Ag dual metal electrochemical chip detector integrated with a microfluidic electrochemical illicit drug aptasensors has the advantages of simplicity, sensitivity, and selectivity and there is great potential for it to be further applied in multiplex illicit drug analysis with high integration, high throughput, and high automation.

4.2.2. Paper-based platforms

Paper materials hold the merits of abundance, low cost, simple fabrication, and portability and act as an ideal supporting material for developing sensor devices with a wide range of applications, particularly in the field of point of care diagnostics. Hashemian et al. developed a paper device for codeine detection through an aptamer immobilized on cellulose paper for thin-film microextraction of codeine from practical samples followed by electrospray ionization for ion mobility spectrometry [128]. Immobilization was based on the covalent linking of an amino-modified codeine aptamer to the aldehyde groups of the oxidized cellulose paper. The comparison of the results between their method and high-performance liquid chromatography validated the accuracy of this paper device, which
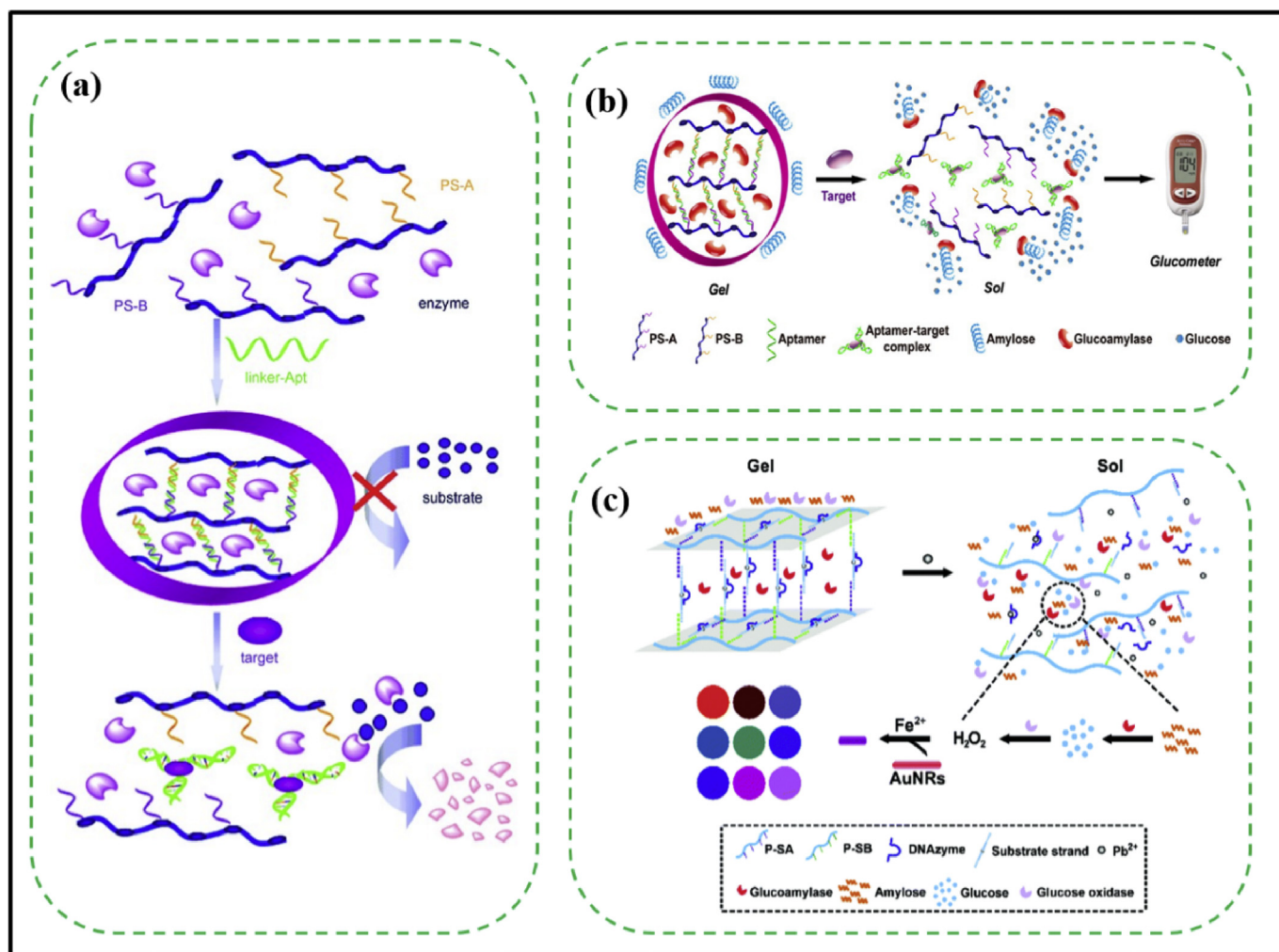
Fig. 5. Working principle of the DNA cross-linked hydrogel for illicit drug detection. (a) An aptamer cross-linked hydrogel as a colorimetric platform for visual cocaine detection [117]; (b) a target-responsive hydrogel with a glucometer readout for portable and quantitative detection of cocaine [118]; and (c) a portable visual cocaine detection method based on a target-responsive DNA hydrogel and the colour change of gold nanorods [119].

could become a choice for simple and rapid codeine analysis in a complex matrix. Fig. 6B also describes an aptamer-based paper microfluidic device coupled with AuNPs for a colorimetric determination of seized cocaine samples [123]. The mechanism was a paper strip that produced a colour change due to the aggregation of AuNPs that was induced by salt in the presence of the drug. These methods based on paper materials not only are easy to operate and have a rapid response without expensive instrumentation but also all of the paper materials utilized in the device are safe and environmentally friendly, which is a huge benefit in analysing illicit drugs in different samples.

4.2.3. Commercial portable device

Aptasensors are often integrated with some commercial miniaturized devices for on-site analysis. Portable devices with characteristics of rapid detection, easy-to-use, and low cost have been employed over a wide range of applications in daily life. A glucometer, as the most successful portable device, is widely used due to its portable size, simple manipulation, low cost and quantitative analysis. However, typically glucometers can only directly monitor blood glucose. To use glucometers for the analysis of more targets, some experts successfully combined glucometers with aptamers to analyse a variety of non-glucose targets, including illicit drug

analysis [118]. Fig. 6C shows a novel determination method using personal glucose meters and aptamers to quantify cocaine [124]. However, only a few quantized portable devices have been successful in commercialization, and many more commercial portable devices should be explored for illicit drug analysis in the future.

5. The potential of illicit drug aptasensors for WBE

Drug abuse is a global concern, and effective evaluation and control are urgently needed to fight against drug abuse. WBE is a low-cost but effective approach to evaluate drug consumption in a certain area in comparison with traditional population surveys. As an alternative and powerful analytical tool, community sewage sensors have been recently proposed to monitor biomarkers in sewage for evaluation of drug consumption and prediction of infectious disease in our group [15,37]. Compared with the conventional method, the developed sensors have minimal sample pretreatment, fast response time, and potential for on-site monitoring. A wide range of community sewage sensors have been recently reported for various target analyses in wastewater, such as illicit drugs [37], prevalence of cancer-associated prostate-specific antigens [37,129], and population biomarkers [15,130]. In particular, our group has developed a lateral flow device through a
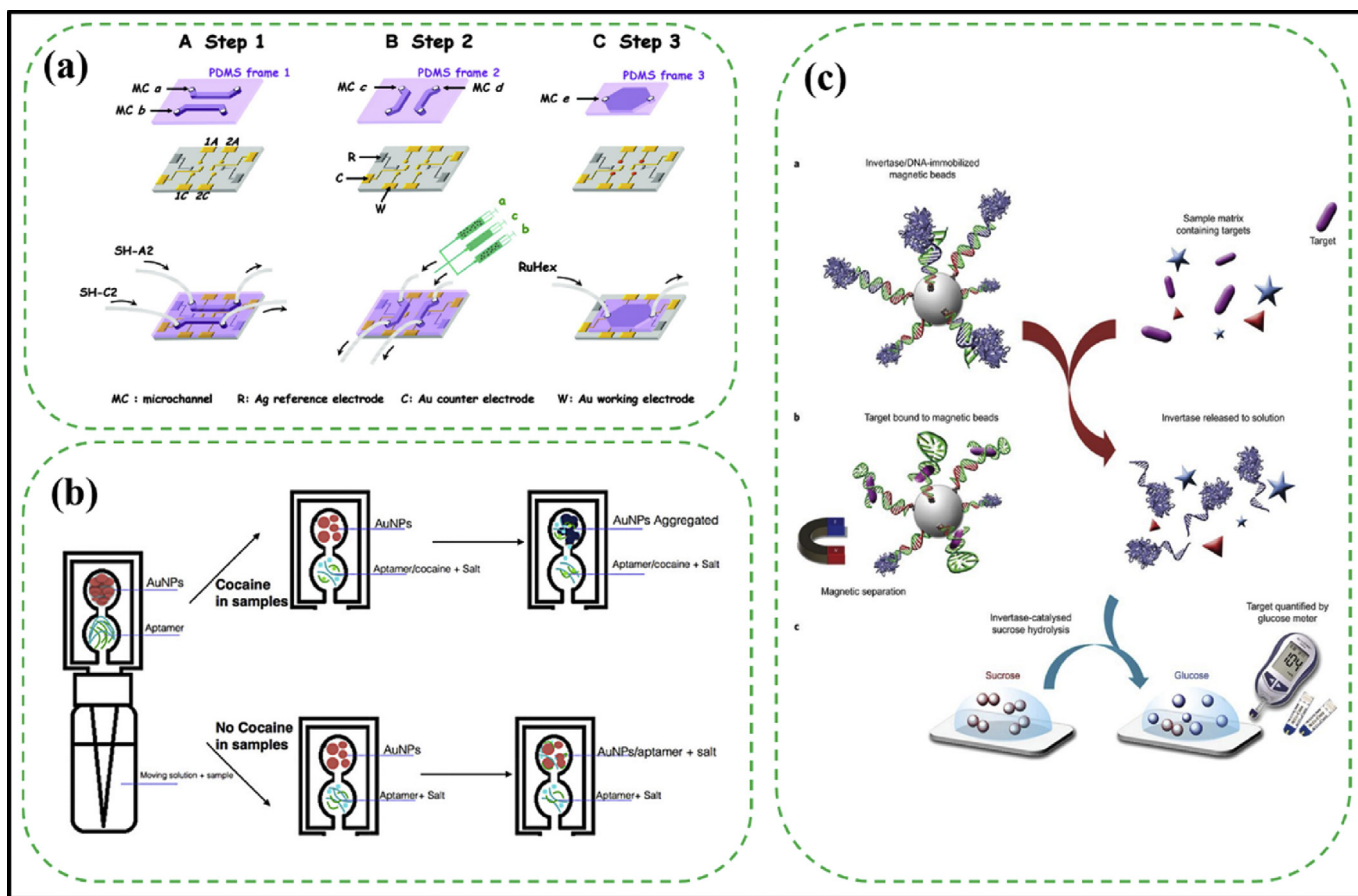
Fig. 6. Device integration used in illicit drug aptasensors. (a) Microfluidic electrochemical aptasensor integrated on-chip for the amplified analysis of cocaine [122]; (b) an aptamer-based paper device for the colorimetric determination of cocaine [123]; and (c) using personal glucose meters and functional DNA sensors to quantify cocaine [124].

combination of loop-mediated isothermal amplification methods as a portable tool to determinate human nucleic-disease markers in sewage, which has allowed for on-site monitoring of genetic pollution biomarkers for public health assessments [131,132]. Aiming at the epidemic situation of new coronavirus that is raging all over the world, we also put forward an important innovative view that paper-based biosensor depending on WBE is used to screen potential new coronavirus patients [39]. This technology overcomes the traditional detection method's inability to quickly and large-scale screen potential new coronavirus patients, and the inability to detect through PCR in areas with limited resources, which holds great significance for the timely diagnosis and early warning of new coronavirus. These works demonstrate that community sewage sensors hold great promise in playing a significant role in wastewater analysis, particularly for field testing, to rapidly inform drug consumption patterns and epidemiology studies at the community level.

As mentioned, aptasensors have attracted increasing interests in a variety of illicit drug analyses, such as methamphetamine, cocaine, ketamine, and codeine [18,59,65,69,94]. Aptasensor signals from the binding between an aptamer and drug can be output as colorimetric signals [133], fluorescence signals [58], SERS signals [59], electrochemical signals [37], and other techniques [24,134]. However, we are not sure that these aptasensors can be used for illicit drug evaluation in wastewater as an alternative and cheap community sewage sensor because of the complex composition of wastewater and low concentrations of the target drug.

To address this challenge, we have designed a novel biosensor, namely DNA-directed immobilization of aptamer sensors (DDIAS) for the assessment of cocaine consumption in wastewater [37]. As shown in Fig. 7A, thiolated ssDNA was first hybridized with an aptamer to form dsDNA and then modified on gold electrodes to control the surface density of dsDNA on the electrode and make it effectively bind with cocaine; additionally, 6-mercapto-hexane was also modified on the surface of the electrode. After optimization, DDIAS was further applied to quantify the cocaine concentration in wastewater samples from a wastewater treatment plant over seven consecutive days. The concentration pattern of the sampling week was comparable with that of mass spectrometry. Based on the determination results of DDIAS, we found cocaine consumption trends with a weekend peak based on samples collected in a wastewater treatment plant in the southwest of England [37]. The results showed that DDIAS can be quickly and economically employed in drug abuse assessment and may become an efficient approach to monitor sewage on-site and in real-time with non-technical personnel.

Another important thing is that various drugs usually are widely distributed and transported in natural water and wastewater [10,31], thus, multiple drugs can be present in the same sample. Although the aptasensors mentioned above demonstrate the ability to monitor a single drug, there are no reports of multiple illicit drug analyses. Although one could utilize several biosensors for different target determinations separately, this could be expensive and time-consuming. Considering that cocaine and methamphetamine are two of the most widely abused drugs worldwide [2,18], we developed
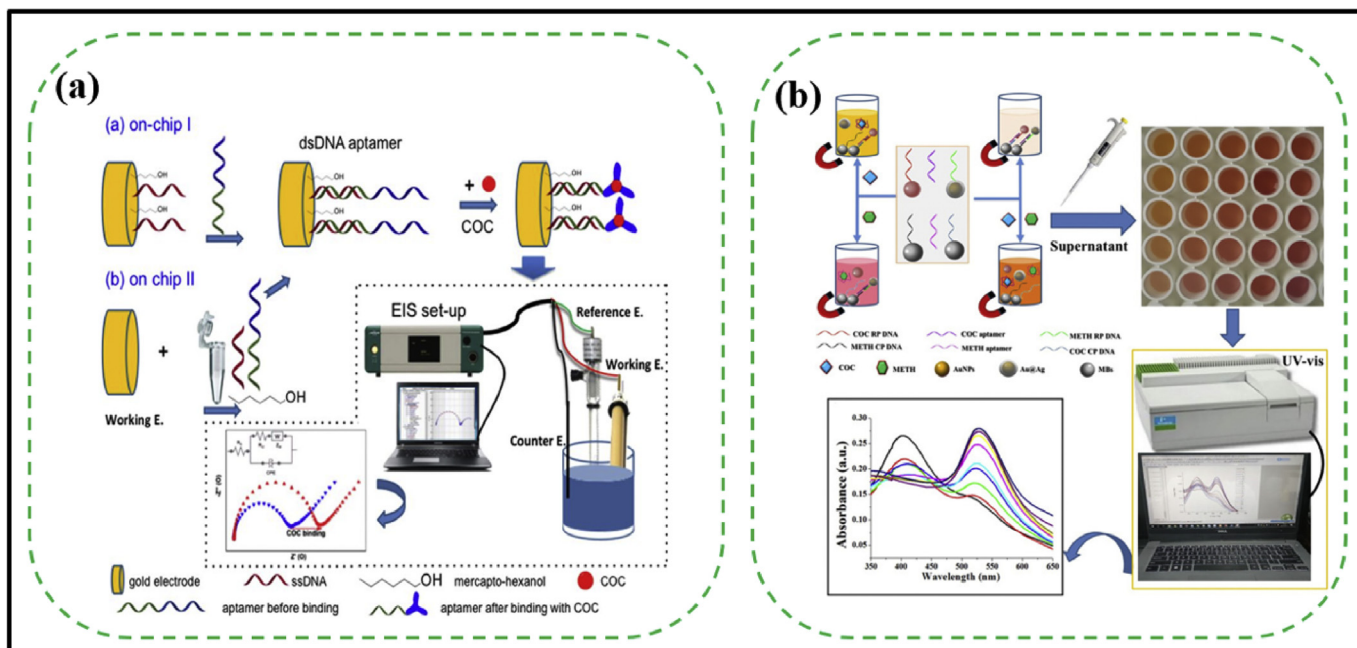
Fig. 7. Illicit drug aptasensor application in WBE. (a) Community sewage sensor towards the evaluation of drug use trends and detection of cocaine with DNA-directed immobilization aptamer sensors in wastewater [37]; (b) rapid duplexed detection of illicit drugs using a gold nanoparticle conjugated aptasensor in wastewater [62].

a single colorimetric aptasensor depending on gold nanomaterials conjugated aptamers for duplexed determination of methamphetamine and cocaine in sewage [62]. Fig. 7B shows that AuNPs and Au@Ag have been synthesized and modified with reporter probe nucleic acids for cocaine and methamphetamine, respectively. The magnetic beads were conjugated with two capture probe nucleic acids that separately targeted cocaine and methamphetamine. The respective reporter probes and capture probes were hybridized with each illicit drug-binding aptamer and then formed a sandwich structure, which could be removed by an external magnetic field. However, in the presence of illicit drugs, the special structure would be disassembled due to the high affinity of the aptamers with the illicit drugs, which caused a colour change of the supernatant. The biosensor showed the capability of duplex detection of methamphetamine and cocaine after a non-negative matrix factorization algorithm process.

We demonstrated that the sewage aptasensors can act as a potential monitoring approach for the assessment of drug consumption in a certain area by WBE. Thus, the use of biosensors instead of advanced analytical method such as LC-MS/MS has profound potential in WBE. However, there are a few challenges in addition to the biosensor development itself. These include the uncertainty associated with the continuous monitoring, instead of using daily composite samples or even grab samples. The uncertainty will increase because of more frequent data monitoring. What's worse, low analyte concentrations in combination with the complexity and unknown composition of the wastewater matrix might hamper not only the sensitive and accurate quantification but also a sound identification. For the biosensor itself, how it can cope with the complex components in wastewater is a big issue. The analytical tools for drug use trends utilize mass spectrometry-based techniques due to their robustness, sensitivity and selectivity. Various illicit drugs or their metabolites presented in wastewater can be quantified using mass spectrometry system (Tab S1). However, troublesome sample purification, costly measurements and the requirement for well-trained personnel may burden the assessment process. Alternatively, sensors may play a

important role as novel analytical tools to perform a rapid and on-site analysis of wastewater with minimal sample processing by unskilled personnel. Though sensors may have a shortage of sensitivity for the lower concentration of drugs in wastewater as well as the stability of sensors for long-term monitoring, we need to improve the performance of the sensor by utilizing a range of above-mentioned strategies. One can use signal amplification (e.g. nano-material, nanotechnology and molecular amplification) to enhance the signal to meet the target analytes. There is also an alternative to integrate a sample processing technique either with a simple enrichment of illicit drug using microfluidic techniques, for example, using a paper-microfluidic technique which does not need power and laborious facilities, to purify wastewater samples and integrate with sensors for the rapid test. This will also provide a potential to perform the assay in the field. Furthermore, the ultimate goal of WBE is to provide results in real-time, which is challenging for large-scale LC-MS/MS instrument. This data can be achieved with biosensors due to its simplicity, rapidity and portability. There are, however, several issues that need to be addressed as mentioned above, mainly including the relatively low sensitivity of biosensors, most probably low selectivity and other drawbacks to overcome. Herein, much more studies should be carried out on these aspects to make any biosensors useful in the wastewater environment. Due to fast advancements in the field, we believe that wastewater biosensor will provide a novel avenue to analyse the abuse of illicit drugs and even discover illicit drug manufacturing bases along sewage pipe networks.

6. Conclusion and perspective

The challenge of drug abuse worldwide is that it takes both time and money to combat. An aptamer is a good option for drug sensor construction due to its high specificity and highly selective binding with the target. The good stability of the aptamer offers possible uses in different environments when compared to that of an antibody-based biosensor. Combinations of various nanomaterials with aptamers produce excellent sensors for illicit drugs and

increase the analytical performance. Recent developments and applications of aptasensors for illicit drug detection have been summarized in this review, especially those using fluorescence, colorimetry, SERS, and electrochemical methods. The utilization of some new technologies, such as DNA amplification, DNA hydrogel, and nanopore technology, can improve the sensitivity and specificity, and visualization of the analytical performance of illicit drug aptasensors. Combinations of aptasensors and new integrated devices, such as paper microfluidic, and commercial biosensors devices, are conducive to the miniaturization and portability of illicit drug aptasensors, which can be easily used in complex environments. We also present several recent publications about community sewage sensors for illicit drug analysis in wastewater. Therefore, we are hoping that most of those developed aptasensors can be implanted as an alternative method to analyse wastewater for drug consumption evaluation for WBE.

However, commercial illicit drug aptasensors for wastewater have yet to be achieved. Practically, the complex matrix in real wastewater samples has a significant effect on the sensitivity and selectivity of aptasensors. The selectivity of the sensors in wastewater matrices has not been widely evaluated but is often relatively poor given the concentrations of analogues in natural waters. Apart from the interference found in wastewater samples, the aptasensor is not sensitive enough to monitor illicit drugs at the low concentrations present in wastewaters. All of these limitations hinder the further wide application of illicit drug aptasensors in WBE. Moreover, apart from the selectivity and sensitivity, one also needs to take into consideration the stability, reproducibility, robustness, ease-to-use, portability, and cost before they can be applied in practical use or for commercialization potential.

Therefore, more efforts are needed to address the major challenge of screening illicit drug aptamers with excellent sensitivity and selectivity in wastewater. In the future, we deem the following work needs to be done in the following areas: aptamers, nanomaterials, new technologies and finally commercial applications. For example, for the identification of the aptamer probes, it's essential to understand the intrinsic properties and functions between the aptamer and illicit drugs, such as binding structure [135] and kinetic effects [136], and these should be studied for further optimization. Then, according to the characteristics of the aptamer, the selectivity of the aptasensor can be improved by optimizing the stem length and different bases of aptamer [137]. Another avenue is that many more aptamers need to be developed and implemented for more drug detection because the drug market is very large, and many new drugs appear every year. For the implementation with nanomaterials and nanotechnology, the characteristics of existing materials should be further explored for better use in drug aptasensors; on the other hand, depending on the needs of drug aptasensor design, we need to synthesize new specific nanomaterials that hold wonderful optical, electrochemical, and other necessary characteristics, which ultimately will contribute to improving the analytical performance of sensors. The current progress in the field of nanotechnology shows great potential in improving some aspects of biosensors and may open up new opportunities for improvement of sensing performance. Although we reviewed some new technology, such as DNA technology and device integration technology, used in the design of illicit drug aptasensors, we further recommend incorporating other promising technologies, such as 3D printing technology, into aptasensor designs to improve their miniaturization along with their portability, robustness, stability, reusability, and/or reproducibility.

The last and most important consideration is a practical application of illicit drug aptasensors. Although the reported aptasensors are highly selective and sensitive and have excellent reproducibility and repeatability in a buffer, they may not work well when considering the complex matrix and presence of multiple drugs for in situ detection in wastewater. Therefore, more efficient and useful aptasensors for the on-site detection of illicit drugs in wastewater are an urgent need in the future. Such demand for illicit drug aptasensors before the practical or commercial application may be satisfied by paying special attention to the development of (i) a single analytical device for detecting multiple illicit drugs, (ii) robust aptasensors that can be applied in complex environments, (iii) highly selective aptasensors for illicit drugs, and (iv) portable aptasensors for on-site monitoring. Because of the rapid progress of versatile nanomaterial syntheses and the rapid occurrence of new technology, we believe that the integration of these new technologies and versatile nanomaterials into aptasensors will promote tremendous progress in illicit drug analysis to be marketed and applied in WBE in the near future.

Author contribution

Kang Mao: Conceptualization, Writing-original draft, Writing-review & editing. **Hua Zhang:** Project administration, Writing - review & editing. **Yuwei Pan:** Writing - review & editing. **Kuankuan Zhang:** Writing - review & editing. **Haorui Cao:** Writing - review & editing. **Xiqing Li:** Writing - review & editing. **Zhugen Yang:** Conceptualization, Writing - original draft, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declared no conflict of interest.

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Appendix A. Supplementary data

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