European Journal of Pharmaceutics and Biopharmaceutics 179 (2022) 79–94

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

# Nanobiosensors for detection of opioids: A review of latest advancements

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ARTICLE INFO

*Keywords:*
Detection
Opioids
Nanotechnology
Nanosensor
LOD

ABSTRACT

Opioids are generally used as analgesics in pain treatment. Like many drugs, they have side effects when overdosed and can cause addiction problems. Illegal drug use and misuse are becoming a major concern for authorities worldwide; thus, it is critical to have precise procedures for detecting them in confiscated samples, biological fluids, and wastewaters. Routine blood and urine tests are insufficient for highly selective determinations and can cause cross-reactivities. For this purpose, nanomaterial-based biosensors are great tools to determine opioid intakes, continuously monitoring the drugs with high sensitivity and selectivity even at very low sample volumes. Nanobiosensors generally comprise a signal transducer nanostructure in which a biological recognition molecule is immobilized onto its surface. Lately, nanobiosensors have been extensively utilized for the molecular detection of opioids. The usage of novel nanomaterials in biosensing has impressed researchers who work on developing biosensors. Nanomaterials with a large surface area have been used to develop nanobiosensors with shorter reaction times and higher sensitivity than conventional biosensors. Colorimetric and fluorescence sensing methods are two kinds of optical sensor systems based on nanomaterials. Noble metal nanoparticles (NPs), such as silver and gold, are the most frequently applied nanomaterials in colorimetric techniques, owing to their unique optical feature of surface plasmon resonance. Despite the progress of an extensive spectrum of nanobiosensors over the last two decades, the future purpose of low-cost, high-throughput, multiplexed clinical diagnostic Lab-on-a-Chip instruments has yet to be fulfilled. In this review, a concise overview of opioids (such as tramadol and buprenorphine, oxycodone and fentanyl, methadone and morphine) is provided as well as information on their classification, mechanism of action, routine tests, and new opioid sensing technologies based on various NPs. In order to highlight the trend of nanostructure development in biosensor applications for opioids, recent literature examples with the nanomaterial type, target molecules, and their limits of detection are discussed.

## 1. Introduction

Opioids (opiates) are a class of analgesics extensively used for pain treatment in medicine [1]. Opioid overdose is responsible for the deaths of thousands of people every year [2]. Pain relief or euphoria are the main effects of opioids as a class of controlled and illicit medications (narcotics). Opioid dependence and abuse are an epidemic, as revealed by a dramatic rise in fatal overdoses [3]. A total of 22 opioids and 22 opioid metabolites can be frequently found in the effluent of wastewater treatment plants as well as surface waters all over the world. Analytical methods make it possible to identify opioids such as morphine, codeine, methadone, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrilidine

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https://doi.org/10.1016/j.ejpb.2022.08.017
Received 7 June 2022; Received in revised form 28 July 2022; Accepted 27 August 2022
Available online 5 September 2022

(EDDP), which is a metabolite of methadone [3].

As a result of their activities at the opioid receptors located within the respiratory neuronal network of the brainstem, opioids can potentially cause life-threatening respiratory depression and even death [4]. Opioid receptors can be divided into three types; μ, κ, and δ opioid (MOP, KOP, and DOP). The newly discovered nociceptin/orphanin FQ opioidpeptide receptor (NOP) is regarded to be a non-opioid branch of the opioid receptor family [5]. Alkaloids, semi-synthetic, and synthetic substances are the three types of opioids defined by their synthesis mechanism [6]. Opioids may be classified by their actions as agonists, partial agonists, or antagonists. Agonists could interfere with a receptor to maximize the receptor's reaction [7]. Antagonists attach to receptors but do not induce any functional response, and inhibit an agonist from reacting with that receptor [8]. The general classification of opioids is summarized in Fig. 1.

Current methods for opioid monitoring are commonly carried out by urine or blood tests. However, saliva and hair are also utilized as biological sources. Dipstick tests, microcrystalline tests, immunoassays, and the main spectroscopic techniques such as mass, Raman, infrared, ultraviolet (UV), and some chromatographical procedures are currently used for opioid detection [9,10]. Urine opioid tests are immunoassays that detect morphine and codeine in general. These tests identify additional opioid substances depending on the cross-reactivity of the individual antibodies used in the assay, which varies significantly among manufacturers and laboratories [10,11]. Laboratory testing is critical in identifying opioid use and evaluating individuals with opioid intoxication. To achieve drug specificity and high detection sensitivity, laboratories combine immunoassay and chromatographic methods [12].

Nanotechnology is a part of our daily and scientific life by developing smart and novel nanosized structures for different approaches [13–16]. In a particular way, nanotechnological materials are the transducer surfaces of the developed biosensors to enhance their sensitivity and selectivity with the aid of their high specific surface area to volume ratios. These nanomaterials-based biosensors could be named nanobiosensors or nanosensors in the literature [17–19]. Depending on whether these nanomaterials are functionalized or not, they have a wide range of uses, making them ideal for drug detection. Because of their large surface area, functional groups, and unique properties that allow for sensitive and selective sensing, such functionalized nanoscale materials have lately benefited from identifying illegal drug fingerprints [20,21]. Nanoparticle (NP)-assisted biosensors [surface-enhanced Raman scattering (SERS), optical, electrochemical, etc.] have many advantages over the existing test procedures with label-free, real-time, multimodal, easy fabricated, faster, inexpensive, and low detection limit analysis that allows for adapting clinical laboratory routines [22–24]. However, there are still several hurdles that researchers in the field of nanobiosensing must overcome before this vision can become a reality [25]. To begin with, there is presently no excellent biosensing or nanobiosensing approach poised to become the gold standard for drug monitoring, and the several examples shown here represent a diverse range of technologies and sensing strategies for varied applications.

Despite these drawbacks, there are various advantages to using these detecting platforms, and sensor techniques, as high sensitivity, selectivity, and the ability to analyze crude biofluids are all factors to consider. The medical business, specifically therapeutic medication monitoring, needs a high degree of standardization, control, validation, and translation into the clinical. Because systemic, chronic, or local toxicities can affect the efficacy of nanobiosensors in drug monitoring applications [26,27]. These limitations should be considered in developing nanomaterial-based biosensors for commercialization and

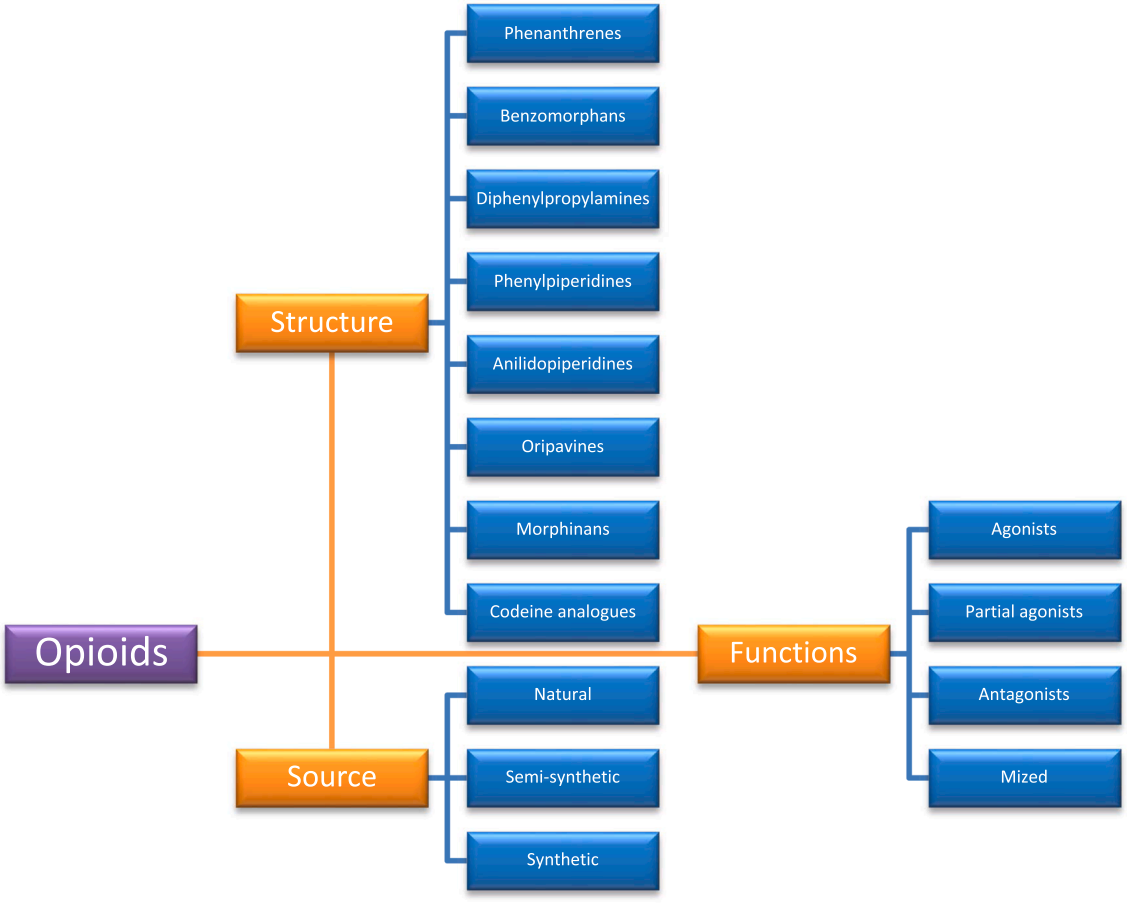


**Fig. 1.** Classification of opioids regarding their structure, source, and functions.

translation into the clinical. Given this background, this review aims to emphasize general information, routine monitoring techniques, and novel nanotechnological developments in opioid detection.

## 2. Opioids

### 2.1. Structure, Classification, and mechanism of action

Opioids are considered a class of compounds derived from the poppy plant (*Papaver somniferum*)[28]. This pharmaceutical class includes prescription pain relievers and criminal substances like heroin. Some opioids are derived from plants, such as the opium poppy (opiates). In contrast, others are synthetic or 'man-made.' Buprenorphine (Suboxone® or Subutex®), fentanyl, oxycodone (OxyContin® or Endone®), morphine, tramadol, methadone (Biodone Forte® and Methadone Syrup®), Codeine (Panadeine Forte®, Nurofen Plus®, and Panadeine®), and heroin are examples of common opioids (Fig. 2). O-desmethyl tramadol, U-47700, MT-45, AH-7921, furanyl-fentanyl, and acetyl fentanyl are examples of recently developed psychoactive opioid compounds [29–32].

Opioids can be classified based on their origin (*Natural*, *Semi-synthetic* and *Synthetic)* [8,33] and possible interaction with target receptors [34]. Opioids can bind to and activate different classes of GPCRs (G-protein coupled receptors), including the μ (named after morphine, as it binds with it), δ (named after vas deferens, the tissue from which it is isolated), and κ (named after ketocyclazocine, as it binds with it) receptors.

### 2.2. Mechanism of action

Different genes control the three types of receptors associated with opioids. Upon the receptor's activation by binding a ligand, a part of G-protein is released, diffuses in the target cell membrane, and reaches its target. After the activation, cyclic adenosine monophosphate (cAMP) (secondary messenger) gets inhibited, and in response to that, the target cell activates the protein kinases and gene transcription factors, causing a wide range of physiological effects, including analgesia [35]. The varying affinity of opioids towards various receptors is the reason for the variable effect of opioids. Opioids have also shown antagonistic action towards NMDA (N-Methyl-D-Aspartate) receptors. Opioids activate the pain pathways of serotonin and noradrenaline from the brain stem. The stimulation of NMDA receptors leads to tolerance development and neuropathic pain [36,37].

Both μ and δ opiates suppress the spinal nociceptive reflex, reducing the spine's neuronal activity via noxious stimuli. This leads to manipulating the pain behavior that is organized by the supraspinal region [38,39]. As a classic opioid, morphine has been used to manage pain caused by malignant tumors for a long time, and has been the topic of many clinical tests. It has also been used to treat pain not caused by cancer, and serves as a comparator for evaluating the performance of more modern opioids, which are proven equally effective. Morphine undergoes a metabolic process in the liver. Morphine-6-glucuronide, also known as M6G, is an effective painkiller, whereas morphine-3-glucuronide, named as M3G, can induce neuroexcitatory effects. After the final dose, these metabolites are excreted through the bile, then by the feces, and the urine. Deposition of metabolites in people with renal impairment may create side effects that require adjusting the dose or switching to a different opioid [40]. A novel opioid, tapentadol, acts on both neuronal reuptakes of noradrenaline and μ receptors. This medication has no identified analgesically active metabolites, which is a feature that confers several benefits, in particular when compared with tramadol [40,41]. Therefore, when selecting the most appropriate opioid and procedure for individuals, it is necessary to consider a number of factors. Drug efficacy can be affected by pharmacokinetics and pharmacodynamics, as well as various physiological barriers, side-effect profiles, administration routes, and patient-related factors [35].

## 3. Opioids toxicity and monitoring

### 3.1. Opioids toxicity and complications

Opioid drugs may be implemented intravenously (IV), intramuscularly (IM), orally, topically, and inhaled. Overdose of opioids can cause toxicity problems globally. Once ingested, the liver converts opiates into inactive molecules, which are then eliminated by the kidneys. Because opiates like buprenorphine and fentanyl are lipid-soluble, they tend to

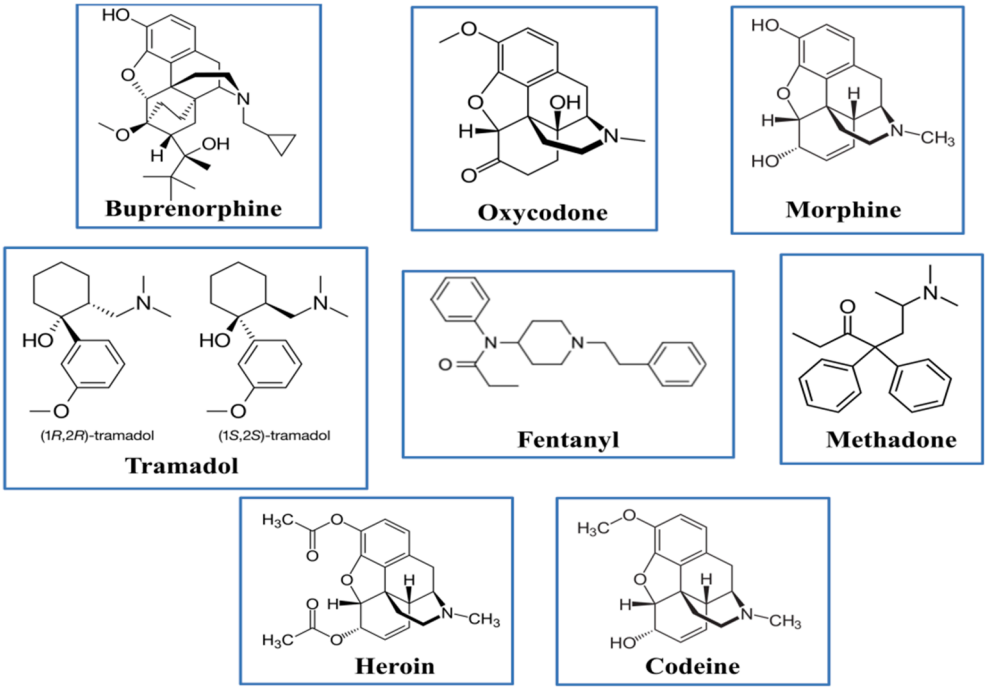


**Fig. 2.** Chemical structure of different types of opioids.

redistribute into fatty tissues and have a longer half-life. For example, the hepatic microsomal CYP2D6 enzyme converts codeine to the active metabolite morphine [42,43]. Failure in this metabolic pathway can result in the accumulation of oxycodone and hepatic or renal impairments. Methadone could be accumulated in plasma with limited ranges due to the unaffected excretion [44].

On the other hand, opioids are also known for their neurotoxic effects due to overdoses. Opioid-induced neurotoxicities result in a multifactorial syndrome that develops within a few days and could be ended with death. To suppress opioid-induced neurotoxicity, rehydration should be implemented [45]. A prospective investigation of demographic profile and drug consumption habits in patients with chronic pain, which lasted for 2 years, revealed that 90 of the 100 patients were using opioids [46]. Due to this usage, it was found that substance abuse is higher in the population suffering from chronic pain than in the general population [46,47]. Such evidences demonstrate that enhanced prescription for opioids in patients for pain management is responsible for increased drug abuse, its toxic effects, and other side effects.

Another major concern with opioid usage as the drug is the difficulty in maintaining its efficacy for the long duration of treatment due to induced tolerance in the person [48,49]. Opioids have shown important immunomodulatory effects, such as suppression of cellular immune function, low resistance towards infection, suppression of cytokine expression, phagocytes' activity, antibody, and natural killer cells [50,51]. Opioid usage by men also impairs their hormonal activity in them, as they suffer from various sexual dysfunction symptoms (such as erectile dysfunction), low energy levels, and depression. This may also be associated with hypogonadism [52,53]. Similar effects are also observed in females, such as a decline in mineral density of bone, depression, and sexual dysfunction [54].

Hyperalgesia (enhanced pain sensitivity) is also an outcome of long-term usage of opioids. This effect is also associated with the apoptosis induced by opioids, which leads to the decline in the population of gamma-aminobutyric acid (GABA) neurons and results in the impairment of neural circuits of the spine [55]. Bradycardia is also induced by the parasympathetic stimulation caused by opioid usage [56]. Because opioids cause molecules to accumulate in various body organs over time, it is likely that their chronic use had life-threatening consequences [57]. A study in Australia showed that synthetic opioids caused pulmonary edema in 82.6% of cases, aspiration of vomitus in 30.4%, and acute bronchopneumonia in 17.4% of cases [58]. Also, opioids are one the most crucial reason for respiratory depression and death from their actions at the opioid receptors within the brainstem respiratory neuronal network [4]. As a result, it is critical to take opioid misuse seriously and devise effective detection measures to limit its potentially lethal effects.

### 3.2. Opioid detection

The structural classification of opioids reveals that most of them share the morphine skeleton. Therefore, developing methods to determine these compounds accurately is very difficult. In addition, due to the low molecular weight of opioids, the immunocomplex created by opioids on the sensor's surface does not induce a noticeable response on the transducer device [59–61]. Hence, to develop immunosensor for opioids, broadly specific antibodies with higher sensitivity are required; thus, immunosensor development is difficult for opioids. Multiple detection platforms evolved to detect clinical, biological analytes, such as lateral flow immunoassay, magnetic nanoparticles (MNPs), chemiluminescent materials, fluorescent microspheres, etc. [62–64]. Each of them is affected by some disadvantages; for example, colloidal gold particles can only be employed for semi-quantitative estimation, despite their cost-effectiveness and stability [65].

Many nanosensors have been lately developed for the detection of opioids. For example, a carbon nanotube (CNT)-based immunosensor was fabricated to detect heroin (abused opioid) in real-time. The developed nanosensor could detect heroin at very low concentrations, with a limit of detection (LOD) of about 15 pg $mL^{-1}$ [66]. A glassy carbon electrode was coated with carbon nanotubes to fabricate an immunosensor to detect noscapine and morphine [67,68]. Aptameric sensors were also developed for opioid detection based on electrogenerated chemiluminescence and show a LOD of 1.0 nM [69]. Additionally, a colorimetric sensor array based on unmodified gold nanoparticles (AuNPs) was developed in order to accurately detect a variety of structurally distinct opioids in aqueous media, including morphine, codeine, oxycodone, noroxycodone, thebaine, tramadol, and methadone [70]. Also, a new electrochemical sensor based on a selective carbon paste electrode was applied for norepinephrine detection. The sensor was modified with 6-amino-4-(3,4-dihydroxyphenyl)-3-methyl-1,4-dihydropyrano[2,3-c],pyrazole-5-carbonitrile (ADPC) assisted $Fe_2O_3$@$CeO_2$ core–shell NPs. To investigate its electrochemical properties and analyze the redox properties of the modified electrode, chronoamperometry, differential pulse voltammetry, and cyclic voltammetry were conducted [71]. Nowadays, giant magnetoresistive sensors coupled with super paramagnetic NPs offer a sensitive, low-cost, and quantitative solution for morphine monitoring in saliva at the point of care (POC). The results can be transmitted via a smartphone-controlled platform, delivering a dynamic range up to 500 ng/mL and a low LOD of 3.78 ng/mL [72].

## 4. Nanotechnology for opioid sensing

The combination of biosensors and nanotechnology opens new windows toward optimizing the performance of a biosensors. Nanobiosensors have many potential benefits over traditional sensing devices, one of which is greatly enhanced sensitivity. To compare these detectors more effectively, it is necessary to define sensitivity; in fact, the ability of a sensor to detect an analyte is influenced by several significant factors. The internal sensitivity of a sensor is calculated as the ratio of the change in the sensor output signal to the change in the measured property (probably due to the presence of the analyte linked to the sensor) [73,74]. The sensitivity is related to the lowest analyte concentration that can be detected, known as the limit of detection (LOD) or minimum detectable concentration (MDC) (or mass). It is the is the lowest quantity of a substance that can be distinguished from the noise level [73].

It is challenging to detect drugs since they have a short half-life in the human body. Drug detection and quantification become increasingly complex over time. Although traditional spectroscopic and chromatographic procedures such as mass spectrometry (MS), (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE), may detect illegal substances in the system reliably [75], there are still many limitations to using these methods; all the above-mentioned analytical methods, due to the use of complex and heavy equipment, are restricted to laboratories. For decades, GC and MS techniques have been used to identify and quantify opioids in clinical laboratories. MS is usually coupled with GC to improve its performance, selectivity, and accuracy (GC–MS) [76,77]. Expensiveness is one of the major issues that prevents using this technique for drug identification, such as opioids. Also, GC–MS cannot distinguish between drugs with similarly fragmented ions. The results obtained in these cases are often very unreliable [78].

Another technique widely used in laboratories for drug detection is HPLC. Despite its higher accuracy compared to other techniques, its expensive equipment and high maintenance cost limit its use for drug detection [79]. Besides, the sensitivity of HPLC for certain compounds is often low, and some cannot be monitored as they are irreversibly adsorbed [80]. Recently, CE has emerged as an economical, promising, and practical approach for monitoring opioids and their metabolites in biological samples. Compared to GC–MS, CE instrumentation is simpler to operate and less expensive. Compared to HPLC, CE shows higher efficiency and resolution, easier sample preparation, uses a smaller sample amount and nonpolluting chemicals, and is cheaper [81]. One of the

most important limitations of CE is that its sensitivity is somewhat lower than the other mentioned analytical techniques used for opioid analysis in biological samples [82]. Furthermore, these methods have a variety of drawbacks in terms of selectivity, sensitivity, and stability, they are inconvenient, pricey, and time-consuming.

Biosensors, on the other hand, can overcome the disadvantages above. The development of affinity nanobiosensors has accelerated in recent years, and their use in forensic investigation has been proven through various applications [83]. Owed to their outstanding optical and electrical characteristics, NPs can be used to develop various analytical methods, including colorimetry, SERS, chemiluminescence, and scanometry [84]. Despite developing a broad spectrum of nanobiosensors over the last two decades, the future aim of high-throughput, low-cost, and multiplexed clinical diagnostic lab-on-a-chip instruments has yet to be fulfilled. When the analyte is applied to the sensor system, the phenomenon of SPR allows for easier visual observation of their color shift. Nanomaterials with tunable luminescent emission, for instance, QDs, carbon quantum dot (CQD), graphene quantum dots (GQDs), upconversion nanoparticles (UCNPs), carbon dots (CDs), and nanoclusters (NCs), can replace traditional fluorescent indicators like cyanine-3 (Cy3), fluorescein (FAM), carboxy-X-rhodamine (ROX), carboxytetramethylrhodamine (TAMRA), and others (Fig. 3) [85–88].

Some advanced, efficient, and state-of-the-art physicochemical analysis methods like SERS, colorimetric, optical, electrochemical, fluorescence, lateral-flow assay (LFA), and enzyme-linked immunosorbent assay (ELISA) have been used to detect opioids [83,89]. A summary of the newly (last 4 years) nanosensors described in the literature to detect opioids is given in Table 1.

### 4.1. Oxycodones

Since 1917, oxycodone ($C_{18}H_{21}NO_4$) has been administered in clinical studies. Parenteral oxycodone was primarily administered to treat severe postoperative pain, while oxycodone and acetaminophen combinations, such as oxycodone and acetaminophen, were prescribed to manage mild pain. Controlled-release oxycodone has been used to treat cancer-related pain as well as chronic non-cancer-related pain since its inception [102].

The use of $CoFe_2O_4$ NPs modified carbon paste electrode (CFCPE) has been proposed as a quick and responsive electrochemical sensor for the concurrent determination of codeine and oxycodone. Results demonstrate that the CFCPE significantly improves the electrocatalytic operation of the electrode surface for codeine and/or oxycodone oxidation. The concentrations of codeine and oxycodone were measured using differential pulse voltammetry (DPV) under optimal circumstances, with detection limits of 0.02 μmol $L^{-1}$ and 0.05 μmol $L^{-1}$, respectively, and a linear range of 0.06–38 μmol $L^{-1}$. The developed sensor showed a rapid reaction time (90 s) and a high selectivity in the presence of $Mg^{2+}$, $K^{+}$, $Na^{+}$, $Ca^{2+}$, $SO_4^{2-}$, $Cl^{-}$, $PO_4^{3-}$, $NO^{3-}$, sucrose, citric acid, glucose, uric acid, morphine, and methadone. The electrode was suitable for examining diverse genuine samples due to its excellent stability, sensitivity, and low detection limit [103]. Oxycodone is a powerful opioid that is commonly administered as an analgesic. Despite its efficacy in treating mild to severe chronic pain and tumor pain, oxycodone usage carries the risk of addiction, overdose, and death. In emergency treatment, rapid and reliable assessment of oxycodone blood concentration would allow for individualized analgesic dosage and monitoring and rapid diagnoses of suspected overdose. However, following the dosage of oxycodone, many metabolites are always present in the blood, and no electrochemical information on any of these metabolites has been reported so far. For the first time, oxycodone and its two primary metabolites, oxymorphone and noroxycodone, were studied electrochemically using Nafion-coated SWCNT and SWCNT electrodes. Both electrodes were able to selectively determine oxycodone in the presence of oxymorphone and noroxycodone. The Nafion/SWCNT sensor had a LOD of 85 nM to detect oxycodone and a linear

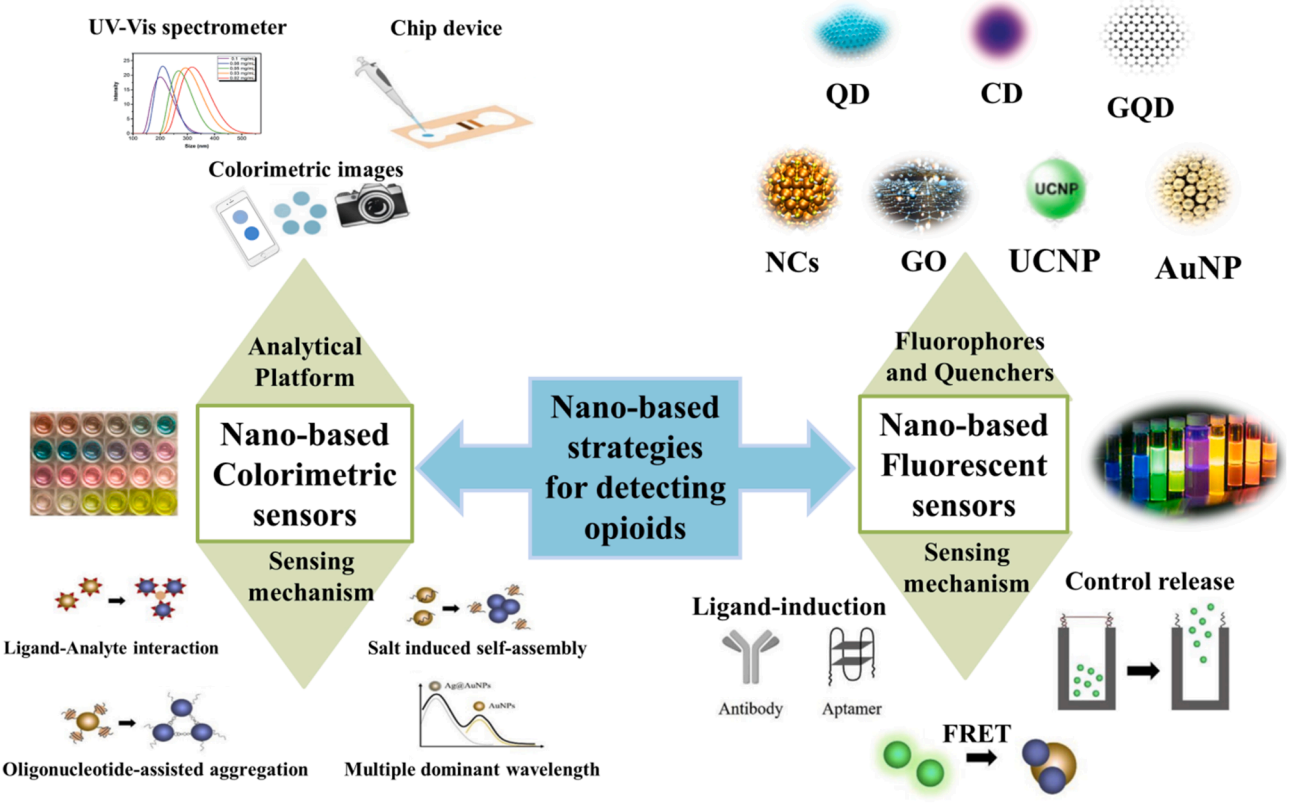


**Fig. 3.** Different nanostrategies for the detection of opioids.

**Table 1**
Newly developed nanosensors for opioid detection in the last 4 years (2018–2022).

| Nanomaterial | Sensor Type | Target | LOD | Reference |
|---|---|---|---|---|
| monolayer molybdenum disulfide (MoS2) | Optoelectronic | μ-opioid receptor | Lower than 1 nM | [24] |
| $BaFe_{12}O_{19}$ NPs | Voltammetric | Morphine | 0.02 μM | [90] |
| Pt-Pd-doped NiO NPs | Electrochemical | Nalbuphine, tramadol | Nalbuphine: 0.9 nM Tramadol: 50.0 nM | [91] |
| Molecular imprinted polymeric nanofilm | Plasmonic nanosensor | Cocaine | 0.1 μg/L | [92] |
| AuNPs | SERS | caffeine, oxycodone, methadone, and morphine | 5 pg $mL^{-1}$ | [22] |
| $CeO_2/Al_2O_3$ Nanocomposite | TRD-PM-$CeO_2/Al_2O_3$ | tramadol hydrochloride | $5.0 \times 10^{-11}$ mol $L^{-1}$ | [93] |
| CuO decorated polyaniline nanocomposite | Electrochemical impedance spectroscopy | butorphanol | 1.38 μg/L | [94] |
| NiO-CNTs nanocomposite on the glassy carbon electrode | Electrochemical | Fentanyl | 0.01 μM | [95] |
| Silver NPs on zinc oxide | SERS | Oxycodone | 90 ng $mL^{-1}$ | [96] |
| Molecularly Imprinted Polymer Zn/$La^{3+}$ MOFs | Electrochemical | Buprenorphine | 1.08 ng/mL | [97] |
| Transdermal microneedle integrated with platinum (Pt) and silver (Ag) wires | Electrochemical | Fentanyl | 27.8 μM | [98] |
| Graphene oxide and DNA aptamers | DNA Aptasensor | Tramadol hydrochloride | 1.04 nM: serum and 2.56 nM: phosphate buffer saline (10 mM PBS) | [99] |
| nanoporous gold | square wave voltammetry | Cocaine | 21 nM | [100] |
| ZnO/$Fe_3O_4$/Carbon MNPs-based hexagonal nanotubes | Electrochemical | oxymorphone and heroin | 3.5 nM for oxymorphone and 4.7 nM for heroin | [101] |

range of 0.5–10 μM in buffer solution [104].

To accelerate the development of molecular imprinting innovation in the separation and analysis of oxycodone, researchers developed a molecularly imprinted polymer (MIP) based on magnetic graphene oxide (MGO) and carbon dot nanoparticles (CDs), MIP@MGO/CDs, using ultrasonic-assisted dispersive solid-phase microextraction and HPLC. The adsorption isotherms of MIP@MGO/CDs followed the Langmuir model, with a highest adsorption capacity of 99 mg/g and a 3.39 imprinting factor. The LOD (calculated as 3x (S/N)) was 0.80 ng/mL in the linear range of 1–2000 ng/mL under the optimum circumstances attained by a central composite design utilizing the response surface technique. Average oxycodone recoveries ranged from 92.50 to 103.20% in human urine samples, with a RSD lower than 3.65% [105].

### 4.2. Morphine and its derivatives

Morphine ($C_{17}H_{19}NO_3$) is the most prevalent alkaloid in opium. Morphine is the gold standard or benchmark of analgesics administered to alleviate severe or acute pain and suffering in therapeutic practice. Morphine, on the other hand, is prohibited from athletic doping. Morphine can reduce discomfort throughout a high-intensity sporting event, which might affect performance [106].

In a study, GQDs functionalized with antimorphine were applied to develop a novel turn-on fluorescence nanosensor to detect morphine. The covalent functionalization of GQDs with antimorphine yielded antimorphine-GQDs. Upon morphine addition, the fluorescence intensity of antimorphine-GQDs increased, leading to a low LOD of 0.06 μM [107].

Due to its outstanding features and advantages, graphene quantum dots (GQDs), a novel carbon-based nanomaterial, have received much interest. Their features include chemical inertness, fluorescence properties, easiness of functionalization with biological molecules, low toxicity, and biocompatibility with excellent photostability and photoluminescence [108–110].

Fluorescent GQDs have been synthesized and used as nanosensors to distinguish and determine two of the most frequently used narcotic substances, morphine and methamphetamine, with detection limits of 1.48 and 0.5 μg/ml, respectively. This low-cost sensing device has several advantages, including a quick reaction time (less than1 min), nontoxicity, and a low LOD [111].

The sol–gel process was used to prepare alumina NPs and a film of nanoaggregate aluminum atoms on a silicon surface, which were then thermally grown. These NPs exhibit high electron conductivity on a carbon paste electrode surface, hence were used to develop a morphine nanosensor. The linear response range and LOD were determined to be 0.1–550 and 0.03 μmol $L^{-1}$, respectively. The suggested sensor was effectively applied to determine morphine in real samples like human urine and drug [112].

In another study, square wave voltammetry (SWV) was applied for the voltammetric detection of morphine by utilizing a new ionic liquid modified NiO/CNTs carbon paste electrode (CPE). Using SWV, the LOD of morphine was determined to be 0.01 μM [113]. On the other hand, Zare et al. synthesized MgO/SWCNTs via a simple precipitation process. A CPE was modified with the MgO/SWCNTs nanocomposite and 1-methyl-3-octylimidazolium tetrafluoroborate (MOCITFB) and then used for the determination of morphine in drug samples. At the surface of the modified electrode, MgO/SWCNTs/MOCITFB/CPE, the morphine oxidation signal followed a linear calibration curve, displaying the linear range from 3.0 nM to 320 μM, with a LOD of 0.8 nM. Furthermore, the MgO/SWCNTs/MOCITFB/CPE system was effectively employed for injected samples to determine morphine, with a recovery rate ranging from 98.41 to 102.49% [114].

To detect morphine, a sensitive and green CQDs-based fluorescence immunoassay (C-Dots-FLISA) was established. Anti-morphine antibody was conjugated with C-dots containing amine using glutaraldehyde as a coupling reagent. The linear range was from $3.2 \times 10^{-4}$ to 10 mg/L ($R^2 = 0.992$) under optimum circumstances, with a LOD of $3 \times 10^{-4}$ mg/L [115].

Afsharmenesh et al. developed a nanocomposite comprising ZnO/CNT and 1-methyl-3-butylimidazolium bromide that acted as supersensitive detector on a CPE for the voltammetric measurement of morphine. Using cyclic voltammetry, a sensitive analytical technique was devised based on the direct relationship between the morphine concentration and the oxidation peak current. The LOD obtained for morphine was 0.06 μmol $L^{-1}$, with a linear range from 0.1 to 700 μmol $L^{-1}$ [116].

Bastami et al. described a new colorimetric test based on silver citrate-coated Au@Ag NPs (Au@Ag) as a nanosensor to determine morphine with the naked eye (Fig. 4). The new sensing system based on optical methods relies on the Au@Ag NPs aggregation in the presence of morphine, leading to a visible change in color from light yellow to brown. Utilizing low and high power ultrasonic irradiation, Au@Ag NPs were synthesized via two different procedures, and they led to linear ranges of 0–30 and 0–50 μg/mL, with LODs of 0.055 and 0.100 μg/mL, respectively [117].

A morphine detection dipstick was designed based on an AuNP-labeled single-chain fragment variable (scFv) antibody. The scFv antibodies for morphine were developed in Escherichia coli HB2151

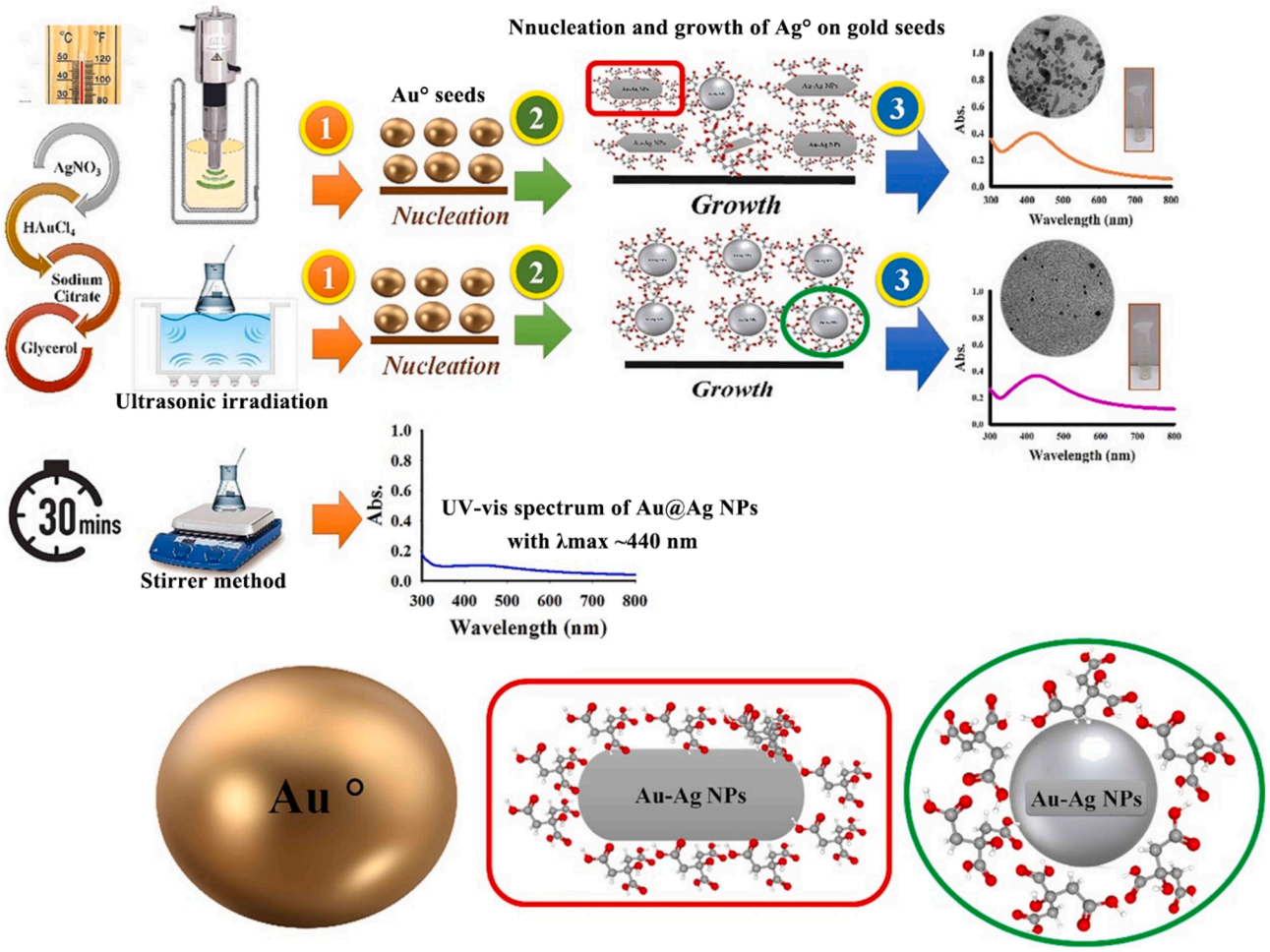


**Fig. 4.** Synthesis approach for Au@Ag NPs. First step: Au nucleation like a seed; Second step: Ag nucleation and growth on the gold seeds'' surface. Third step: Au@Ag NPs'' UV–vis spectrum and max 440 nm (no noticeable peak was seen in the lack of ultrasonic irradiation (stirrer technique)). Reproduced from ref [117] under the terms of the creative Commons Attribution License (available at https://creativecommons.org/licenses/by/4.0/).

utilizing a phage display-based antibody library. The AuNP-labeled morphine scFv was employed as an optical immunoprobe in a dipstick. The scFv capacity to identify free morphine was determined using a competitive dipstick test. The detection range was found to be 1–1000 ng $mL^{-1}$ with a LOD of 5 ng $mL^{-1}$, while the $IC_{50}$ value for morphine was 14 ng $mL^{-1}$. This scFv antibody optical dipstick kit could precisely attach to free and analogs morphine in a solution in less than 5 min, hence it has great potential for on-site screening of a genuine sample in saliva, urine, and blood [118].

A nanosensor based on the fluorescence quenching of functionalized CdS-QDs via differences in the chirality of the diverse functionalization types was proposed as a new way of chiral molecule identification. L-morphine and D-methamphetamine were detected and determined using D- and L-cysteine functionalized CdS-QDs as fluorescent probes. It was found that upon addition of D-methamphetamine and L-morphine to D- and L-cysteine functionalized CdS-QDs, their fluorescence was effectively suppressed. The magnitude of the fluorescence quenching was proportional to the concentration of L-morphine and D-methamphetamine solutions, following the Stern-Volmer equation. Consequently, functionalized CdS-QDs may be employed as a straightforward, rapid, inexpensive, and responsive nanosensor for morphine and methamphetamine detection [119].

In another work, carbon nanohorns with high surface area and coated with Pt-NPs (CNHs@PtNPs) were used to develop a highly sensitive electrochemical sensor to measure MDMA and morphine simultaneously. The CNHs@PtNPs nanosystem with 3D structure showed strong electrocatalytic activity against MDMA and morphine under the optimum conditions. The electrode displayed good sensitivity for MDMA and morphine with a broad linear range (0.05–25.4 μmol $L^{-1}$) and a LOD of 0.018 μmol $L^{-1}$ and 0.02 μmol $L^{-1}$, respectively, at potentials of 1.0 V and 0.2 V (vs. SCE) [106].

Soltanabadi et al. developed a morphine detector based on a quartz@Au-layer biosensor. For such purpose, a modified CPE was prepared via mixing a hydrogel as an absorbent polymeric matrix. The sensor showed a LOD of 0.25 ng $mL^{-1}$ and a quantification limit (LOQ or QL) of 0.25–2500 ng $mL^{-1}$. This detection platform is quick (460 s) and requires a minimal sample volume (1 μL), hence can be applied in clinical tests [120].

To quantitatively assess morphine, a magnetic resistance sensor system with good stability and high sensitivity (LOD of 0.1 ng/ml) was fabricated [121]. Moreover, to quantify monoactyl morphine, a competitive indirect fluorescence-based immunoassay with high sensitivity was employed [59]. A similar type of sensor was also developed to detect papaverine and morphine by employing a Column-based immunoaffinity approach [122]. Besides, a morphine detection platform was designed based on an electrochemiluminescence, label-free method. This sensor utilized the voltammetric principle, DNA as probe and gold as electrode [123].

### 4.3. Cocaine

Cocaine is a stimulant of the central nervous system (CNS) and the

world's most widely misused drug after cannabis. The pressure on law enforcement agencies (LEAs) and healthcare systems and the accompanying social and economic concerns make illicit drug-related crimes a major concern [124].

Mao et al. developed a non-aggregation core–shell Au@Ag NP-based colorimetric biosensor to detect cocaine and methamphetamine. The nanobiosensor included a reporting probe coated on Au@Ag NPs with a particular single-stranded DNA sequence, a capture probe linked with magnetic beads, and an illegal drug-binding DNA aptamer. With little interference from other illegal substances, this sensing technology could determine methamphetamine at a concentration as low as 0.1 nM (14.9 ng $L^{-1}$). Methamphetamine in various concentrations was spiked into urines, and the biosensor recovered 83.1% of the time. Likewise, the biosensor showed a high sensitivity for detecting cocaine as well [125].

Tavakkoli et al. developed a nanoporous gold (NPG) electrode as a sensitive nanobiosensor for detecting cocaine via immobilizing the 5′-disulfide-functionalized end of an aptamer sequence on NPG, and then conjugating the 3′amino-functionalized end to 2,5-dihydroxybenzoic acid (DHBA) as the redox probe. The conformational changes of the aptamer from an open unfolded to a closed conformation in cocaine reduced the space between the electrode surface and DHBA, hence increased electron transfer efficiency. The developed aptasensor showed linear responses in the concentration ranges of 0.05–1 and 1–35 μM, with a very low LOD of 21 nM, when measured through the square wave voltammetry (SWV) method [100].

Many applications, for instance, initial diagnosis and testing of the drug, need selective and ultrasensitive molecular detection at the sub-nanomolar or nano level. Wang et al. designed a high-sensitivity sensor for cocaine based on specific DNA aptamers and a nanochannel. The single nanochannel-aptamer-based biosensor can selectively detect cocaine in a broad concentration range, from 1 nM to 10 μM, in which a linear relationship between the goal concentration of cocaine and the output ionic current was found [126].

Bouvarel et al. described the online coupling of a monolithic molecularly imprinted polymer (MIP) with nano-liquid chromatography to selectively detect cocaine and its primary metabolite, benzoylecgonine, in complicated living specimens. After evaluating several synthesis conditions, a monolithic MIP was generated into a 100 μm internal diameter fused silica capillary utilizing trimethylolpropane trimethacrylate as a cross-linker, methacrylic acid as a functional monomer, and cocaine as a template. A linear calibration was established between 100 and 2000 ng $mL^{-1}$. In plasma and urine samples, LOQ values of 14.5 ng $mL^{-1}$ and 6.1 ng $mL^{-1}$ were attained, respectively [127].

Another study discussed the development of a sensor for detecting trace quantities of cocaine using electrochemical impedance spectroscopy (EIS) and nanoMIPs (Fig. 5). The nanoMIPs sensor detected cocaine with a LOD of 0.24 ng $mL^{-1}$ (0.70 nM) and in a linear range from 100 pg $mL^{-1}$ to 50 ng $mL^{-1}$ [128].

A straightforward method for developing paper-based analytical devices (PADs) for the simultaneous SERS and electrochemical analysis of cocaine specimens has also been described. For such purpose, a 2-μm-thick Au film was grown by depositing AuNPs on office paper to generate nanosized gold tracks, constituted mainly by Au (1 1 1) fcc planes as SERS transducers and electrodes. Compared to a standard Au electrode, the improved device had a Raman-scattering enhancement factor of $3 \times 10^6$, a 15-fold larger electroactive area, and a 2.6-fold lower charge-transfer resistance. Furthermore, these PADs have been effectively employed in forensics to monitor and evaluate a confiscated sample of street cocaine, analyze its chemical profile, and simultaneously detect paracetamol, caffeine, and levamisole adulterants [127].

GO has been employed as a nanomaterial in biological detection by quenching the fluorescence of aptamers. For instance, to detect cocaine, an aptamer containing poly-cytosine (poly-C) DNA can be adsorbed on the surface of GO. Cocaine may be adsorbed on the outsider of naked GO

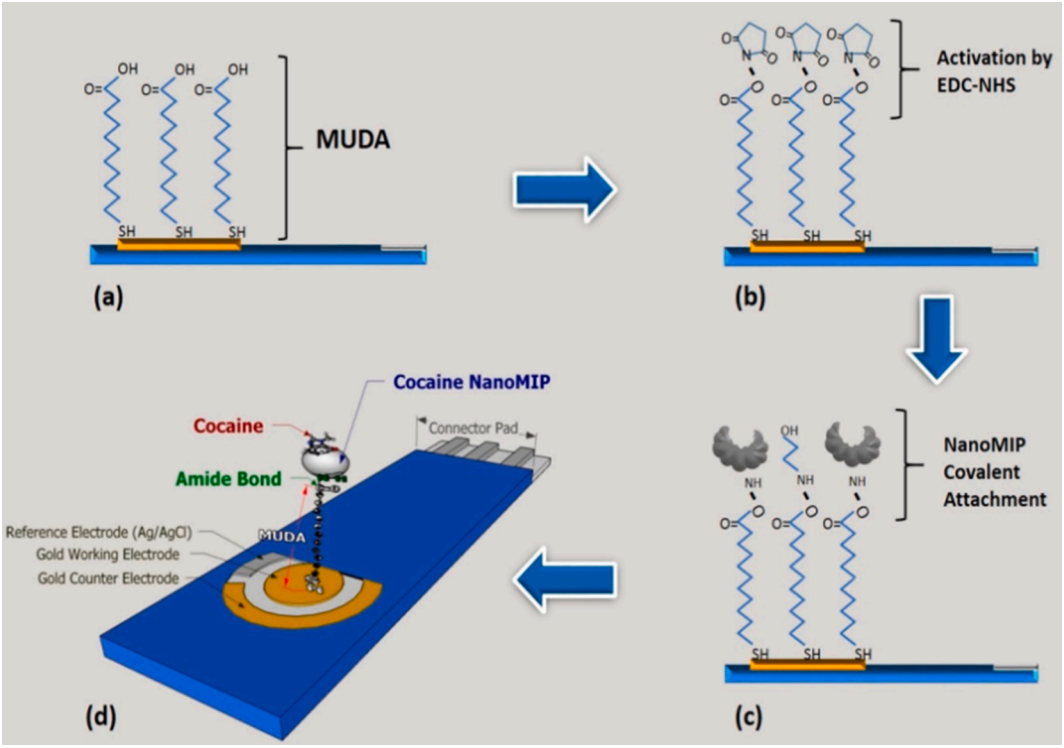


**Fig. 5.** Steps for mounting 11-mercaptodecanoic acid (MUDA) molecularly imprinted polymer NPs (nanoMIPs) to the gold surface of the working electrode (DPR C220AT, DropSens). a) synthesis of self-assembly monolayer (SAM); b) activation of the carboxylic group by 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-Hydroxysuccinimide (EDC-NHS) group; c) covalent attachment of nanoMIPs through the coupling of amine; (d) A three-dimensional representation of the completed nanoMIPs sensor for cocaine detection. Reprinted from ref [128] under the terms of the creative Commons Attribution License (available at https://creativecommons.org/licenses/by/4.0/).

to restrict the enhancement of sensitivity. Nevertheless, some issues remain, since physical adsorption methods hinder future improvements in detection sensitivity, and erroneous signals impact the results. In this regard, a nanobiosensor for cocaine detection has been fabricated by Shi et al. using a GO-aptamer and tween 20, a nonionic surfactant with a lipophilic hydrocarbon group interacting strongly with GO. To prevent cocaine from binding nonspecifically, Tween 20-protected GO was utilized, and the LOD increased to 2.45 pM. Their results demonstrate that the developed method is promising for such analytical purposes [129].

In another study, nanoporous anodic alumina (NAA) was used for implementing "molecular gates" for detecting cocaine. The NAA support was functionalized with a short single-stranded DNA (ssDNA) and loaded with a fluorescent reporter (rhodamine B). The pores were subsequently occluded via hybridization of a particular cocaine aptamer. The calculated LOD for this nanosystem was $5 \times 10^{-7}$ M [130].

In another research, a sensitive aptamer-based impedimetric sensor for cocaine detection was developed. A nanocomposite comprising AgNPs and a dendrimer was employed to change the screen-printed electrode (SPE) surface. The cocaine-binding aptamer was then coupled to the modified dendrimer/AgNP/SPE, resulting in a highly sensitive and specific layer for the detection of this drug. A LOD of 333 amol $L^{-1}$ and linearity from 1 fmol $L^{-1}$ to 100 nmol $L^{-1}$ (with two linear ranges) were obtained. Its excellent sensing qualities indicate that this aptamer-based assay could also be used to detect other targets in this field [131].

A potentiometric sensor based on MIP NPs prepared via solid-phase imprinting process was also used for cocaine detection. The MIP NPs were synthesized via polymerization in water and UV-initiated in organic solvent, and all of them displayed a strong affinity for cocaine, with dissociation constants between 0.6 and 5.3 nM. This biosensor can detect cocaine in blood serum samples at concentrations ranging from 1 nM to 1 mM [132].

Researchers used a target-induced strand releasing technique to create a label-free nanopore biosensor for quick and extremely sensitive cocaine identification in saliva and human serum samples. A prehybridized cocaine aptamer was used in this bioassay. A linear relationship between the concentration of target cocaine and output frequency of DNA nucleotides was observed in the range from 50 nM to 100 μM, with a low LOD of 50 nM [133].

Azizi et al. described an aptasensor generated through the physical vapor deposition method, with captured DNA trapped on the surface of an indium tin oxide (ITO) electrode modified with AuNPs. To monitor cocaine, DNA was coupled with carbon dots, and the developed system was treated with thionine to make an oxidation–reduction redox sensor. The developed nanobiosensor demonstrated a dynamic detection range from 10 to 70 pM and a LOD of 0.26 pM [134].

A novel fluoro-graphene-plasmonic nanohybrid aptamer-based fluorescent nanoprobe for cocaine has also been developed. The LOD was found to be 4.6 nM (1.56 $ng.mL^{-1}$), the reaction time was about 2 min, and the selectivity was outstanding against cocaine metabolites and other drugs [135]. To accelerate the chemical reaction and eliminate the matrix effects, another aptasensor was designed based on the monitoring capability of the sensor surface and alternating current electrokinetics effects. The analysis has a sample-to-result time of 30 s, a LOD of 7.8 fM and a linear response for cocaine in a standard buffer from 14.5fM to 14.5 pM; also, for serum cocaine detection, a special buffer was utilized, and a LOD of 13.4 fM for cocaine spiked in human serum was experimentally proven (equivalent to 1.34 pM cocaine in the serum sample) [136].

In another experiment, a self-assembled 2D AuNPs film was synthesized via seed growth and functionalized with CTAB surfactant to be used as a SERS substrate. A rapid and efficient pretreatment strategy to isolate and purify cocaine in human urine in 3 min was developed based on Ultra Performance Liquid Chromatography (UPLC), which was demonstrated to minimize the interference of complex biological components, enabling to detect cocaine concentrations ranging from 10 ppm to 0.1 ppm [137].

Thioflavin (T) is a small-molecule fluorescence indicator that can be linked to the anti-cocaine aptamer to boost the fluorescence signal. Cocaine detection can be performed in a few seconds using a "mix-and-detect" method based on the fluorescence shift caused by competitive binding between the two molecules. This label-free approach was highly sensitive to cocaine, with a LOD of 250 nM [138]. Similarly, Mao et al. synthesized core–shell Au@AgNPs via seeds growth method for methylamphetamine (MAMP) SERS detection. The new biosensor had an excellent logarithm linear correlation with MAMP concentrations ranging from 0.5 to 40 ppb and a low LOD of 0.16 ppb [139].

A colorimetric sensor based on the combination of AuNPs and aptamers, named GAPTA, was also developed. It exhibited exceptionally promising performance in terms of cocaine detection, with a sensitivity of 0.2 nM. The optimal electrolyte and aptamer concentrations were found to be 55 mM and 118 nM, respectively, and the GAPTA sensor had a LOD of 0.97 nM [140].

Another aptamer-based biosensor was developed using an evanescent wave fiber substrate. The semi-log calibration curve showed a working range of 10–5000 μM, with a LOD of around 10.5 μM. The entire process took 16.5 min, while the interval of detection was 6.5 min (Fig. 6) [141].

### 4.4. Codeine

Codeine (3-methylmorphine) is an opioid and a mu-opioid receptor agonist (MOR), and serves an analgesic effect through hyperpolarizing CNS neurons, followed by suppression of the release of nociceptive neurotransmitters and greater pain tolerance owing to lower neuronal excitability [142,143].

Nia and colleagues developed a lanthanum doped feathers-type ZnO nano-flower. An innovative, cost-effective, and straightforward technology was provided to concurrently detect diclofenac and codeine utilizing a modified carbon paste electrode ($La^{3+}$-ZnO/CPE). The electro-analytical determination of codeine was investigated using $La^{3+}$-ZnO/CPE over a wide concentration range with a LOD of 0.01 μM [144].

In another study, researchers combined HPLC with $Fe_3O_4$/rGO/Ag nanosystem (as a nano-sorbent) to detect codeine and morphine in biological samples. For codeine and morphine, the LOD were 2.1 ng $L^{-1}$ and 1.8 ng $L^{-1}$, respectively, with an enrichment factor of 1000 [145].

A disposable Nafion-coated SWCNT network electrode was also developed, which demonstrated an easy charge separation and electron transfer between interfering anions and positively charged opioids, as well as a significantly decreased matrix effect in human plasma. For codeine and morphine, LODs of 0.277 and 0.071 μM were found, respectively [146].

Multi-walled carbon nanotubes (MWCNTs) were carboxylated with $H_2SO_4$/$HNO_3$ (3:1) and then functionalized with *Ferula gummosa* aqueous extract. The functionalized nanomaterial was tested as a sorbent for the simultaneous microextraction of morphine and codeine, and the LOD and LOQ were determined to be 1.0 and 3.3 ng/mL, as well as 0.75 and 2.47 ng/mL, respectively [147].

A novel electrochemical platform based on carbon black paste electrode modified with hierarchical porous carbon and α-cyclodextrin was developed for the simultaneous measurement of codeine and dipyrone. Linear concentration ranges from 0.5 to 38 μmol $L^{-1}$ and from 0.46 to 35 μmol $L^{-1}$ were found using the SWV assay method, with a very low LOD of about 9.5 nmol $L^{-1}$ and 9.0 nmol $L^{-1}$, respectively [148].

A sensor for both codeine and acetaminophen was developed using a simple galvanic substitution process among metal ions and porous silicon (Psi). In the presence of 0.1 M hydrofluoric acid solution, a bimetallic nanostructure of Au-CuNPs was adsorbed on the porous silica surface. Dynamic linear ranges from 0.06 to 0.6 μM were found, with a LOD of 0.30 0.35 μM for acetaminophen and codeine, respectively. Furthermore, recovery experiments on genuine materials, for instance, human blood plasma, urine, and pills, were performed [149].

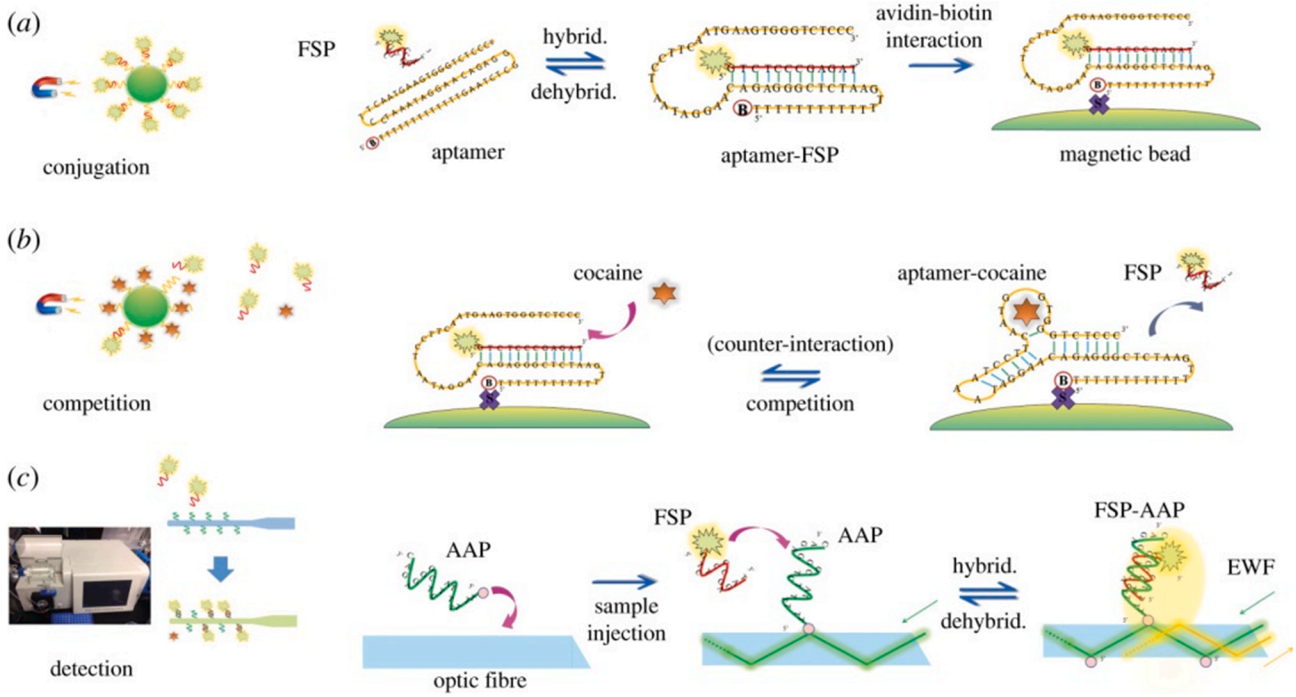


**Fig. 6.** An evanescent wave optic fiber (EWF) biosensor is used in an aptamer-based cocaine bioassay. (a) Magnetic-based fluorescence-labeled short DNA probe (FSP) aptamer formation; (b) competing phase for cocaine detection and generating FSP; (c) sensing process to measure the produced FSP by fluorescence via evanescent wave excitation on the fiber's surface. Amino-modified anchor DNA probe (AAP) is a complementary probe to anchor FSP on the fiber surface, while FSP is a fluorescence-labeled sensor for quantification. Reprinted from ref [141] under the terms of the Creative Commons Attribution License (available at https://creativecommons.org/licenses/by/4.0/).
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Khairy et al. reported the simultaneous detection via electroanalytical sensing of acetaminophen, caffeine, and codeine using $CeO_2$NPs modified screen-printed electrodes. $CeO_2$-SPEs were used at different concentrations, leading to a low LOD of 0.051, 0.043, and 2.4 μmol $L^{-1}$ for acetaminophen, codeine and caffeine, respectively [150].

### 4.5. Methadone

Methadone is a synthetic opioid beneficial for treating opioid addiction and alleviating mild-to-acute pain. It is used to relieve chronic pain and addiction to heroin and other opiates [151,152]. In an interesting study, morphine and methadone were simultaneously measured using a highly sensitive electrochemical sensor based on a graphite electrode modified with thioglycolic acid decorated cadmium selenide (CdSe)-doped GO. Under optimal conditions, the current vs. concentration calibration curve showed two linear ranges: from 0.1 to 20 μM and from 20 to 323 μM for methadone, and from 0.05 to 350 μM for morphine, with a LOD of 0.03 and 0.04 μM for methadone and morphine, respectively [153].

Functionalized CQDs were also synthesized and combined with MWCNTs as innovative modifiers to improve the characteristics of a pencil graphite electrode (PGE). The current response and methadone concentration displayed a linear relationship between 0.1 and 225 μM with a LOD of 0.03 μM under ideal experimental conditions [154].

### 4.6. Tramadol

Tramadol ($C_{16}H_{25}NO_2$) is an opioid pain medication used to treat moderate to moderately severe pain. Tolerance to the drug may occur due to frequent or prolonged use. Tramadol inhibits lung and heart function by acting as a CNS depressant. People who use extremely high dosages of tramadol (far more than what it is advised) may cease breathing completely and die from an overdose [155,156]. electrospun carbon nanofibers modified screen-printed electrode (SPE) were used to design a fast, accurate, and selective sensor for tramadol detection. A linear range of 0.05–100 nM was found under optimal conditions, and the LOD and LOQ were determined to be 0.016 nM and 0.05 nM, respectively [157].

To develop an amplified tramadol electrochemical sensor, the surface of pencil graphite electrode (PGE) was modified with CuO-NPs and polypyrrole (pPy). The activity of PGE/CuO-NPs/pPy electro-catalytic for tramadol oxidation determination was outstanding, with a 4.55-fold increase in oxidation current. The PGE/CuO-NPs/pPy was used for tramadol measurement via square wave voltammetric technique. The linear range was found to be 5.0 nM–380 μM, with a LOD of 1.0 nM. In addition, the PGE/CuO-NPs/pPy was successfully applied for tramadol detection in drug samples [158].

The goal of other investigation was to devise a method for finding the most often misused prescription drugs in a lean cocktail. To attain this aim, gas chromatography (GC) with flame ionization detection (FID) was used together with a dilute-and-shoot sample preparation procedure to achieve this goal. All the analytes were well separated within 19 min after the chromatographic technique was optimized and completely verified. The responses were linear over the concentration range from 2.5 to 1000 μg $mL^{-1}$, and the LOQ and LOD ranged from 2.5 to 55.0 μg $mL^{-1}$ and from 1.25 to 2.50 μg $mL^{-1}$, respectively [159].

A novel effective electrochemical sensor for sensitive and quick measurement of nalbuphine and tramadol analgesic drugs was developed based on cobalt oxide/ionic liquid crystal/carbon nanotubes modified-CPE in the presence of SDS surfactant (Fig. 7). Under the optimal conditions, both drugs were simultaneously determined in human urine samples, with a very low LOD of 0.58 nM and 0.62 nM, respectively. The sensor was applied for the determination of nalbuphine in urine samples throughout a linear dynamic range of 9 nM to 80 μM, with a LOQ of 1.61 nM and sensitivity of 0.512 μA/M [160].

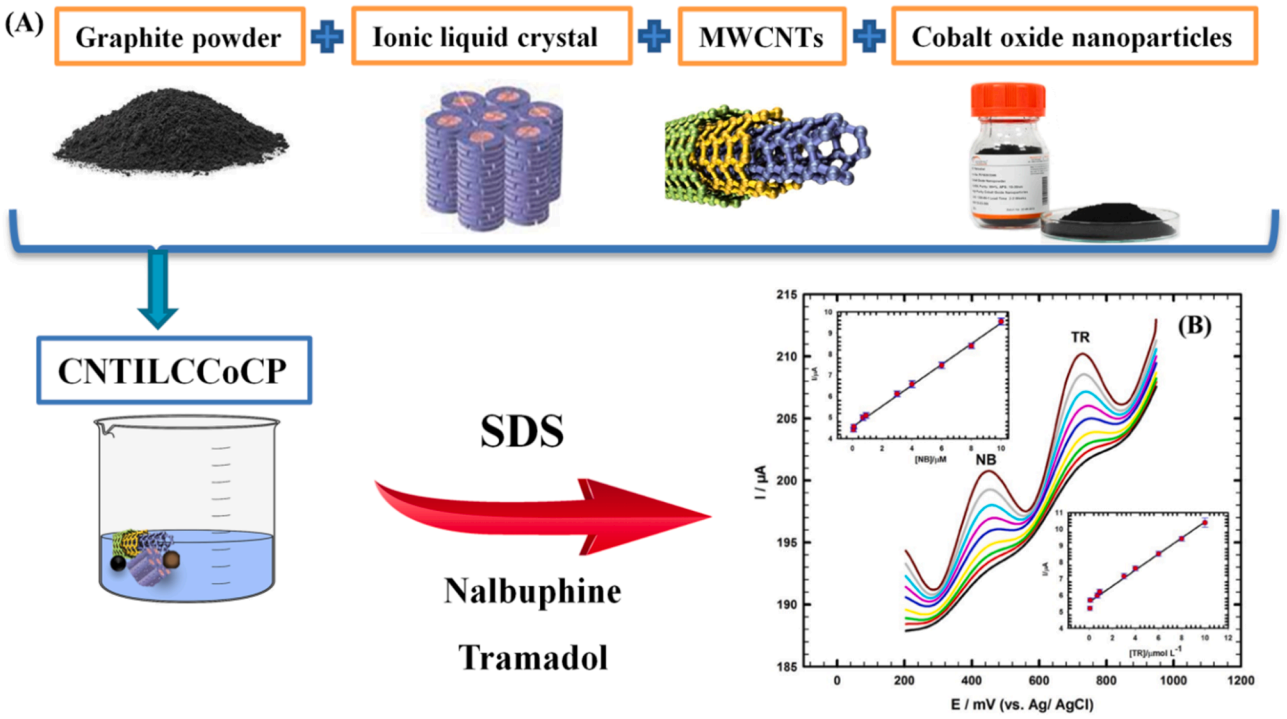


**Fig. 7.** (A) Synthesis process and (B) analytical performance of the CNTILCCoCP-SDS sensor to detect nalbuphine and tramadol.

### *4.7. Fentanyl and other opioids*

Fentanyl ($C_{22}H_{28}N_2O$), a highly strong synthetic opioid that stimulates the mu-receptor (MUR), was first produced in 1960 [161]. Due to its exceedingly unpredictable fatal dosage when coupled with other medicines, fentanyl leads to an extremely high overdose risk in humans. The most recent rises in fentanyl deaths are linked to illicitly manufactured fentanyl combined with or sold as heroin [162].

Lerner et al. prepared a new biosensor based on graphene fentanyl arrays that were functionalized with a water-soluble μ-receptor protein. Microscopic and Raman analysis revealed that arrays of high-quality graphene transistors could be successfully developed via scalable manufacturing methods. The devices could determine naltrexone, an opioid receptor antagonist, at doses as low as 10 pg/mL. An engineered μ-opioid receptor protein, with high binding affinity for opioids, chemically bonded to a graphene field-effect transistor was developed as a novel electronic biosensor for opioids. The biosensor exhibited outstanding sensitivity and selectivity towards naltrexone, over a concentration range of 6 orders of magnitude (10 pg/mL to 10 g/mL). Results were well fitted by a model based on the Hill–Langmuir binding equation. Control investigations confirmed that the sensor response was due to the receptor specific binding to naltrexone, suggesting that the water-soluble MUR retains its physiologically active analyte binding confirmation when covalently linked to graphene [163].

The fabrication of biogenic $Co_3O_4$ NPs using *Duranta repens L.* plant extract was reported for the first time by Memon and colleagues. The addition of $Co_3O_4$ NPs facilitated the tramadol detection process, leading to a very low LOD of 0.001 μM, which corroborates that the modified electrode is more sensitive [164].

*Mentha aquatic* extract was applied to synthesize AuNPs via a biosynthetic approach. A modified CPE containing the AuNPs and 1-butyl-3-methylimidazolium tetrachloroborate (BMTCB) was used to determine tramadol in an aqueous solution, showing good catalytic performance. The Au/BMTCB/CPE exhibited a linear relationship between tramadol oxidation current and concentration in the range of 0.01–400.0 M, with a LOD of 6.0 nM [165].

A novel sensor has been developed based on a CPE decorated with nanographene/tramadol-imprinted polymer NPs/ionic liquid. The nanosensing layer was made by incorporating the tramadol-MIP NPs, acting as an efficient detecting agent, into a CPE matrix constituted of graphite powder, graphene nanosheets decorated with AgNPs, and a ionic liquid as the conductive pasting binder. The LOD of this study was $2.04 \times 10^{-9}$, and the lineal interval ranged from $3.50 \times 10^{-9}$ to $1.00 \times 10^{-2}$ M [166].

## 5. Conclusion and future perspectives

Opioids are one of many therapeutic options for treating chronic nonmalignant pain (CNMP) and are among the most prescribed medications. The lack of alternative nonopioid medications for moderate to severe CNMP that are equally effective and safer than opioids explains a portion of the increase in opioid prescribing for this population. Therefore, their misuse and addictions were the most encountered health issues [167]. It has been reported that in 38 countries, members of the Organization for Economic Co-operation and Development (OECD), opioid usage has skyrocketed, resulting in an epidemic of overdose deaths, abuse, and exaggerated consequences on the society and the economy [168].

Nowadays, urine or blood tests are the most common methods to monitor opioid use. However, other biological sources like saliva and hair are also used. Due to the relatively straightforward collection, urine has traditionally been the fluid used for drug testing. The most widely applied method for drug detection in patients is urine analysis, which can take up to 72 h, excluding sample preparation time. This fluid is typically collected in private, hence, there is a chance of sample tampering by dilution or the addition of substances in order to produce false negative results [12,169]. Urine tests and self-reports make up the current drug testing procedure. However, self-reports might be skewed and are not a real-time solution. In addition, due to the long period required for their completion, they might provide inaccurate results [167,170]. A lot of effort has been focused on developing novel testing procedures for alternative samples that allow an easy and observed collection.

Saliva is one of the most likely samples to meet the aforementioned

requirements. The levels of drugs in oral fluid frequently match those of free drugs in serum. Basic drugs like opioids may even show higher concentrations for longer periods of time after use compared to blood due to a "ion trapping" effect of weakly basic drugs like morphine within mildly acidic saliva [12]. Short-term data on drug addiction can be obtained from blood and urine analysis, whereas long-term medical history must be tracked through hair sample analysis. The analysis of hair samples is frequently used in forensics to document exposure to substances over a long period of time, up to several months. The hair test is complementary to the urine test for the analysis of new psychoactive substances [171]. Detection of opioids after a long time of usage is crucial, hence novel specimens and methods are needed. Given the limitations and challenges described above, a user-friendly, wearable, non-evasive system that can consistently detect and forecast opioid use in real-time is required [170,172].

Currently, quick, presumptive tests, such as color tests, are used to detect illicit drugs in seized street samples; however, these tests lack selectivity and must be confirmed by expensive and time-consuming chromatography-mass spectrometry (GC–MS) methods. As a result, it is essential to develop new sensing technologies that enable the rapid, sensitive, and selective detection of illicit drugs in the field [173]. With significant clinical needs, the opioid epidemic gives new chances for POC drug testing. Compared to gold standard tests, commercially available opioid POC testing has lower analytical sensitivity, specificity, and cross-reactivity. In response to unmet clinical needs, novel methods such as microfluidics and miniature mass spectrometry have emerged in the research literature. Prospects for the future include the development of quantitative POC devices as well as more innovative and real-time drug testing [174]. Detection of opioids from biological fluids includes some limitations, such as pretreatment steps, incorporation of drugs, and long waiting times for analysis [175]. Novel strategies such as microneedle arrays may overcome these challenges with their capacity to continuously detect a wide scale of clinically significant analytes [176].

Nanomaterials have unique properties including large surface area and easiness of modification, and can be used in a variety of applications, including the development of biosensors and sample pretreatment, which makes it easier to monitor illegal drugs [177,178]. The sample pretreatment procedure is crucial for obtaining ideal detection performance, which is necessary to effectively monitor illicit drug levels. However, since the detection of illicit drugs is typically carried out in bodily fluids, foods, and beverages containing various compounds, it may be challenging to eliminate interferences from complex matrices. By applying an external magnetic field, sample pretreatment combined with nanometer-sized MNPs can provide faster phase separation and be simpler to reuse. In addition, MNPs can be used to produce an easy extraction process with high selectivity that uses less reagent than traditional sample pretreatment techniques [179]. Nanomaterials can be used as signal reporters that offer quick and accurate detection in addition to sample pretreatment techniques[177].

Although nanomaterial-based biosensors have many advantages over opioid detection, these techniques are difficult to bring outside of the lab for point-of-care and field analysis; simpler techniques such as rapid colorimetric testing would be sufficient. However, challenges remain in developing simple yet integrated platforms for analyzing opioids in various sample matrices [180]. Since clinical validation of developed novel nanobiosensors is still a long way off, the researchers need to identify the technologies validation process of therapeutic drug monitoring and work closely with industry partners to advance these methods to the high level of development necessary for regulatory [181]. The lab must ensure that the assay being used is as accurate and precise as possible and that the necessary quality control is being carried out. Method validation is becoming more widely recognized as crucial. In this regard, precision, LOD, LOQ, linear dynamic range, reproducibility, repeatability, and robustness parameters should be assayed and documented [182]. Overall, these parameters need to be clarified, and the newly developed system should be compared with the gold standard or commercially used test kits to ensure system performance [183]. In order to put newly generated nanobiosensors into commercial use, several critical procedures must be undertaken. Besides, these nanomaterial-based biosensor systems would monitor a particular tissue, organ, or even the entire body (transdermal needles, wearable sensors, etc.), and toxicity analyses ought to be carried out before integrating them into clinical practice, since systemic, long-term, or local toxicities may reduce the effectiveness of nanobiosensors for drug monitoring [184,185]. Necessary steps include market analysis, definition of market requirements, financial return and investment opportunities, and risk assessment. Before finalizing initial design requirements, sensor developers and manufacturers must define a clear regulatory and commercialization plan that identifies the difficulties, risks, costs, and timelines of bringing such technologies to market [186,187].

Nanobiosensors are still a long way from clinical validation, hence the next task will be to clarify the technologies best suited to the specific requirements of therapeutic drug monitoring and to collaborate closely with industry partners to advance these methods to the high level of development required for regulatory clearance of these novel opioid monitoring assays [185,188]. It is evident that there is still a huge requirement to improve a more efficient, real-time, selective, cost-effective, portable, and environmentally friendly opioid analysis system. It would be possible to develop novel nanomaterials in multidisciplinary teams that include engineers, basic scientists, and medical doctors to provide selective detection of opioids. Given that developed materials should be examined with human samples, prototypes should be miniaturized and combined with artificial intelligence or machine learning technologies to provide reliable monitoring of the results from health specialists, forensic scientists, and even polices. As a means of achieving these goals, governments or foundations should invest in these kinds of institutions and researchers. Also, detailed research on the market and feasibility analysis should be performed, and suitable investors should be found to commercialize these systems.

## Funding

Financial support from the Community of Madrid within the framework of the Multiyear Agreement with the University of Alcalá in the line of action "Stimulus to Excellence for Permanent University Professors", Ref. EPU-INV/2020/012, is gratefully acknowledged.

## Data Availability Statement

This article's data sharing is not applicable as no new data were created or analyzed in this study.

## Ethical approval

Not applicable.
Consent to participate
Not applicable.
Consent for publication
None.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Acknowledgments

Financial support from the University of Alcalá for the publication of this article is acknowledged.

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