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Sensing Beyond Senses : An Overview of Recent Developments in Nano enabled Sensor for Analysis of Illicit Drugs

Sandeep K Vaishnav¹, H. S. Bhawara², Rajesh Mishra³

Senior Scientific officer, State Forensic Science Laboratory, Police Line Campus, Tikrapara, Raipur,
Chhattisgarh, India¹

Joint Director, State Forensic Science Laboratory, Police Line Campus, Tikrapara, Raipur, Chhattisgarh, India²

Director, State Forensic Science Laboratory, Police Line Campus, Tikrapara, Raipur, Chhattisgarh, India³

ABSTRACT: The multidisciplinary field of nano-enabled sensing is growing and has dynamic multifunctional detecting abilities. A significant area of contemporary analytical chemistry is the detection and analysis of drugs, which has numerous consequences for legal and societal issues in forensic cases. Drug misuse has been more widespread in recent years and is linked to problems with crime and public health. Nanodevices can therefore play a significant role as effective instruments in this sector given the necessity to identify illicit drugs and their well-known benefits of sensitivity, selectivity, ease of use, affordability, and capability of downsizing and automation. As a result, sensors with nanotechnology capabilities can replace more complex instruments already in use. In the domain of analytical chemistry, the employment of nanomaterials recently served as inspiration for the creation of several novel detection techniques. The limited number of publications covering nanoprobess, nanosensors, and nanochips for forensic analysis has demonstrated the relatively limited application of nanodevices in forensic. This review will thus give a brief indication of the potential of this cutting-edge field of study, which sought to present novel methods for "on site" analysis of illicit drugs at the trace level which will transform as a future weapon in crime scene investigation. Additionally, the use of nanomaterials in the forensic opens up new possibilities for the creation of detection system and enables the monitoring of illicit substances in various complicated matrices.

KEYWORDS: Nanoprobess, Nanosensor, Nanochips, Nanomaterials, nano-enabled; multifunctional nanomaterial, Illicit drugs.

I. INTRODUCTION

In recent years, the incidence of drug usage has escalated into a grave public health concern and a significant criminal problem on a global scale. [1] Drugs classified as depressants, stimulants, opioids, or hallucinogens are illegal substances that cause addiction, organ malfunction, and death at high dosages [2]. The physical or mental health of users may gradually worsen as a result of drug usage. Due to the fact that illegal drugs are rarely present in their purest form in the sample that is submitted for analysis, many forensic experts find it difficult to identify them. The inability of scientists to find the substance in traces in biological samples is another problem. Blood, saliva, semen, and urine are among the various biological samples a forensic scientist might get from a crime scene.

Screening and confirmation are the two ways through which drugs have been tested in Forensic. Once drugs are detected through screening by spot test kits like immunoassays, Marquis test, etc., After drugs are found using screening spot test kits, samples are then seized and submitted to forensic labs for further testing and confirmation. High sensitivity and selectivity for medicines and their metabolites are required for confirmation, which is often done using chromatographic and spectroscopic methods [3-5]. These methods are only employed in labs. These methods also have some shortcomings in regards of sensitivity, selectivity, and stability. Additionally, they are expensive, time-consuming, and laborious. Therefore, the utilization of biosensors can address the problems outlined above. Affinity biosensors' development has accelerated over the past few years, and a huge number of pertinent applications have shown how beneficial they are for forensic investigation [6]. DNA based sensors have irrupted recently in the field of forensic toxicological analysis showing their high usefulness, for example, in the detection of pathogen micro-

organisms [7], toxicological behavior of drug, etc. [8-11]. Additionally, aptamer based aptasensor have been also established for various forensic applications [6]. Enzyme biosensors have been recognised to have a limited number of typical applications in forensic analysis, despite their extensive usage in other (bio) chemical analysis fields. The complicated biological samples that are presently being evaluated in forensic laboratories have only seldom been subjected to these approaches. Nevertheless, a number of enzymes may be used to create biosensors that have been successfully used to identify, among other things, inorganic poisons like cyanide and arsenic as well as chemical and biological war weapons. [6]

Due to their exceptional optical, electrical, thermal, and catalytic capabilities, nanostructured materials are ideal signal amplifiers for chemical and biological sensing. [12] A shift in this behaviour may be anticipated given the rising significance of nanosensors in applications linked with forensic work as it will be proven in this article, even though the use of nanosensors in forensic practise is still quite restricted as indicated by the small number of studies on this topic that reported on biosensors. In this review, we examine the use of nanosensors for forensic investigation, focusing on the applications that have gained the greatest attention in recent years. Optical, electrochemical and various other kinds of detection approaches are taken into consideration. This review highlights the most important analytical characteristics of the suggested methodologies. Despite their shown value, there is always a need to improve and expand current analytical techniques in order to detect and identify current and emerging illicit substances with greater sensitivity and specificity. Consequently, the purpose of this review is to explain how nanotechnology can increase the sensitivity and selectivity of the illicit drug analysis.

II. SIGNIFICANCE OF NANOTECHNOLOGY

Nanotechnology has been crucial in fields including biological science, environmental research, pharmaceuticals, material science, and electronics. [13] Nanostructured materials, including nanoparticles, nanotubes, nanorods, nanofibre, supramolecular materials, and others, have demonstrated excellent electrical and chemical properties. Because of its nanoscale dimensions it has a large surface area. [14–18] Nanodevices with excellent sensitivity and selectivity, such as nanobiosensors, nanochips, nanoprobe, etc., have been developed for a variety of applications in the life sciences field, including disease diagnosis [19-21], interaction studies [22-23], hybridization assays [24-27], and metal detection [28, 29]. Therefore, forensic analysis using these nanodevices is now possible, greatly improving sensitivity and selectivity. (Figure1) Nanotechnology enables the miniaturization and the integration of receptor biomolecule transduction systems, microfluidics and electronics in intricate nanobiosensor designs, enabling fabrication of lab-on-chip devices for quick and affordable screening of illicit drugs and their metabolites, including patient material. [12, 30-34]

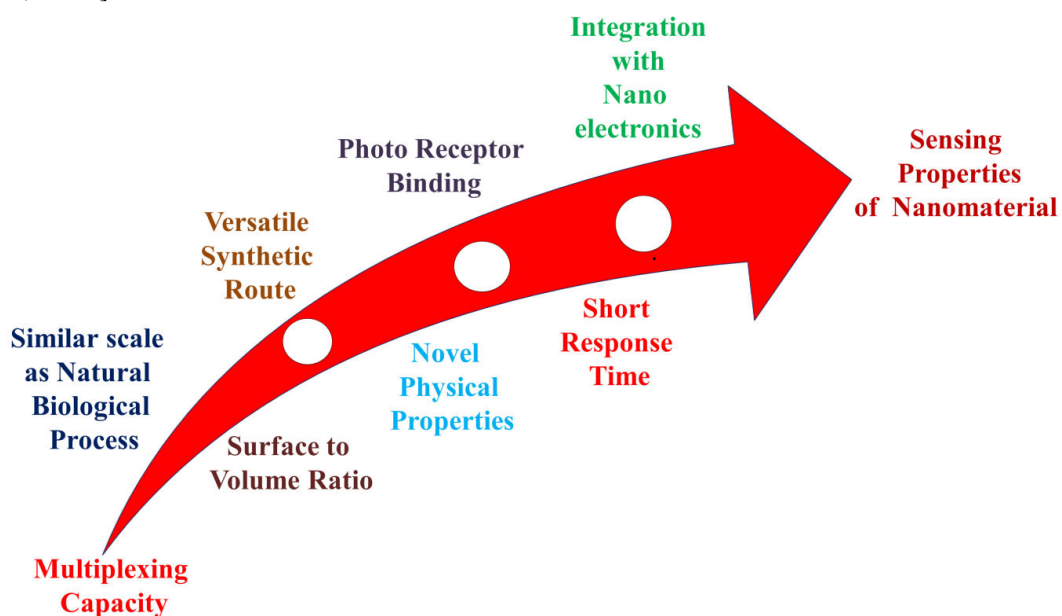


Figure 1 Relevant properties of nanomaterial in view of sensing ability.

III. NANOENABLED PROBE AND CHIP FOR DETECTION OF ILLICIT DRUG

Research on the creation of nanosensors in numerous sectors has advanced tremendously [35–38]. Nanosensors are made from materials with nanostructures. In order to create a nanobiosensor, a probe is first modified with nanomaterial, such as nanoparticles, nanowires, nanoshells, nanotubes, nanorods, nanofiber, etc. Biomolecules are then immobilized to this nanostructured material. The surface of the transducer that has been nanostructured can immobilise this biomolecule. Due to their electrical characteristics and large surface area, biosensors based mostly on nanoparticles and nanotubes have been developed. They are also excellent catalysts and have good biocompatibility. [39–43] Additionally, the development of nanochips might provide a fantastic platform for the detection of illicit drugs in biological matrices. Time and money are saved. Biochips have been developed for pathogen detection, medical diagnostics, blood cell lysis, viral genotyping, immunoassays, cell transfection assays, etc. However, very little work has been done on the creation of nanochips for the detection of illicit narcotics up to this point. Therefore, the development of nanochips for the detection of illicit narcotics is a very interesting topic. In Figure 2, an example of a nanochip with a nanostructured material covering the chip platform is shown. The biomolecule is then immobilised on nanochip. The development of nanochips will undoubtedly increase the sensitivity and selectivity of drug detection. Additionally, it saves money and time.

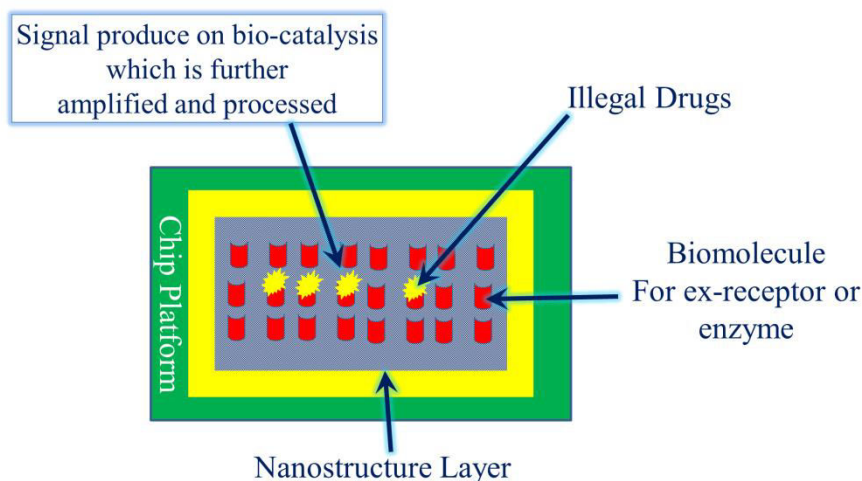


Figure 2 Schematic representation of nanochip: Chip platform covered by nanostructure followed by bimolecular receptor

III. I CODEINE DETECTION

The opiate family of drugs, including codeine (COD), which is an analgesic and antitussive, is naturally present in the poppy plant. To relieve pain and pyrexia, this medication is widely accessible as pharmaceutical tablets, either alone or in conjunction with other analgesics such as acetylsalicylic acid, ibuprofen, and caffeine. If misused, it can also result in drug addiction and mental harm, which may then lead to societal problems. For the purpose of determining COD at MWCNT/GCE (Multiwall Carbon Nanotubes/Glassy Carbon Electrode), a sensor was reported. [44] The simultaneous measurement of COD and paracetamol was done with detection limit $0.19\mu\text{M}$. Employing a boron-doped diamond (BDD) film electrode is a quick and accurate electrochemical approach for detecting COD in pharmaceutical formulations and human urine. [45] The analysis of COD was unaffected by ascorbic acid, uric acid, or glucose, indicating that the approach had high selectivity. Voltammetric analysis of COD employing a carbon-ceramic electrode constructed with single-walled carbon nanotubes was performed and revealed a LOD of 110 nM. However, it was also researched to simultaneously measure COD and caffeine in tea, cola, and pepsin samples. [46]

Recently, a strategy was put forth that is based on the structural changes that codeine and morphine cause in dsDNA, which result in different electrochemical oxidation signals for both drugs. On the basis of this result, a biosensor constructed on a pencil graphite electrode (PGE) augmented with MWCNTs distributed in poly (diallyldimethylammonium) (PDDA) was created. [47] The electrostatic attraction for codeine or complexation for morphine caused depressed currents in the presence of DNA and shifted to more positive and negative potentials, respectively. This allowed for a clear distinction between the peaks for both analytes. Blood serum, urine samples, and

pharmaceutical formulations employed for the validation of this biosensor. Anand et al. developed a "smart" system for the on-the-spot, simple, portable, rapid, ultrasensitive nano aggregation colorimetric detection of codeine sulphate levels using a smartphone, taking advantage of the special characteristics of citrate stabilised gold nanoparticles (AuNPs) as a probe (Figure 3).[48] This system was motivated by the need to detect narcotics. The suggested approaches show that the citrate-capped gold nano probe may be employed as a viable new platform for the creation of a quick, ultrasensitive, on-site semi-quantitative field test for the measurement of narcotics/codeine sulphate from post-mortem blood, skeletal remains, and soil.

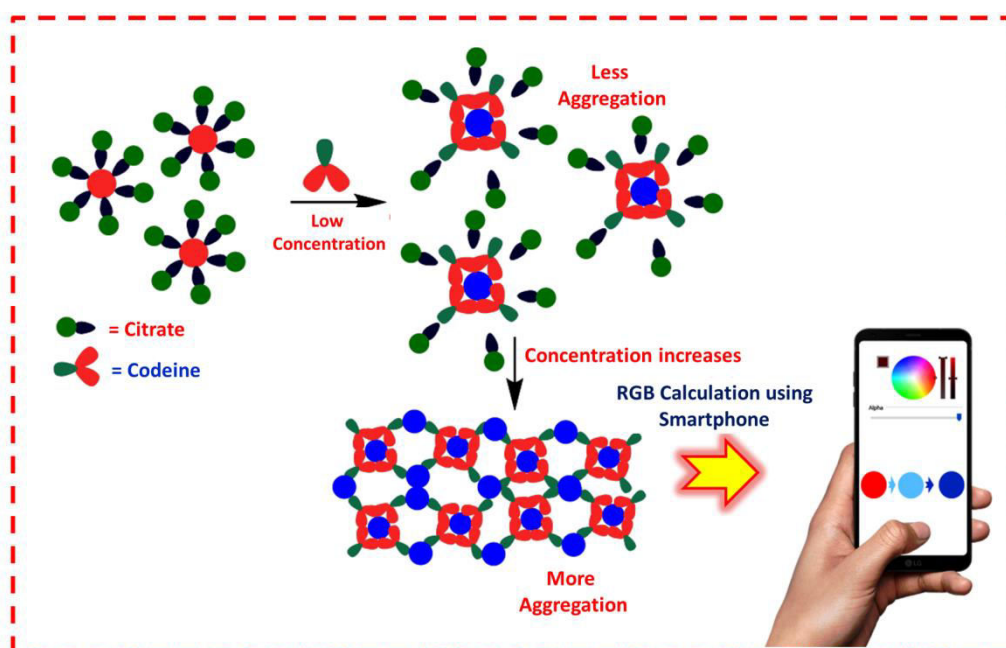


Figure 3 Schematic representations of Codeine and gold nanoparticle. [77]

III. II MORPHINE DETECTION

Being the prototype opioid and an efficient opiate analgesic, morphine has a risk of destabilising the central nervous system. It works by dulling the brain's pain perception centre and has been used to treat individuals who are experiencing extreme pain, especially those undergoing surgery. Numerous opioids, including heroin and codeine, are produced using morphine as a precursor. A class of medications known as narcotic painkillers includes morphine. Our society's biggest concern is that it might lead to drug addiction and mental harm if taken improperly. Numerous analytical techniques have been created as of late, as detailed below. An AuNP and Nafion modified CPE (Carbon paste electrode) was created by Atta's group for the voltammetric detection of morphine (Figure 4). [49] In standard solution, the Limit of Detection (LOD) was determined to be 1.33 nM. However, due to the interferences present in the urine matrix, the LOD of morphine in the urine sample was 87.2 nM, indicating that this approach is not selective. For the detection of morphine, the use of an ionic liquid, 1-butyl-3-methylimidazolium hexafluoro phosphate, and MWCNTs on a carbon paste electrode (CPE) was demonstrated. [50] The morphine LOD was determined to be 0.14 M, with a significant linear range of 0.45 to 450 μ M. [50] In a different approach, a glassy carbon electrode (GCE) modified with N-hexyl-3-methylimidazolium and MWCNT was investigated for the voltammetric measurement of morphine in urine and injection sample with a detection limit of 20 nM. [51] However, since the authors spiked the morphine concentration to μ M in biological fluids, it is likely that this approach is not sensitive to morphine at values of nM in actual biological samples. Moreover, the simultaneous measurement of morphine, noscapine, and heroin has been performed using a glass chemical electrode coated with graphene nanosheets (GN). With the peaks for noscapine and heroin, the DPVs produced for a combination of these three compounds showed rather modest signal levels. However, when the glass chemical electrodes were modified with GN, the peak current was increased and a distinct distinction between the peaks for morphine, noscapine, and heroin was seen. The detection limit for morphine, noscapine, and heroin, respectively, is 0.4, 0.2, and 0.5 μ M, indicating that the sensitivity was not very high. [52] In another detection system of morphine, a

multiwalled carbon nanotube and vinyl ferrocene modified carbon paste electrode (VFc/MWCNT/CPE) was created. The LOD was discovered to be 90 nM, showing that the approach is sufficiently sensitive to detect morphine. [53]

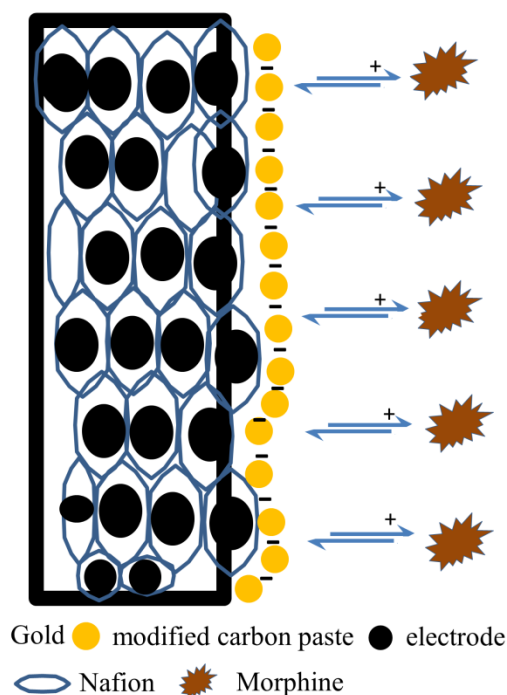


Figure 4 Schematic representation of Nafion modified carbon paste gold coated electrode for determination of morphine. [49]

III. III COCAINE DETECTION

After cannabis, cocaine is the substance that is most often misused worldwide. It is categorised as a central nervous system stimulant. Cocaine is one of the most frequently confiscated illicit substances in the world, with over 655 tonnes apprehended each year [1]; there are more than 17.5 million users in European nations, where it is one of the main recreational narcotics traded unlawfully [2]. Criminal activity with illicit substances is extremely concerning because it puts a strain on healthcare and law enforcement systems and causes related social and economic issues. As mentioned below, several analytical techniques have been created to date. A single-Quantum Dot-based aptameric fluorescence sensor was created by Johnson et al. in order to detect the presence of cocaine in both signal-off and signal-on modes. [54] The detection threshold is 0.5 μM . This single-QD-based aptameric sensor has a number of important benefits over other aptameric sensors, including easy sample preparation, excellent sensitivity, and incredibly low sample consumption. In another method, Milan N. Stojanovic and Donald W. Landry created an aptamer-based colorimetric probe. [55] Cocaine binds to the anti-cocaine aptamer MNS-4.1 through a hydrophobic pocket, which causes an alteration in absorbance. We were able to visually identify these solutions after 12 hours thanks to the development of a blue precipitate and partial solution decolorization in mixtures containing cocaine. Supramolecular-Aptamer complex and metallic or quantum dots were used in the cocaine detection methods published by Itamar Willner and his colleagues investigated Surface Plasmon Resonance (SPR) sensor based on the utilisation of two anticocaine aptamer subunits, aptasensors. [56] A second aptamer subunit is labelled with Pt-NPs, CdS-NPs, or Au-NPs after the first subunit, which serves as an electrode or an SPR-active surface, is assembled on an Au support. In order to quantify cocaine quantitatively, supramolecular complexes of cocaine and NPs-labeled aptamer subunits form on the metallic surface when cocaine is introduced to the different aptasensor configurations. Cocaine may be detected by the electrocatalyzed reduction of H_2O_2 by the supramolecular platinum nanoparticles-aptamer subunits-cocaine complex. The photoelectrochemical detection of cocaine is made possible by the Cadmium sulphide nanoparticles (CdS-NPs) tagged aptamer subunits-cocaine complex in the presence of triethanol amine as a hole scavenger. All aptasensor configurations allow for the evaluation of cocaine with a 10^{-6} to 10^{-5} M detection limit. [56] The sensing platform's main benefit is the absence of background interference signals. Additionally, Roberta D'Aurelio and a colleague created

a molecularly imprinted nanoparticle-based drug detection sensor with a linear range between 100 pg mL⁻¹ and 50 ng mL⁻¹ and a limit of detection of 0.24 ng mL⁻¹ (0.70 nM). (Figure 5) [57]

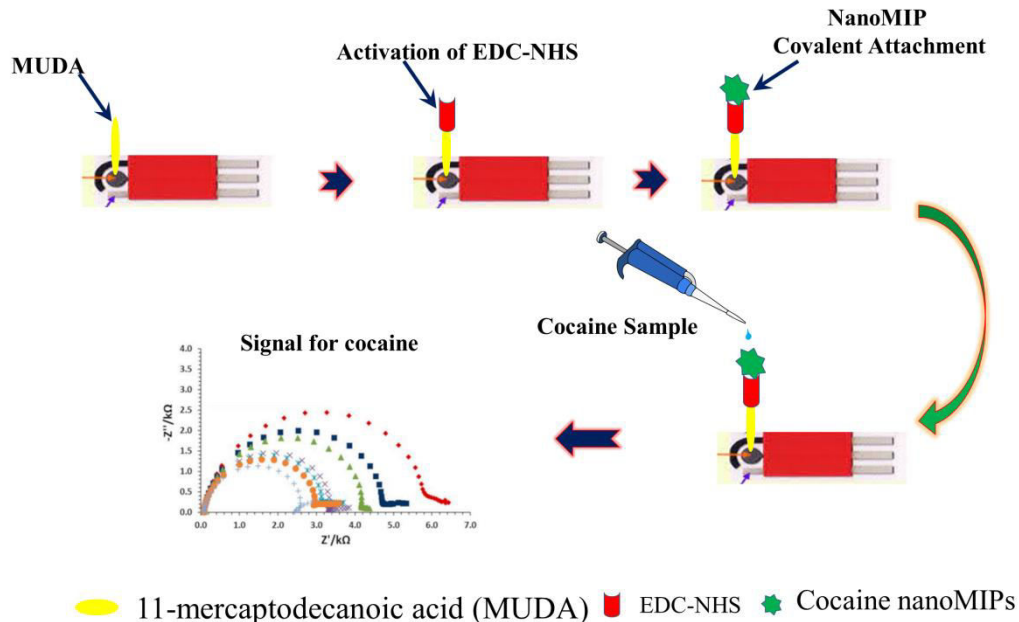


Figure 5 Scheme of the nanoMIPs sensor for cocaine detection.

Additionally, Ruqin Yu and his team created an aptamer-based substrate for surface enhanced Raman spectroscopic (SERS) sensor for cocaine detection. The 3-mercaptopropionic acid (MPA) monolayer was combined with an oligonucleotide aptamer having a tetramethylrhodamine (TMR) moiety at the 5' end to create the biosensor chip. The surface-tethered aptamer on the binding target in undergoes a conformational shift that brings a specific Raman reporter in close vicinity to the SERS substrate, boosting the Raman scattering signal as a result of the local improvement of environmental of SERS. [58]

III. IV METHAMPHETAMINE DETECTION

A SPR immunosensor that fixed a methamphetamine-bovine serum albumin (MA-BSA) conjugate on the Au thin film of the sensor chip by physical adsorption enabled the extremely sensitive and selective identification of methamphetamine (MA). With increasing concentrations of anti-MA-BSA antibody up to 20 mg mL⁻¹, the incidence angle of the MA-BSA-immobilized sensor increased. The inhibition effect of MA, which allowed the determination of MA in the concentration range of 0.1-1000 ng mL⁻¹, as well as in phosphate buffer saline solution comprising 1% human urine, was found to allow the determination of MA in those solutions without any significant change in incident angle shift due to nonspecific adsorption. [59] Additionally, Carl D. Meinhart and a partner created a Microfluidic device based on silver nanoparticles for quick drug usage detection in saliva. [60] They created a microfluidic system that uses SERS to quickly detect minute amounts of methamphetamine in saliva. In order to regulate the couplings between the analyte, silver nanoparticles (Ag-NPs), and a salt, the detection method takes advantage of chemical concentration gradients that are encouraged by the laminar flow inside the device. In order to explain the kinetics of the colloid accumulation response and to pinpoint the best site in the channel for SERS interrogation, the spatial variation of the SERS signals was utilised to map the degree of species diffusion and nanoparticle aggregation in the channel. [60] Furthermore ShohreRouhani and group developed novel fluorescence nanosensor based on 1, 8-naphthalimide-thiophene doped silica nanoparticles for the determination of methamphetamine. [61] (Figure 6)

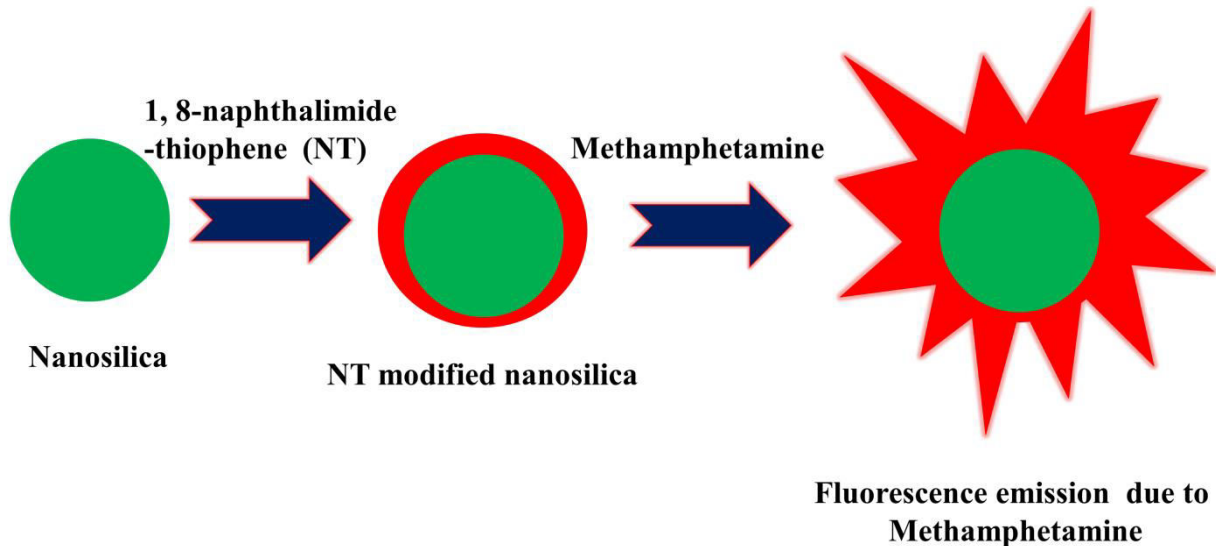


Figure 6 Schematic view of Fluorescence detection of Methamphetamine

III. V CLONAZEPAM DETECTION

In sexual assaults and other crimes when drugs are used as a tool, benzodiazepines are frequently used. Skeletal remains and other bodily fluids are routinely found at crime scenes, but analytical tools and procedures to identify substances used during drug-facilitated crimes from these materials are few. Anand et al. provide a very effective, cost-efficient, and specific approach for the detection of clonazepam in tiny levels when melamine is present (Figure 7). [62] Clonazepam and melamine had hydrogen bonding interactions, which caused AuNPs to aggregate and alter colour from wine red to blue. According to the findings, clonazepam concentrations between 10×10^{-8} and 1.0×10^{-9} M had an absorption ratio (A_{636}/A_{552}) that was linear. The detection limit was 8.9×10^{-10} M ($S/N = 3$), which was substantially lower than the detection limit of the majority of known techniques. The determination of clonazepam was unaffected by coexisting drugs such as alprazolam, diazepam, nitrazepam, and lorazepam. The sensor created by this novel methodology offers a promising alternative method for on-site, real-time screening of clonazepam and might be utilised as a spot test. This suggested plan was further backed by a series of studies that demonstrated the practicality of the constructed nanosensor using actual samples like blood and skeletal remains. [62]

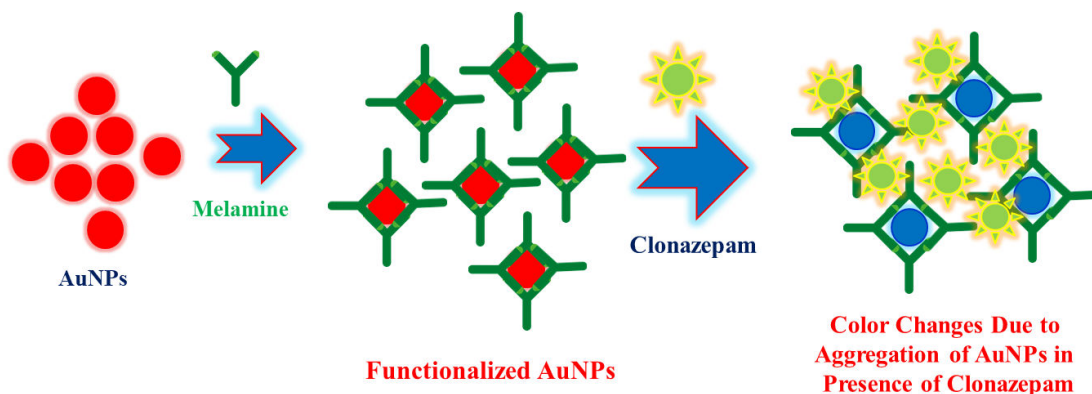


Figure 7 Schematic diagram of gold nanoparticles interaction with clonazepam. [99]

III. VI γ -HYDROXYBUTYRATE (GHB) DETECTION

γ -Hydroxybutyrate (GHB) is now one of the most hazardous illegal substances. The misuse of γ -hydroxybutyrate (GHB), a central nervous system depressant that, depending on the amount used, can stimulate, elicit euphoria, anxiolysis, or induce hypnosis, is becoming more and more common. The substance is used in low amounts for



recreational purposes and is also linked to drug-enabled sex crimes. GHB is frequently replaced by its unscheduled precursors γ -butyrolactone (GBL) and 1,4-butanediol (BD), which the body swiftly metabolises into GHB. [63] A chitosan/porous silicon biosensing platform was created and described for the detection of medicines containing carboxylic acids in water. Forensic analysis-compatible quantities of GHB, a model molecule for the illegal rape drug GHB, were detected using optical interferometry measurements on the chitosan/porous silicon substrate. [64] It has been claimed that a protein array chip may use imaging surface plasmon resonance to identify the distinct components of a combination of four common drugs (cocaine, ecstasy, heroin, and amphetamine) without the need of labels. The chip is created by injecting a combination of antibodies into the supporting substrate, where they are then allowed to attach to proteins that are already present in a predetermined pattern called narcotic analogue coupled proteins. The optical contrast and subsequent observable SPR and/or ellipsometric signal were obtained via an indirect detection approach in which antibodies are expelled from the surface upon identification of their matching drugs. The limit of detection for heroin, cocaine, and the other two drugs was in the range of $0.5 \text{ pg } \mu\text{L}^{-1}$, $2.5 \text{ pg } \mu\text{L}^{-1}$, and $5 \text{ pg } \mu\text{L}^{-1}$, respectively. [65]

III. VII MULTIPLEXED DRUG DETECTION

Optomagnetic technique was created by M. W. J. Prins and their collaborators, enabling very sensitive one-step tests in blood serum/plasma and entire saliva. Within total test duration of one minute, abuse-related drugs are found at sub-nanogram per millilitre values. They created a chip platform based on nanoparticles in a fluid sample that is stationary that are magnetically activated and optically sensed. The dried nanoparticles are redispersed and bind to the morphine molecules in the sample. With the lower magnet, nanoparticles are drawn to the sensor surface in the second phase. The higher magnet is used in the third phase to remove loose and weakly bound particles using a magnetic force that is directed away from the surface. Anti-morphine antibodies and BSA-morphine are coated on the nanoparticles' sensor surface. The developed detected five drugs—amphetamine (AMP: 80 ng mL^{-1}), morphine (OPI: 48 ng mL^{-1}), methamphetamine (MAMP: 80 ng mL^{-1}), tetrahydrocannabinol (THC: 800 ng mL^{-1}), and cocaine (COC: 48 ng mL^{-1}) in 100% saliva. [66] Moreover, nanostructured silicon (nSi) surface aided laser desorption/ionization mass spectrometry (nSi-MS) has recently been described by Chia-Wen Tsao and his colleagues as a method for determining misused chemicals such as amphetamine, methamphetamine, codeine, morphine, and ketamine. The performance of the nSi-MS, including detection sensitivity, limit of detection, linearity, and repeatability, was assessed using drug standard tests applied to the nSi chip platform. Drugs were effectively discovered from extremely trace urine volumes ($\sim 10 \text{ } \mu\text{L}$) and a quick analytical technique (< 15 minutes) in real urine samples taken from drug addict inmates. [67]

IV. CONCLUSION AND FUTURE PROSPECTS

In several forensic investigation fields, nanotechnologies have become more important in the development of tools including sensors, chips, and probes. This review article makes it abundantly clear that nano enabled sensors have the potential to be used as quick, dependable, and cost-effective analytical tools in this area of contemporary analytical chemistry that is distinguished by the variety of analytes that can be studied and the legal context that can affect both the work's implementation and outcomes. It is evident from a few chosen examples that there is a wide variety of intriguing nanosensor designs being created. However, the widespread application of nanodevices in forensic practise is still quite restricted, perhaps as a result of the fact that many of the claimed advancements were not implemented to samples with the degree of complexity that is absolutely necessary. In order to improve the reliability and robustness required for a true application to this sector, significant research attempts must be conducted in the coming years to enable the development of nanodevices better suited for these samples.

Greater sensitivity, lower production costs, and reduction to nanoscale size are just a few of the new potential capabilities that nanodevices might provide. Through the provision of gadgets that are compact, transportable, and simple to use in daily activities, functionality will be considerably enhanced. The domains of virtopsy, crime scene investigation, identification, forensic ballistics, and toxicology are going to be completely transformed by this technology as future armour.

According to published works, the most often misused drugs globally have included codeine, morphine, cocaine, etc. With this review, we intend to open up hitherto unexplored areas of 'less frequent' drug abuse for academics all across the world. Numerous medications have not yet been examined in terms of nanotechnology. We also observed that gold nanoparticles (NPs) have been widely used to prepare chemically modified electrodes, whereas silver, platinum,



palladium, or metal oxide nanoparticles have not been widely used in the preparation of nanomaterials for certain nanomaterials, primarily carbon nanotubes and other carbon species. Future research in the area of nanomaterial-based abuse drug detection devices will undoubtedly focus on the use of such nanoparticles as modifiers, and this is to be expected.

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