Functionalization of metal Nanoparticles for SERS-based Detections of illicit drugs in biological samples

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Abstract

Surface-enhanced Raman scattering (SERS) has been widely used for the detection of illicit drugs due to its excellent chemical fingerprint information, high sensitivity by plasmon-enhanced excitation and scattering, and independence of aqueous solution impact. Even though it has been more than one decade since the first SERS was synthesized, extensive effort has recently been undertaken to improve hand-held Raman analyzer and make SERS a practical point-of-care (POC) device for the detection of illicit drugs in real samples. Lately, there has been a fast growth in improving methods for precise control over metal nanoparticle size and modification of their detection performance with functionalizing agents, which will dramatically enhance their application in various biomedical applications.

In this study, a general view on the background of the SERS and several basic concepts and focuses are discussed. Then we put forward a summary of the progress in trace determination of illicit drugs using various functionalization methods for enhancing characteristics of the substrate toward a more selective detection of the analytes.

Keywords: Surface-enhanced Raman Scattering, Localized surface plasma resonance, Noble metals, Functionalized SERS surfaces.

1.Introduction

In recent decades, there has been a growing tendency to improve health-care access through POC technologies. One of the prime applications of POC diagnostics is in immunosensor for illicit drug monitoring. Illicit drug abuse is a public health issue and has unintended consequences, such as addiction, nausea, dizziness, sedation, vomiting, constipation, lightheadedness, and allergic reaction.[1] Therefore, harm reduction agencies are searching for rapid screening methods to test, identify, and where possible, quantify their constituents, allowing their users to make wise decisions.[2] In recent decades, there were numerous efforts to establish a safe method for the detection of illicit drugs such as Gas Chromatography (GC)[3], mass spectroscopy[4], nuclear magnetic resonance[5], liquid-liquid extraction[6], and High-Performance Liquid Chromatography (HPLC).[7] Although helpful, all the mentioned methods have some disadvantageous: 1) These methods need expensive instrumentation and trained personnel in laboratories. 2) The process of sample preparation is complicated and time-consuming. Drug detection technology should be 1) portable and rapid, allowing POC detection in different situations. 2) sensitive, so it can detect limit concentrations. 3) user-friendly and straightforward for ordinary users. 4) selective such that false negatives are avoided.

In recent years, SERS technology has proven to be a powerful replacement for conventional analytical devices and a powerful method for differentiation and detection of a wide variety of analytes. SERS is a versatile vibrational spectroscopy technique that provides a distinctive spectral fingerprint of an analyte via inelastic light scattering from the sample.[8] Due to the amplification of electromagnetic (EM) field generated by the localized surface plasmons, SERS allows for highly sensitive structural detection of low concentration analytes. SERS finds reliable applications in

medical diagnostics, therapeutic monitoring, pharmaceuticals, food safety analysis, and possibly, if integrated into a microcapsule, as ingestible sensors for therapeutic drug monitoring and health analysis.[9-13]

In SERS, when a molecule is adsorbed onto nano-roughened noble metal surfaces such as silver and gold, Raman spectra will considerably be amplified. Nanoplasmonics allow the manipulation and confinement of electromagnetic waves around nanometallic structures, providing an exceptional possibility of optical control beyond the diffraction limit. When the laser impinges at the dielectric and metal interfaces, the delocalized conduction electrons of the metal nanostructures are driven into collective oscillation, which is called surface plasmon resonance (SPR). In metal nanostructures, the SPR is highly localized to a specific position, which is termed localized surface plasma resonance (LSPR). Plasmonic nanoparticles (PNP), which are usually Ag, Au, and Cu, show strong SPR in the visible to the infrared region and can generate a strong LSPR effect. Hence, at the nanoparticle surface, the incident light energy will be effectively coupled into the metal nanoparticles resulting in a considerable enhancement in the local electromagnetic field intensity, which is the key to the enormous improvement in SERS.[14] This enhancement is possibly due to the large electromagnetic field generated by the SPR on metal nanostructures, particularly in hot spots of an ensemble of structures. As depicted in the Fig.1, two different enhancement processes happen in SERS: the enhancement of the Raman scattering (re-radiation of the Raman signal) and an enhancement of the excitation light (enhancement due to the excitation of the plasmon resonance of the electric field at the vicinity of the nanostructure surface).

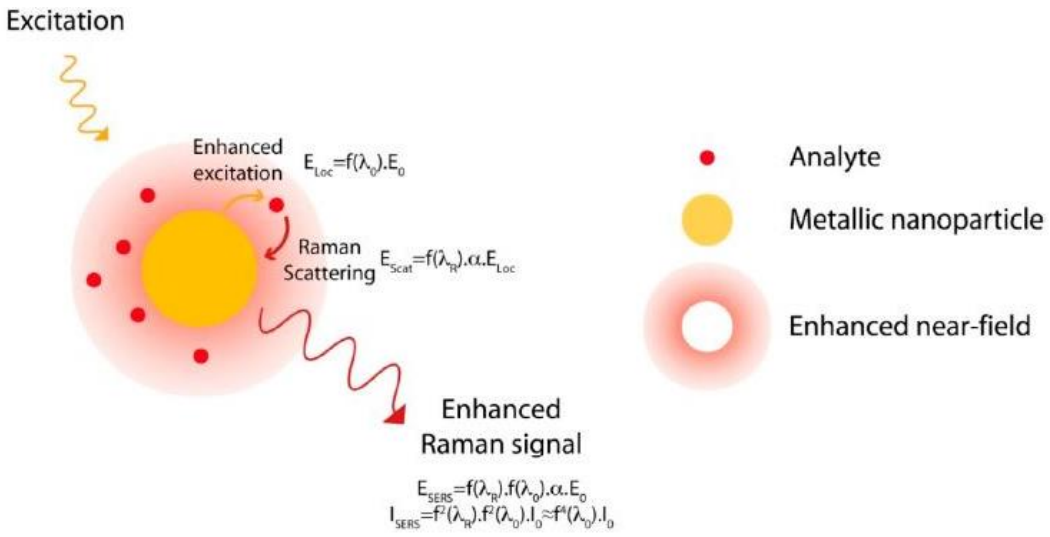

Figure 1. Principle of the Surface-Enhanced Raman Scattering.[15]

Although SERS is a possible method for the detection of a single target molecule, it cannot separate target molecules present in a complex matrix, which is a significant drawback for detecting trace levels of the target analyte in complex samples. In this case, the Raman signals of the target analyte will be concealed by the signals of other ingredients/compounds present in the sample, especially when the analyte is dilute. Therefore, the sensitive and high accuracy analysis of illicit drugs is still in urgent need. Several methods have been used to overcome this effect, such as using modified SERS labels[16-18]or coupling the SERS device to a prior purification method, such as electrophoresis or chromatography.[19–21]

To compensate for the non-specific nature of SERS, other potential methods, such as employing biointerfaces that may exclusively bind certain moieties (i.e., viruses, proteins, and antibodies) are now getting attention. By using SERS together with this strategy, more specific signals can be gained from the capturers that can overlap with the specific target signals. [22] In this review, engineering different binding sites to assemble and tune plasmonic surface structures of gold and silver nanoparticles and their applications in SERS to identify and detect illicit drugs are summarized.

2. chemical analysis with SERS

SERS is a prevailing method and makes it possible to implement different analysis, even if only a trace amount of sample is available. At present, SERS has been employed for qualitative and qualitative detections, which can respond to the need for sensitive, reliable, and rapid analysis. One of the main advantages of SERS over Raman is that due to the low sensitivity, Raman is more applicable for the characterization of molecular structures rather than molecular detection.[23] Figure 2 is a schematic to show the differences between SERS and Raman scattering.

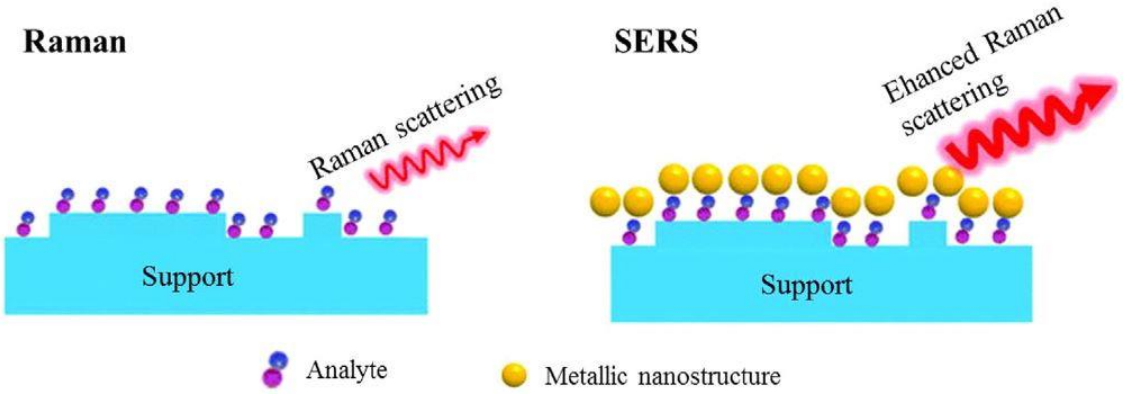

Figure 2. The Schematic of Raman spectroscopy and surface-enhanced Raman.[24]

2.1. SERS substrate development

In recent years, considerable efforts have been devoted to the development of new substrates for SERS. Usually, the emphasis of these works has been on optimizing the enhancement factor by manipulating the structure of the metallic nanoparticles. However, in most cases, the failure of SERS in molecular detection is because the target analyte has not adsorbed on the surface properly; thus, selectivity toward the analyte is not enough. Therefore, it is crucial to have available methods for promoting adsorption of target molecules onto the surface of metal nanoparticle materials. For this purpose, surface functionalization was gradually used to provide a more selective

substrate for analyte detection. Functionalization of SERS substrates leads to higher selectivity, and more specific signals can be thus gained from the interferences that can overlap with the specific target signals. Sometimes the SERS formation may change drastically as a result of interaction between the target molecule and capturer molecules.[25] Taking advantage of the method, a series of works was explored to use surface functionalization to improve sensitivity toward illicit drug detection.

2.2. Functionalization methods

Dopamine is an essential neurotransmitter in the hypothalamus owing to its vital function in the pituitary gland and hypothalamus, which directly affects the emotion in the midbrain. Its level is highly related to some advanced diseases, such as Huntington, Parkinson, and Schizophrenia. Due to the crucial role of dopamine in the disease modulation, it is critical to develop a sensitive approach for monitoring dopamine. Ibuprofen is a pain killer which is frequently used for headaches, muscle aches, and menstrual pain. But over usage of this drug can cause dizziness, headache, nausea, and nervousness. Therefore, it is crucial to have a reliable method for the detection of ibuprofen.

Sebok et al.[26] proposed a selective substrate by adsorption of L-cysteine and L-glutathione on the surface on the gold substrate for selective detection of Ibuprofen and Dopamine, respectively. In this study, The SPR chip was a thin gold layer (50 nm thickness) deposited on a glass substrate. Cysteine and glutathione were immobilized on the surface of SPR gold chips from their aqueous solutions in the concentration range of 0.01-10 mmol dm^{-3}, with the optimum concentration of 0.5 and 1 mmol dm^{-3} bounded on the surface after washing, respectively. Adsorption of L-cysteine and L-glutathione was not fully reversible, and after washing with water, a significant part of the adsorbed amount remained bound at the gold surface. Despite the reversible adsorption of ibuprofen and dopamine on the gold surface, adsorption on the

functionalized surface showed that a significant amount of dopamine and ibuprofen remain bounded to surface (Fig.3). This can be attributed to the strong chemical bonds formed between the molecules and functionalized gold surface, which helps a better capturing of the target molecule on the surface, resulting in a higher level of selectivity.

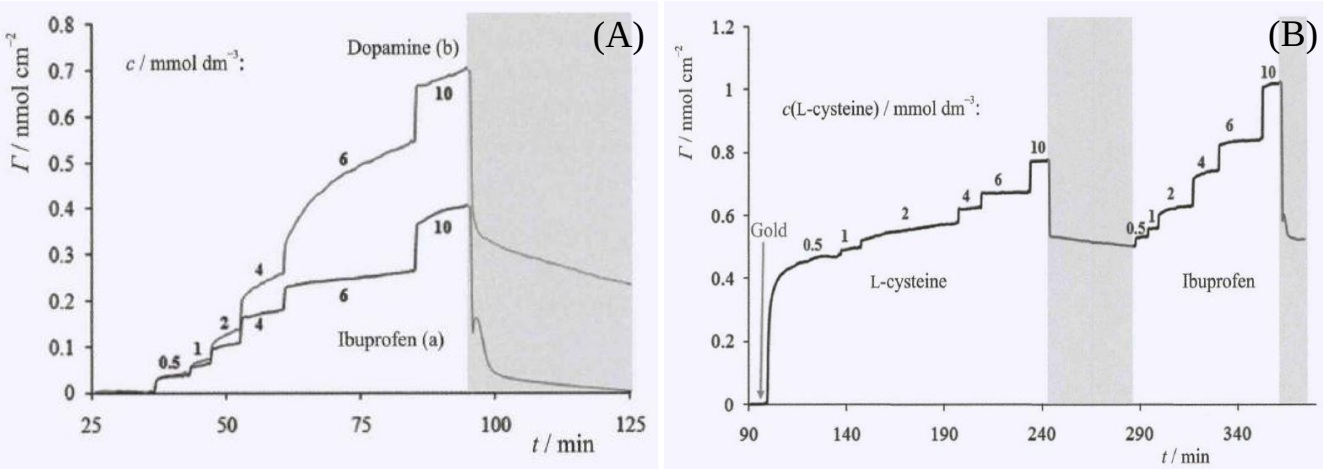

Figure 3. A) Representative SPR curves of adsorption of ibuprofen (a) and dopamine (b) on the gold surface from aqueous solutions at different concentrations 0.5, 1, 2, 4, 6, 10 mmol dm-3). Washing with water is denoted by a gray area. B) Representative SPR curves (sensorgrams) of adsorption of ibuprofen on L-cysteine functionalized gold surface from aqueous solutions, (at

In another study, Yu et al.[27] proposed a method to quantitively monitor the level of dopamine using Ag nanoparticles (NPs). In this method, after the synthesis of Ag NPs in silver nitrate solution, citrate was employed as both the capping agent of Ag NPs and the sensing agent of dopamine. Citrate was self-assembled on the surface of Ag nanoparticle dimers by reacting with carboxylic groups on the surface of Ag NPs, forming a stable amide bond. Ag dimers were fabricated by the aggregation of citrate-capped Ag NPs upon Hexamethylenediamine (HMD) linkers. Dopamine was allowed to infuse into the SERS biotags for a minimum of 4 hours before the SERS

measurement. A high SERS hotspot region with an intense electric filed was generated at the gap of the Ag nanoparticles dimer, allowing highly selective detection of dopamine. A wide linear response range of 30 pM to 300 nM and a limit of detection of 20 pM were obtained for quantitative detection. Figure 4 shows a Schematic illustration of the preparation process of the Ag NP dimer hybrid structure and the detection of dopamine via SERS.

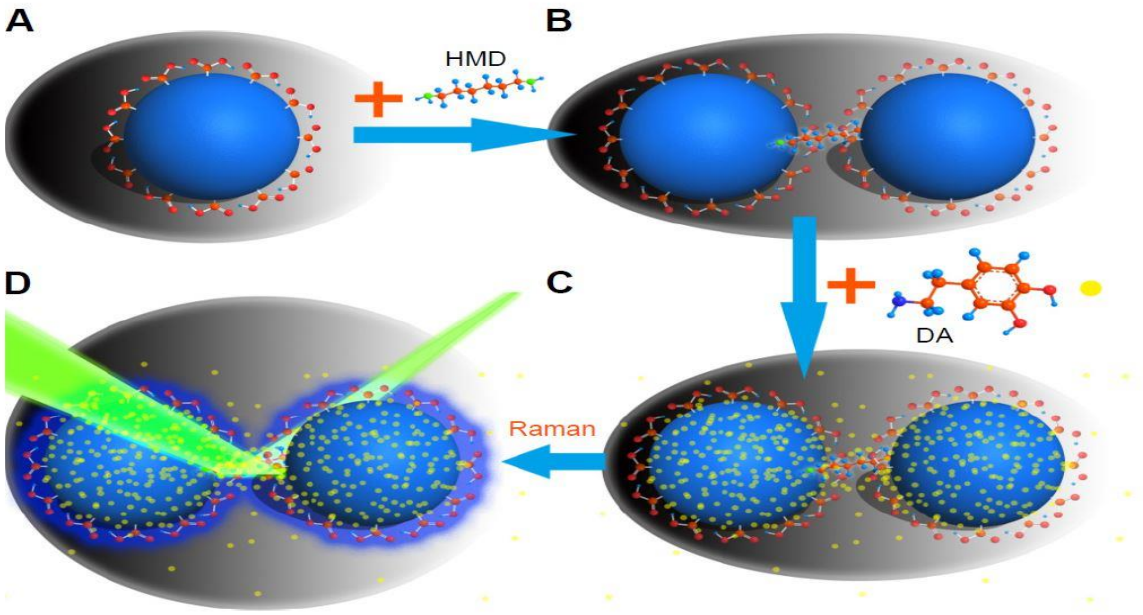

Figure 4. Schematic illustration of the preparation process of the Ag NP dimer hybrid structure and the detection of dopamine via SERS.(A) Ag NPs modified by citric acid; (B) Ag NP dimers further induced by the addition of HMD; (C) dopamine molecules adsorbed on Ag NPs surface by chemical adsorption; (D) the level of dopamine can be monitored via measuring the SERS signal enhanced by Ag NP dimers.

Cocaine is a highly addictive drug that can heighten activity in the body, including heart rate, blood pressure, alertness, and energy. Except for approved medical use, there is no safe way to use cocaine in any form. Some of the side effects of cocaine are increased body temperature, heart rate, blood pressure, weight loss, nausea, abdominal pain, and tremors.

Sanles et al.[28] precisely analyzed the label-free determination and quantification of benzoylecgonine (BCG), a major cocaine metabolite, on SERS substrate. In this work, the determination was based on watching the vibrational changes occurring at the surface of a monoclonal antibody, as the bio-interfaces, on silver-coated carbon nanotubes (CNT@Ag). CNT@Ag provides a stable substrate for supporting the bio-interfaces, with a high density of hot spots. To functionalize the surface with thiol groups, thioglycolic acid was added to 1.0 mL of CNT@Ag and left for 12h so that the thiol was adsorbed on the silver surface. Coupling of the monoclonal antibody to thioglycolic acid-functionalized CNT@Ag was achieved using carbodiimide chemistry to conjugate the primary amines of the antibody with the carboxyl groups of thioglycolic acid. A small amount of BCG (10 mL 10^{-5} M) was added to 0.1 mL solution. The formed substrate had a considerable selectivity toward the detection of BCG. Therefore, it can be used as an indicator of drug abuse. Figure 5 shows a schematic representation of the label-free detection of BCG on CNT@Ag.

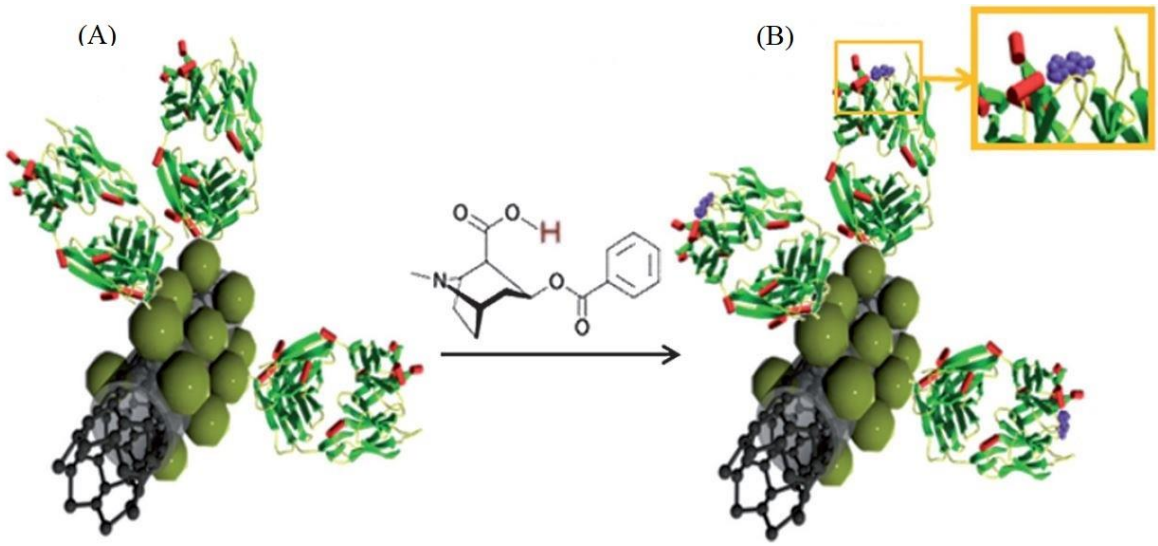

Figure 5. The label-free specific indirect detection of BCG on CNT@Ag. A) corresponds to the fragment antibody region adsorbed on the CNT@Ag substrate. B) complex monoclonal antibody–BCG system.[29]

In another study, Chen et al.[30] introduced a reagentless aptameric sensor based on SERS with “signal-on” architecture using a model target of cocaine. The SERS substrate was arranged by using an Ag colloid film on a polished gold disc. To improve the response performance, the sensor was modified by self-assembly of 3-mercaptopropionic acid (MPA) and a 5’-terminal thiolated oligonucleotide aptamer with a tetramethylrhodamine (TMR) in the presence of cocaine. This immobilization method optimized the aptamer’s orientation on the surface since it offered something similar to “molecular-imprinted” cages for the aptamer and expediated the folding of the aptamer upon binding to the cocaine target. Under the optimum condition, cocaine can be determined at a low concentration of 1μm and in the range of 1 to 3200 mm. Figure 6 shows the preparation of the aptameric sensor.

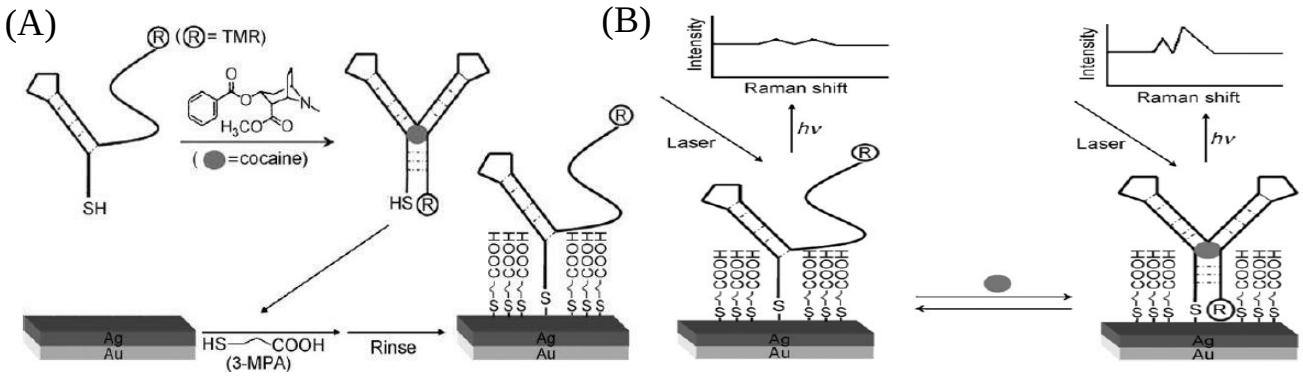

Figure 6. The schematic diagram for the preparation (A) and analytical principle (B) of the aptameric sensor for cocaine.

3,4-Methylenedioxymethamphetamine (MDMA), commonly known as ecstasy (E), is a psychoactive drug primarily used as a recreational drug. The desired effects include altered sensations, increased energy, empathy, and pleasure. But it has adverse side effects including addiction, paranoia, teeth grinding, and rapid heartbeat.

Stewart et al.[31] developed a modified SERS substrate by functionalization of Ag nanoparticles with mixed thiols, which promotes the adsorption and detection of MDMA. MDMA does not adsorb on conventional SERS substrates, even at 10^{-3}M,

which makes its detection difficult. To promote MDMA adsorption, colloids were modified with self-assembled monolayers of sodium mercapto propane sulfonate (MPS) and benzyl mercaptan (BZM) to introduce thiols groups to the surface and form a system with an intermediate level of selectivity. In order to get the best modification result, different ratios of MPS: BZM were prepared and applied for surface modification. This was done by the addition of a mix of the solution of modifiers to the conventional hydroxylamine-reduced silver colloid. This allows high selection detection of MDMA in complex mixtures with a detection limit of < 50 ppm.

3. summary and outlook

In this review, the development and application of functionalized SERS substrates for selective detection of common illicit drugs were summarized. Sometimes, it is hard to get the signal of target analyte directly when it is in a complex matrix. One way to improve the selectivity toward the detection of an analyte in the presence of interferences is to modify the surface and by capturing the target analyte on the surface, amplify its signal and enhance the detection performance. It is a versatile and robust method and a powerful diagnosis method for the analysis of illicit drugs. The results showed that this approach facilitates the generation of portable sensors for fast quantification and detection of illicit drugs and makes this method quite promising for the real-time and non-invasive detection of drugs for specific clinical applications.

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