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Rapid duplexed detection of illicit drugs in wastewater using gold nanoparticle conjugated aptamer sensors

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Abstract: The abuse of illicit drug addiction is a worldwide public health and social problem. In this paper, we reported on a simple and rapid colorimetric biosensor for duplexed detection of methamphetamine (METH) and cocaine in a single assay. Gold nanoparticles (AuNPs) and Au@Ag NPs were synthesized and functionalized with DNA reporter probes (RPs) for METH and cocaine, respectively. The magnetic beads (MBs) were conjugated with two capture probes (CPs) respective to METH and cocaine. The respective RPs and CPs were designed to hybridize with each illicit drug-binding DNA aptamers through DNA-DNA hybridization, forming a sandwich structure. This MBs-based sandwich structure could be removed with an external magnetic field. However, due to the higher affinity of DNA aptamers with illicit drugs, the sandwich structure was disassembled when illicit drugs are introduced into the solution, leading to the colour changes of the supernatant. Utilizing a non-negative matrix factorization (NMF) algorithm to process the data, we demonstrated the ability

of our biosensor for the simultaneous quantification of two illicit drugs. Under the optimal condition, our sensors were able to detect both METH and cocaine at the nM level with a wide dynamic range. This sensing platform provides a huge potential on drug consumption evaluation at the community level for wastewater-based epidemiology.

Keywords: illicit drug of abuse, wastewater-based epidemiology, colorimetric biosensor

1. Introduction

Illicit drug abuse has become a global concern considering its close relationship with the increasing incidence of crimes and severe social problems (Cyranoski, 2018; Galanie et al., 2015; Merz, 2018). Methamphetamine (METH) and cocaine (COC) and are two of the most widely abused illegal drugs in the world (EMCDDA, 2016; Merz, 2018; Mokhtarzadeh et al., 2015; Xu et al., 2017). To evaluate the pattern of illicit drug consumption, a new approach namely wastewater-based epidemiology (WBE) has emerged in the past decade. Usually, the drug residues and their metabolites would be excreted by humans in a given area (e.g., a city and a community) pool in wastewater at a certain wastewater treatment plants. WBE is conducted by collecting wastewater samples and analysing the concentration of targets in wastewater, and thereby can be used to monitor the community-wide drug consumption taking into account population served, stability of drug residues, excretion rates, and wastewater volumes (Cyranoski, 2018; Du et al., 2017; Du et al., 2018; van Nuijs et al., 2011; Zuccato et al., 2005). Compared to the conventional population survey method, WBE holds several advantages such as more objective and much less time-consuming (Du et al., 2018; Ettore et al., 2008), and thus this strategy

has been widely used for the monitoring of illicit drugs in many countries (Du et al., 2017; EMCDDA, 2016; Thomas et al., 2012), including America (Foppe et al., 2018), Australia (Bade et al., 2019) and many European countries (Castrignanò et al., 2018; EMCDDA, 2016). To get the concentration of wastewater, the widely employed analytical instrument is mass spectrometry (MS) coupled with high-performance liquid chromatography (HPLC), which is robust, highly sensitive and selective (Du et al., 2015; Gao et al., 2017). But these methods usually need a sample preparation before illicit drug analysis, such as the purification of raw wastewater with solid phase extraction. In addition, the compulsory sample preparation usually requires the well-trained personnel to operate an instrument for analysis and then interpret the data in the central laboratory. In order to evaluate illicit drugs of abuse, preferably at the site of sample collection, there is an urgent need to develop rapid, simple and inexpensive tools for detection illicit drugs in complex environmental samples.

As an rapid and cost-effective analytical tool, aptamer biosensor (aptasensor) has attracted increasing attention for determination of illicit drugs, such as cocaine, methamphetamine, codeine and ketamine (Asghary et al., 2018; Lodha et al., 2014; Mao et al., 2018; Mokhtarzadeh et al., 2015; Roncancio et al., 2014). The signal of sensors from the binding between DNA aptamers and illicit drugs can be detected with colorimetry (Li et al., 2013), fluorescence (Stojanovic et al., 2000) and Surface-Enhanced Raman Spectrometry (SERS) (Mao et al., 2018), electrochemistry (Yang et al., 2016), and other techniques (Neves et al., 2015; Ya et al., 2015). We have recently developed electrochemical aptamer sensors for the determination of cocaine in wastewater, which demonstrated the cocaine consumption trends with a weekend peak based on the sampling in a wastewater treatment plant in the Southwest of England (Yang et al., 2016). For METH determination, we have developed a new

colorimetric strategy using G-quadruplex DNAzyme for the determination of METH (Mao et al., 2016). Although these assays mentioned above show the great capability for single illicit drug determination, there are no reports on biosensors for simultaneous determination of multiple illicit drugs so far. In fact, a variety of illicit drugs (such as METH, cocaine, et al.) are usually present in the sample at the same time, which are widely distributed and transported in natural water and wastewater (EMCDDA, 2016). Although one can use several biosensors for the determination of various targets separately, it is costly and time-consuming. Thus, there is a great need for the development of simultaneous determination of multiplexed illicit drugs within a single assay. This may provide opportunities for the monitoring of illicit drugs at a trace level in the environment matrixes.

The rapid development of nanomaterial-related technologies (Tang et al., 2018) provides a huge opportunity for designing selective and sensitive aptasensors for the determination of illicit drugs. For example, recent development and applications of nanomaterial-based illicit drug aptasensors by using optical and electrochemical analytical techniques for illicit drugs determination have been reviewed (Kumar et al., 2018; Mokhtarzadeh et al., 2015). In these nanomaterials, AuNPs has been widely used to improve the sensitivity due to their unique properties such as facile preparation methods and excellent optical properties (Shen et al., 2010; Xie et al., 2011). Magnetic beads, have been widely applied for determination and separation due to their wonderful magnetic separation characteristics, excellent binding kinetics, good maneuverability, and biological targeting properties (Song et al., 2014; Wen et al., 2014). However, to our knowledge, the analytical method based on the combination of gold nanoparticles and MBs for the multiplexed determination of illicit drugs has not yet been reported.

Hence, we designed a new biosensing strategy for the simultaneous multiplexed determination of illicit drugs in a single assay, which offers a rapid signal response of targets through simply mixing DNA aptamers for signal readout. Our platform based on non-aggregated AuNPs together with magnetic beads displayed high selectivity and sensitivity towards the targets, which holds unique advantages by comparing classical colorimetric method and could be extend for more wide application. Because AuNPs coated with silver-Au@Ag show similar determination capability with AuNPs but with different optical signals, we combined Au@Ag NPs with AuNPs together to develop a colorimetric system for multiplexed determination of METH and cocaine. In order to prevent ambiguities related to colour determination by visual assay, we introduced a data algorithm analysis system based on functional non-negative matrix factorization (NMF) for targets quantification, which was consistent with the colorimetric result. According to this finding, the feasibility of non-aggregated metal NPs colorimetric determination of two illicit drugs in a single assay was demonstrated for the first time. We believed that colorimetric analysis based on the combination of a non-aggregated noble metal nanoparticle and NMF analysis has a huge potential as a generic platform for the multiplexed determination of a variety of targets.

2. Materials and Methods

2.1. Materials

All oligonucleotide sequences were purified by HPLC and ordered from Sangon Biotech Co. Ltd. (Shanghai, China) (detailed in Table S1). Carboxyl-modified magnetic beads (MBs, 1.05 μm Dynabeads^{TM} My One™, 10 mg mL^{-1}) were bought from Thermo Fisher Scientific. Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) and silver nitrate ($AgNO_3$) were ordered from Shanghai Chemical Reagent (Shanghai, China) Co., Ltd. All illicit drugs and metabolites, including METH and cocaine were bought from

Cerilliant (Round Rock, USA). The ultrapure water is purified using a Millipore filtration system (18.2 MΩ·cm) in the whole experiments. All experiments were performed in compliance with the relevant laws and institutional guidelines, and all reagents in this experiment have been agreed by the institutional committee (Peking University, the People's Republic of China).

2.2. Instrumentation

A Scanning Electron Microscope (SEM) (ZEISS, SIGMA) was used to obtain the SEM pictures of AuNPs and Au@Ag. A JEM-2100 (HR, Japan) microscope was utilized to obtain the TEM images of AuNPs and Au@Ag NPs. UV-vis spectra of AuNPs and Au@Ag were recorded by Lambda 35 UV-vis spectrometer (Perk Elmer, USA) through 80 μL quartz cells with a path length of 1 cm.

2.3. Synthesis of AuNPs and Au@Ag

In this experiment, AuNPs stabilized by citrate were synthesized as follows: 50 mL $HAuCl_4$ (0.01%, w/w) was reduced by addition 750 μL trisodium citrate (1%, w/w) at 120 °C using magnetic stirring for 25 minutes until the obtained reaction mixtures turned to be wine red. Au@Ag NPs were synthesized in a seed-mediated method following the previous report (Shen et al., 2010). Firstly, 600 μL 0.5 % (w/w) $AgNO_3$ solution was introduced to boiling gold seed solution (100 mL). Then, 1 mL1 % (w/w) sodium citrate solution as the reducing agent was introduced dropwise under stirring. The mixture was boiled for 1 h and Au@Ag was ready to use after cooling down.

2.4. The preparation of RPs and CPs

The RPs were prepared as follows: The two types of NPs were conjugated with the different RP DNA sequences by treating citrate-stabilized nanoparticles with a solution containing thiol-modified oligonucleotides, respectively (Figure S1A). 15

nmol thiolated RP DNA sequence was introduced to 5 ml Au@Ag NPs or AuNPs nanoparticles suspended in PB buffer. After 24 h incubation, 2 M NaCl was introduced to reach the salt concentration at 0.05 M and then increased to 0.1 M after standing for 8 h. The nanoparticles were isolated through centrifugation and washed with PBS-T solution after aging in 0.1 M NaCl for two days.

The CPs were prepared as follows: The carboxylated MBs were conjugated with the CP DNA following the manufacturer's protocol. 2.5 mL carboxylated MBs were washed two times with 2.5 mL MES solution and the MBs were re-suspended in 250 μL MES buffer before immobilization. The mixture of 36.2 nmol amino-modified CP DNA and 36.2 μmol EDC HCl was introduced to MBs solution and incubated at 25 ℃ by gently shaking overnight. In the end, to quench excess activated carboxylic acid groups on the surface of MBs, the MBs were incubated with 50 mM Tris solution for 15 minutes at 25 ^{o}C by gently shaking. The coated MBs were washed three times using 2.5 mL Tris solution and then re-suspended in PBS-T solution for use.

2.5. Elaboration and optimization of biosensors

2.5.1. Elaboration of biosensors

As shown in Scheme 1, this sensing platform consisted of a CP, RP and an aptamer. The aptamer bound CP and RP through hybridization forms a double-stranded DNA (dsDNA). After removing Au@Ag NPs-DNA-MBs or AuNPs-DNA-MBs complex by an external magnetic field, we can observe a decreased absorbance signal of Au@Ag solution. However, the target illicit drugs can prevent the formation of dsDNA due to a higher affinity between the targets and the designed aptamer. The dose of illicit drugs could generate a different signal that can be measured by a UV-vis spectrometer.

To evaluate the feasibility of the biosensing mechanism, a typical non-aggregated

Au@Ag nanosensor for METH determination was acted as a case study. 5 μL METH (cocaine, 5 μM) was mixed with 5 μL of 1.2 μM METH aptamer (or 1 μM cocaine aptamer) solutions, followed through adding 20 μL PBS-T solution. After incubation for 30 minutes, 1 μL CP and 50 μL RP were introduced to the mixture and then hybridized reaction with gentle shaking for 90 minutes. The total volume was kept at 150 μL by added PBS-T solution. After hybridization reaction, the magnetic beads with target-linked AuNPs or Au@Ag NPs together with unreacted MBs were removed by using an external magnetic field. We can measure the supernatant for signal readout after the separation by a UV-vis spectrometer in 80 μL quartz micro-cuvette at 25 ^{o}C.

We also carried out control experiments with following mixtures: CP and RP; CP, RP, and illicit drug; CP, RP, and aptamer. In our experiment, each component was introduced as the same volume and concentration as in the presence of METH or cocaine. A certain volume of PBS-T solution was added into the mixture to obtain a 150 μL constant volume in total.

2.5.2. Optimization of the determination conditions

The MBs concentration had a significant effect on the sensitivity of the assay because the ratio between the CPs and RPs would change. To ensure a highly sensitive analysis, a series of MBs concentration from 3.3 μg L^{-1} to 3.3 $\times 10^{2}$ μg L^{-1} (3.3, 6.6, 3.3 $\times 10^{1}$, 6.6 $\times 10^{1}$ and 3.3 $\times 10^{2}$ μg L^{-1}) were tested under the same conditions to get the optimal concentration of the MBs.

The hybridization time was also a significant parameter for colorimetric assay. The hybridization time was studied through measuring the peak intensities of UV-vis spectra. The time interval was recorded 15 minutes a time ranging 0-105 minutes.

The optimal METH aptamer concentration which may influence the UV-vis spectra

of Au@Ag NPs and AuNPs was determined by recording peak intensities of UV-vis spectra at 400 nm and 520 nm, respectively. The biosensor was optimized with the various aptamer concentrations (METH aptamer: 0, 10, 20, 30, 40, 50, and 60 nM; COC aptamer: 0, 10, 20, 30, 40, and 50 nM).

2.6. Analytical performance evaluation

Under the optimal conditions, we evaluated the sensitivity of the sensor to detect METH by testing various METH concentrations ranging from 0 nM to 200 nM and cocaine from 0 to150 nM.

The selectivity of the sensor was examined by comparing the result with seven common illicit drugs and metabolites, namely cathinone (CAT), methcathinone (MCAT), 3-trifluoromethylphenylpiperazine (BZP), ketamine (KET), morphine (MOR), norketamine (NK), and MDA. The experiment followed the same procedure for the determination of other drugs and metabolites at 1 μM, the response of which was compared with the signal from METH and cocaine at 50 nM.

To explore the performance of our developed biosensors in real applications, effluent wastewater samples (collected from Xiaojiahe Wastewater Plant in Beijing, China) were filtered with 0.22 μm microporous membrane to remove suspended particulate matter (SPM), followed by spiking METH and cocaine into the wastewater for a final concentration at 75 nM. Spiked wastewater was detected by our biosensor.

2.7. Non-negative matrix factorization (NMF) analysis

"Multiplicative" iteration rules-based NMF was a multi-variant analytical system by non-negative constraints for the generation of the approximate data and application in an analytical science (Berry et al., 2007; Xie et al., 2011). The principle of NMF analysis that processed the UV-vis spectra in this experiment as shown in Figure 1: Given one initial non-negative matrix (V), it could find two different non-negative

matrices to approximate the original matrix. The one was the basis matrix (*W*) and the other one was the coefficient matrix (*H*). When conducting data analysis, all UV-vis spectra were combined to form matrix *V*. The number of factors would be set as 2 due to that two nanoparticles (Au@Ag and AuNPs) existed in the solution. Low-rank matrices *H* and *W* automatically started from random matrices and stopped while the matrix doesn't change for 10,000 iterations. Matrix *H* corresponded to the intensity coefficient and *W* corresponded with the basic spectra for pure Au@Ag NPs and AuNPs. UV-vis spectra results were exported from the instrument software and imported into MATLAB where NMF data processing was performed to directly quantify of METH and COC.

3. Results and discussion

3.1. Colorimetric sensing principal for METH and cocaine

The AuNPs and Au@Ag NPs were synthesized and characterized by UV-vis spectrometer, SEM and HR-TEM. As shown in Figure 2, AuNPs and Au@Ag NPs were approximately 40 nm with a uniform size (Figure 2b and Figure 2c). Figure 2 (d) indicated a thinner silver layer surrounds the gold core because of the different contrast between these two materials. Figure 2a demonstrated that the UV-vis spectra of Au@Ag NPs and AuNPs. The UV-vis signal peaks of Au@Ag and AuNPs were 400 nm and 520 nm, respectively. Compared with the absorption peak of AuNPs, the peak of Au@Ag NPs solution was blue-shifted because Au@Ag NPs and AuNPs with the same particle size had different surface plasmon resonance (SPR) frequencies. This will facilitate to reduce the interference caused by the overlapping peaks when simultaneously detecting cocaine and METH.

The working principle of our colorimetric biosensor was shown in Scheme 1. We tried to design two MBs-modified capture probe (CPs), one AuNPs and the other

Au@Ag NPs labelled reporter probe (RPs), and two aptamers which specifically bound with METH and COC, respectively. The CPs were superparamagnetic MBs with a carboxyl coating, and it would facilitate sample separation when activated by an external magnetic field. These MBs were functionalized with aminated oligonucleotides (CP DNA) with EDC·HCl as the linker, which could match fully one part of target sequence but different from the fragment complementary with RP DNA (see Figure S1B). The RPs were either Au@Ag NPs or AuNPs modified with RP DNA sequence which is partially complementary to the aptamer. Herein, each aptamer was complementary with its respective MB-conjugated CP DNA and nanoparticle-conjugated RP DNA, then forming a NPs-dsDNA-MBs sandwich structure, for both METH and cocaine.

To establish the multiplexed sensors for METH and COC determination, the mixtures in solution containing METH RP, METH CP, COC RP, and COC CP, and two DNA aptamers for illicit drugs were introduced into the solution. After the hybridization, an external magnetic field was able to adsorb the MBs-conjugated complexes, leading to the bound metal nanoparticle decreasing and color changes in the supernatant. However, in the presence of the target drugs, NPs-dsDNA-MBs sandwich structure complex can’t be generated because of higher affinity between the aptamer and the target than that from DNA-DNA hybridization. Furthermore, the color of the supernatant depended on the amount of two illicit drugs added to the solution. This interesting color change was useful for simultaneous determination of two targets from a single assay, enabling a novel colorimetric approach for the determination of two illicit drugs. Furthermore, this determination strategy avoided the aggregation between optical noble nanoparticles and the sedimentation, which would be beneficial to minimize the non-specific interference response. Due to that

this method only affected the peak intensity rather than the peak position, the UV-vis spectra signal featured an inherent simplicity for quantification.

3.2. Detection conditions optimization

We optimized a range of detection parameters to improve the sensitivity of our sensors, including the concentration of MBs, the binding time to generate NPs-dsDNA-MBs structure and the aptamer concentration. The optimized concentration of the MBs was determined to be 66.7 μg L^{-1}, showing in Figure S2. The results also suggested that the concentration of magnetic beads used to remove AuNPs is the same as that of Au@Ag.

The reaction time for the formation of NPs-dsDNA-MB was an important parameter for the evaluation of our sensors. Aptamers were introduced and the time set as 0 minutes, and the spectra signal changes are observed. The maximum absorption value of METH and cocaine (Figure S3) were recorded after the addition of DNA aptamers against METH and cocaine, respectively. The incubation was maintained at 25 °C for 110 minutes. Figure S3 demonstrated that the intensity of the UV-vis signal gradually decreased after the addition of the aptamer, and had a minimum value at 60 minutes. Therefore, we used the optimized 60 minutes for our assay.

The optimal concentration of DNA aptamers to quench the UV-vis signal of Au@Ag NPs and AuNPs were detected by obtaining absorbance intensities at 400 nm and 520 nm, respectively. Analysis of the plots in Figure S3c showed that the UV-vis signal of AuNPs decreased with increasing METH aptamer concentrations and nearly complete quenching occurred over 60 nM. In this process, it should be noted that if there is excessive METH aptamers, it may result in non-specific (i.e., no surface plasmon resonance enhancement) binding of the aptamer to the METH. As a result,

40 nM METH aptamer was optimized in this biosensor. Importantly, we observed METH obviously blocking of the quenching of the AuNPs by 40 nM aptamer, returning the UV-vis intensity of Au@Ag NPs up to 89.1% compared with its initial value (Figure S3c). Similarly, 30 nM cocaine aptamer was optimized and chosen for a sensitive assay (Figure S3d).

3.3. **Determination of single illicit drug with our sensors**

To check the feasibility of the sensing platform, the single-target model was firstly to test in our experiment. Firstly, the effects of this analytical method without or with METH, on the UV-vis signal of AuNPs nanoparticles were determined. As shown in the result, in a control experiment, stronger absolute absorbance intensity was observed (Figure 3a, MBs + AuNPs). When METH is introduced, the signal intensity of absorbance had no significant change (Figure 3a, MBs + AuNPs + METH), which indicated that only METH alone had almost no interference to this assay. However, once the addition of METH aptamer in the solution, the signal intensity of absorbance decreased dramatically (Figure 3a, MBs +AuNPs + Apt), due to the formation of the AuNPs-dsDNA-MBs sandwich structure according to the base pair matching principle. The complex would subsequently be eliminated by an external magnetic field. The removal of AuNPs nanoparticles decreased the absorbance intensity. When both METH and its corresponding aptamer were present, the absorbance drastically recovered (Figure 3a, MBs + AuNPs + Apt + METH). The signal was a little lower than the absolute absorbance intensity of AuNPs nanoparticles. The increase in signal intensity of UV-vis spectra with both METH and METH aptamer was due to the formation of METH-aptamer complex, which prevented the formation of the sandwich structure complex and therefore prevented the external magnetic field from removing the AuNPs nanoparticles. Similarly, in the absence of cocaine, Au@Ag NPs

can hybridize with aptamer to form a sandwich structure through DNA-DNA hybridization (Figure 3b, MBs + Au@Ag + Apt). When using an external magnetic field, the original deep yellow colour of the supernatant became light yellow. However, when introducing cocaine into the solution, the absorbance was drastically recovered and confirmed that it's the presence (Figure 3c, MBs + Au@Ag + Apt + COC). Hence, the color change of supernatant by certain illicit drugs demonstrated that it was feasible to use the platform for the duplexed determination of illicit drugs.

The intensity of peaks at 520 nm assigned to UV-vis signal form AuNPs clearly increased with the addition of the METH. Figure 3c showed the linear fit of the signal intensity of the supernatant solution as the function of the concentration of METH. The linear range for the detection of METH spanned from 1.0 to 200 nM under optimized condition. In similar way, when the concentration of cocaine increased, the signal intensity of the peak at 400 nm associated with Au@Ag NPs increase noticeably (Figure 3d), which led to the colour change of the supernatant solution from light yellow to deep yellow different from other reported colorimetric illicit drug detection method (Shi et al., 2015). The UV-vis spectra signal demonstrated a good linear change ranging from 10 nM to 150 nM.

3.4. **Duplexed detection of illicit drugs in a single assay using NMF analysis**

Simultaneous determination of different illicit drugs (METH and cocaine) in a single assay was performed through our biosensors. There were two directions of colour conversion (from orange to yellow or red) in this method, our biosensor thus enabled to run a single assay for the determination of two targets. We evaluated the feasibility of our biosensors for the determination of both METH and cocaine in a single assay and the analytical performance from colour changes. A 5×5 matrix was designed in a microplate, in which each element is a sample containing the different

concentrations of METH and cocaine. Figure 4b presented the 5×5 matrix photo showing the colour change of two illicit drugs at different concentrations in each microplate. Obviously, the colour change had a significant difference, and varied from 5-200 nM for METH and 1-150 nM for cocaine. Also, the performance of the double-colour analytical system was evaluated by UV-vis spectra measurements by constructing a 10×10 matrix varied from 0-200 nM for METH and 0-150 nM for cocaine, respectively. As shown in Figure 4a, both the absorption peaks of UV-vis simultaneously increased when the sample containing both METH and cocaine was introduced. However, the UV-vis spectra of solutions cannot be analyzed as easily as the visual signals due to the interference and overlap between UV-vis spectra of Au@Ag NPs and AuNPs. One of the reasons for such interference is that the UV-vis signal-active nanoparticles are not narrow enough to remove the spectra overlap, which may make it difficult to quantify it. To clearly analyze the UV-vis spectra signal and quantify the METH and cocaine, we made full use of NMF to factorize a series of experimental result and data into two basic data groups.

The concentrations of AuNPs and Au@Ag were proportional to the concentrations of the two corresponding illicit drugs. Therefore, the simultaneous quantification of two illicit drugs would be feasible through measuring the UV-vis spectra of these two noble metal nanoparticles. Through NMF analysis, we established a single quantitative analysis for two illicit drugs that worked even when it was difficult to quantify the targets in a complex sample because of the overlap of the two spectra. The target concentrations were quantified by analyzing the changes in the intensity of the signals of UV-vis spectra acquired by NMF. The interference of the spectral overlap between different UV-vis spectra-active nanoparticles could be solved automatically and easily through this data process system. The multivariate

evaluation scheme used in this assay could reduce the interference of spectral overlap on the scale of the whole UV-vis spectra rather than just a single peak as in their univariate counterparts. By using the relative intensities of the maximum peak intensity of Au@Ag NPs and AuNPs acquired from NMF, the results clearly demonstrated the real intensities of optical biosensor instead of the overlapping spectra (Figure S4). Figure S5 showed the quantitative result of the multiplexed determination of illicit drugs with two-colour colorimetry, which was almost in accord with Figure 3c and Figure 3d. Although the results verified the validity of the NMF system, the data before and after the NMF process echoed with each other and confirmed the feasibility of undisturbed linear analysis. As shown in Figure S5, the limit of detection (LOD) of METH was determined to be 0.5 nM, whereas the LOD of cocaine was 3.3 nM. The LOD of METH and cocaine were both lower than some previous reports (METH 50 nM (Shi et al., 2015) and cocaine 10 nM (Nie et al., 2013)), respectively, further illustrating the high sensitivity of our proposed approach.

In the presence of 7 illicit drugs and metabolites (1 μM) other than METH, absorbance signals were nearly as low as that from the blank. Meanwhile, these signals were much lower than that with METH and cocaine, though the concentrations of all the other illicit drugs and metabolites were much higher than 50 nM METH. What's more, compared with the blank the enhancement in absorbance intensity was not statistically significant among other illicit drugs, indicating that interference of these drugs to METH and cocaine determination was not specific (Figure 5a), which demonstrated that the affinity of illicit drug to certain corresponding aptamer was much better and higher than all other illicit drugs, rendering color array with wonderful specificity toward METH and cocaine. Taking into account the complex matrix of wastewater samples, if we want to use the

biosensor for evaluation the pattern of illicit drugs of abuse for WBE, it's worthwhile testing a large cohort of samples to for the calibration curves and improve reliability of our sensors

3.5 Analysis of illicit drugs in wastewater with our biosensors

To evaluate the ability of our colorimetric method for complex sample (wastewater) detection, we used our biosensors for the determination of METH (75 nM nominal concentration) and cocaine (75 nM nominal concentration) spiked into the wastewater samples. As shown in Figure 5b, the result we detected for the colour signal intensity of wastewater with cocaine and METH was very much close to that from the buffer, while wastewater without cocaine and METH were all under the threshold value, indicating a negligible matrix effect on our sensing platform. Compared with the measured concentration and real concentration of illicit drugs, the average recovery of METH and COC in spiked effluent wastewater sample are 85.5% and 83.9%, respectively. The results showed great potential for our sensors for the multiplexed determination of illicit drugs in wastewater to evaluate of illicit drug consumption at the community level.

4. Conclusions

In summary, we have described non-aggregated noble metal nanoparticles (AuNPs and Au@Ag) based colorimetric method for the sensitive determination of illicit drugs using METH and cocaine determination as a case study. The biosensor consisted of RPs, CPs, and illicit drug-binding DNA aptamers, where the DNA aptamer could hybridize with both RP and CP, generating NPs-dsDNA-MBs sandwich structure. When an external magnetic field is used, the sandwich structure was removed from the solution, leading to the absorbance intensity decreasing. The absorbance intensity was correlated with the concentrations of illicit drugs, enabling the quantification of

the drugs. To solve the spectral overlap in this sensing platform, the information of supernatant was used for the quantification of multiplexed targets with an NMF analysis. Through an automatic NMF analysis, our colorimetric biosensors provided a simple and rapid quantification platform for two illicit drugs analysis at nM level in a single assay. These results showed that our proposed sensors have a huge potential on estimating the consumption of illicit drugs for WBE.

There is a growing requirement to monitor illicit drugs and other emerging contaminants in wastewater. Aptamers are synthetic single-stranded DNA which can specifically bind to a wide range of respective targets with a promising selectivity and sensitivity. This will enable our proposed biosensors to be used as a generic platform to detect a variety of contaminants by simply replacing certain corresponding aptamers, and easy to be improved from duplex assay to highly multiplexed determination for a range of substances. To obtain a reliably detection, it was suggested to test a large cohort of wastewater sample with a proper validation method. We believe that the rapid sensors will play an increasing role of monitoring of illicit drug of abuse and contribute as an alternative way for WBE.

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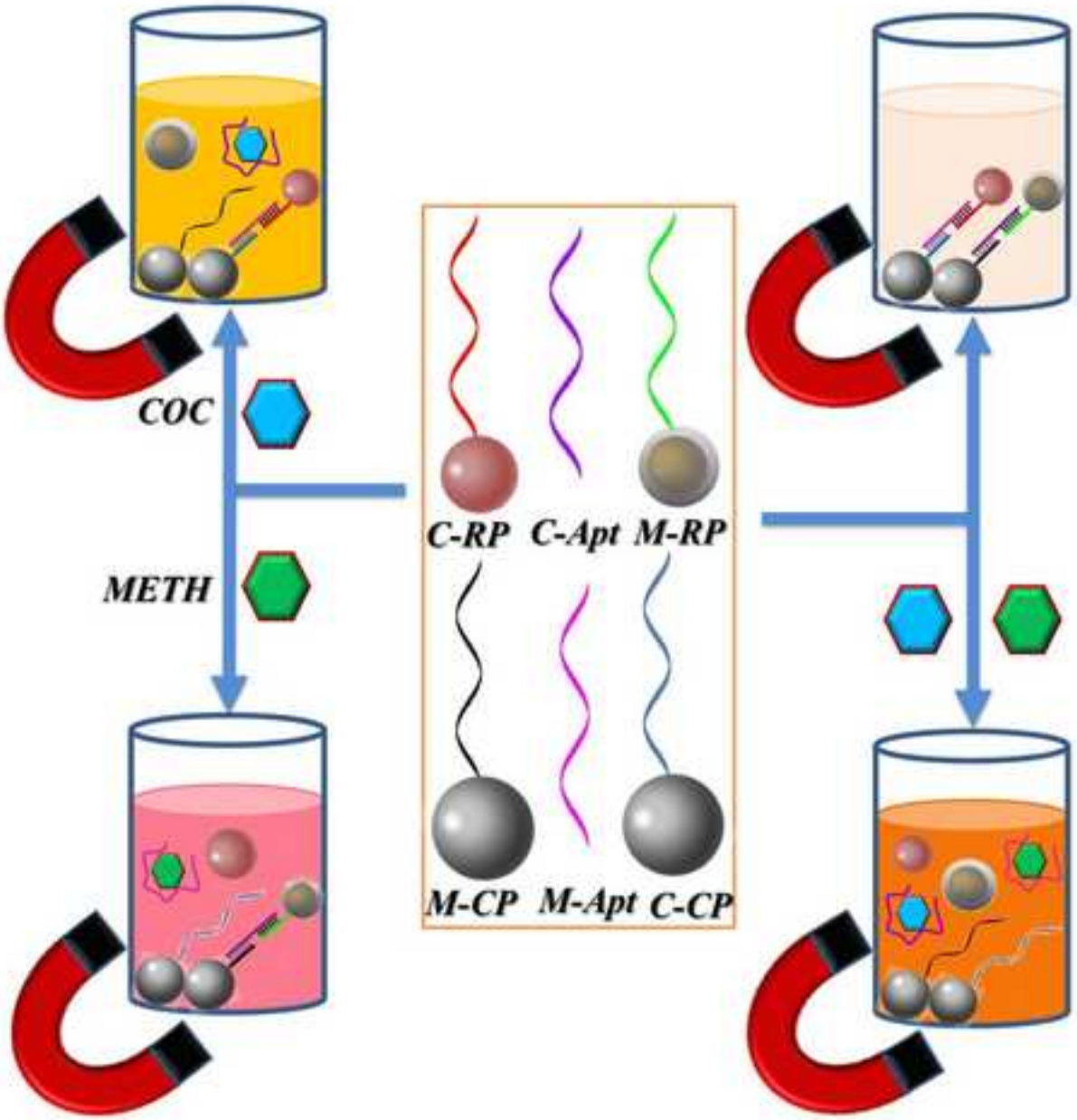
Highlights

- We developed a novel nanoparticles-based biosensor for the detection of illicit drugs.
- High selective and sensitive detection of two illicit drug (LOD of METH is 0.5 nM and cocaine is 3.3 nM) in a single assay.
- The biosensor enables a generic platform for the detection of illicit drugs in wastewater.

*Graphical Abstract



Figure
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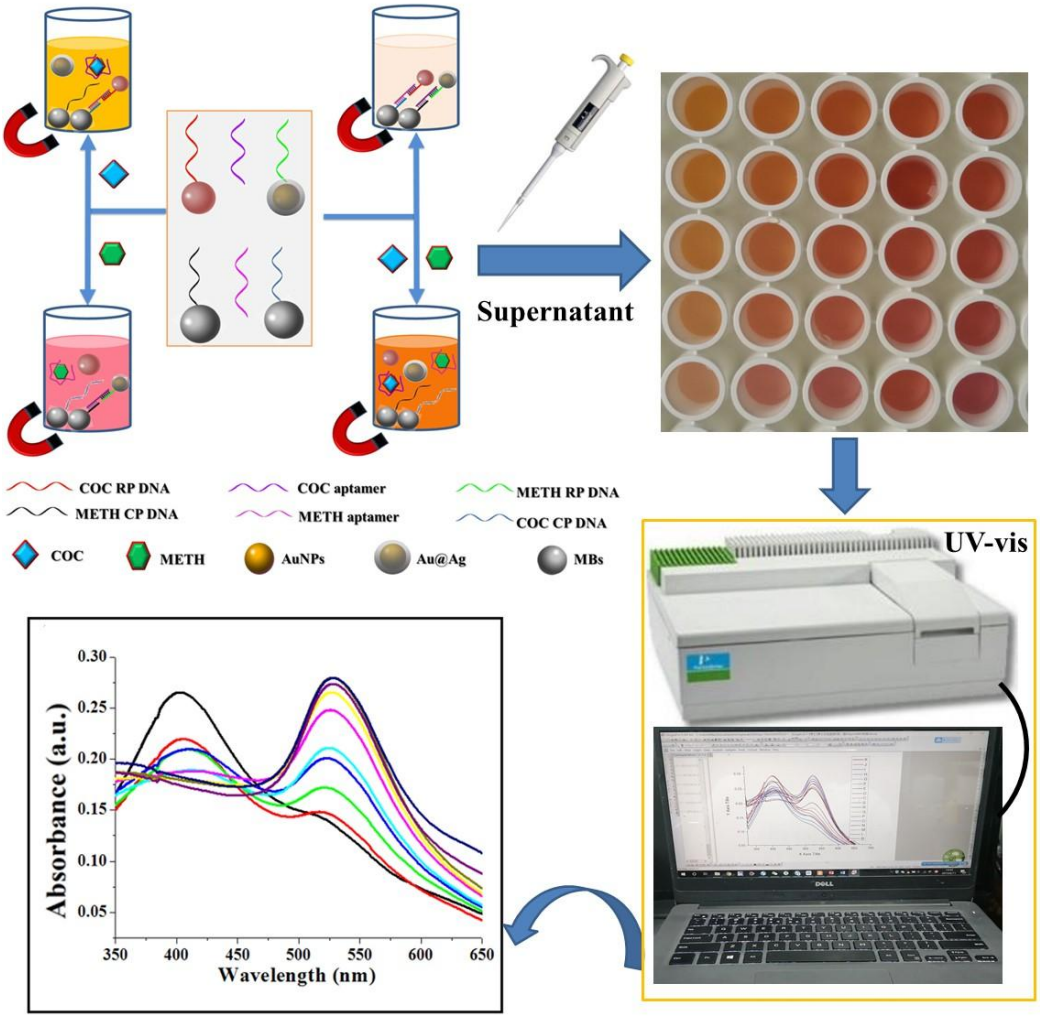

Scheme 1. Schematic illustration of colorimetric detection of METH and cocaine based on non-aggregated nanoparticles (C-RP: cocaine repoter probe, M-RP: methamphetamine repoter probe, M-CP, methamphetamine capture probe, and C-CP: cocaine capture probe).

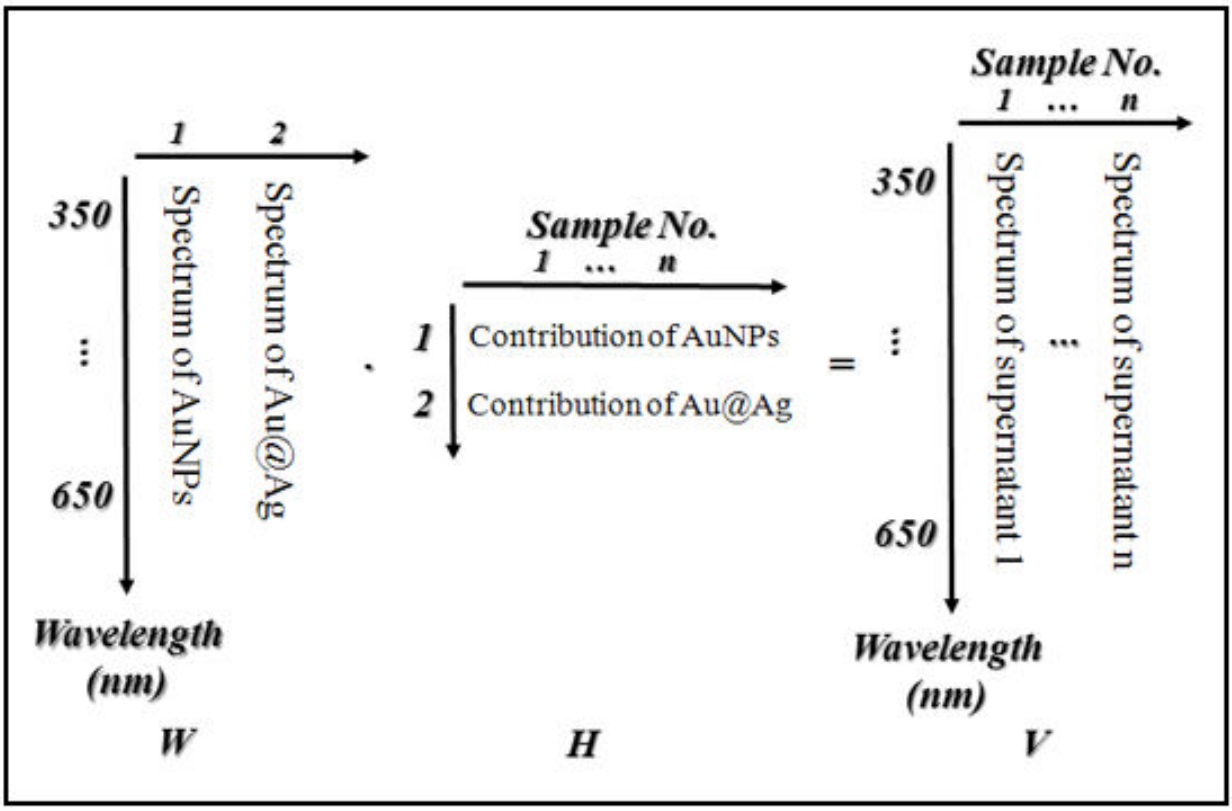

Figure 1. The principle of NMF analysis that processes the UV-vis spectra.

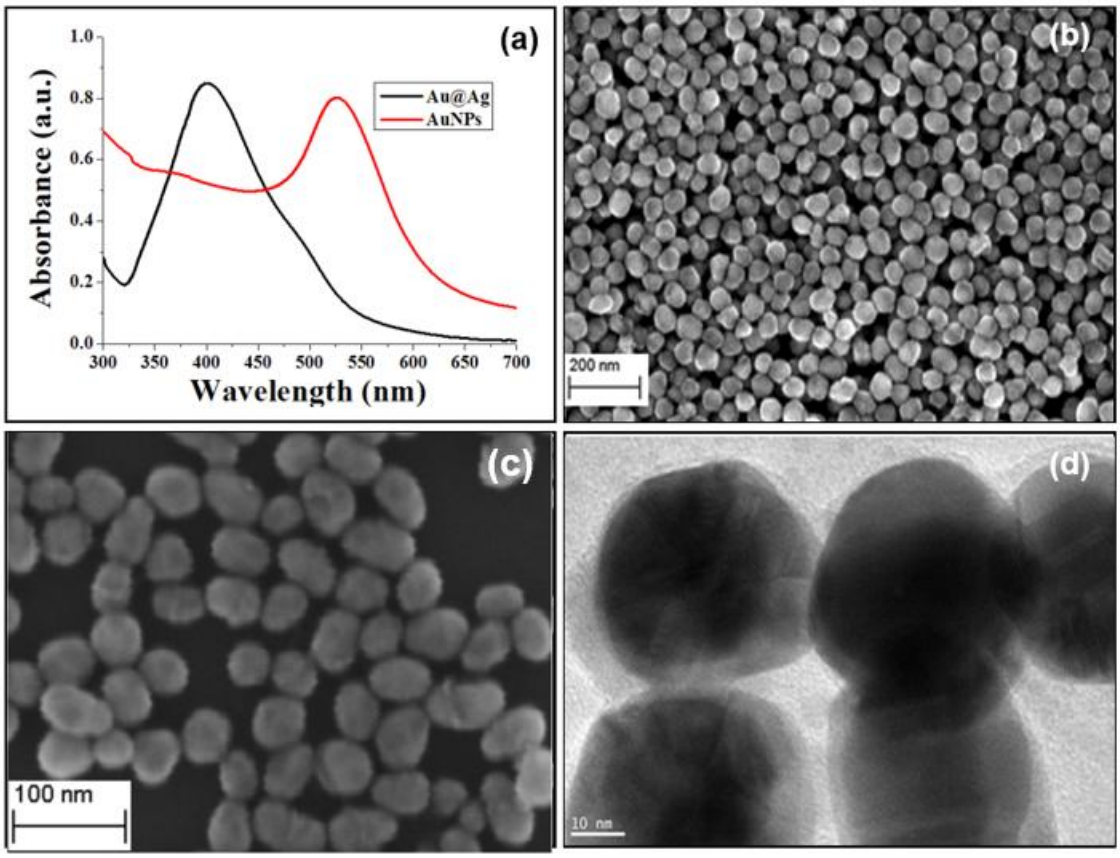

Figure 2. (a) UV-vis spectra of Au@Ag and AuNPs; (b) SEM image of AuNPs; (c) SEM image and (d) high resolution transmission emission microscope (HR-TEM) image of Au@Ag core-shell nanoparticles.

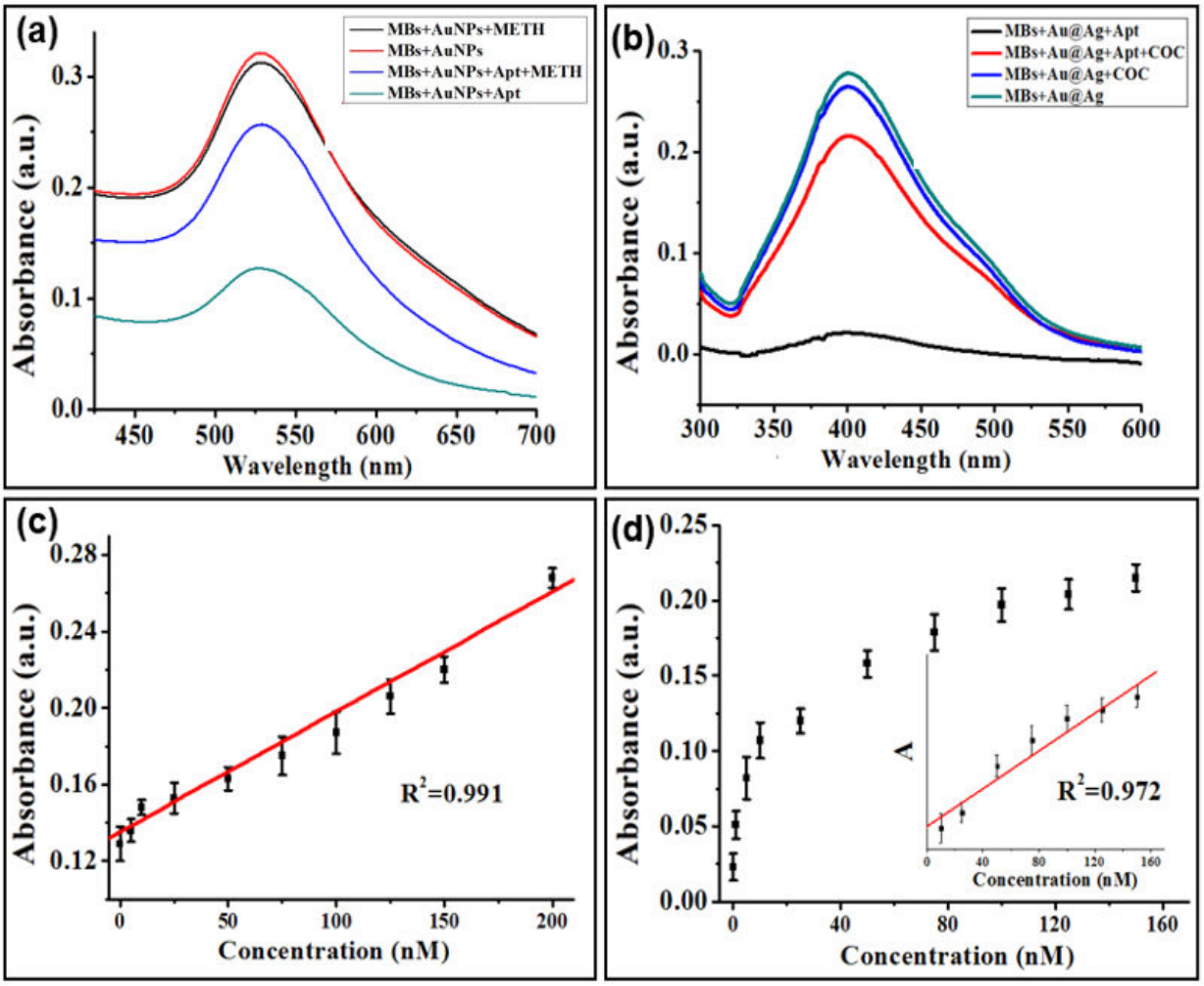

Figure 3. (a) UV-vis spectra of AuNPs in the presence of METH and control experiment; (b) UV-vis spectra of Au@Ag in the presence of COC and control experiment; (c) METH concentration-dependent change of SPR signal intensity (λ_{max}=520 nm) from 0 to 200.0 nM; (d) COC concentration-dependent change of SPR signal intensity (λ_{max}= 400 nm) from 0 to 150.0 nM. The inset shows the linear range concentration from 10.0 to 150.0 nM, error bar representing at least three independent measurments.

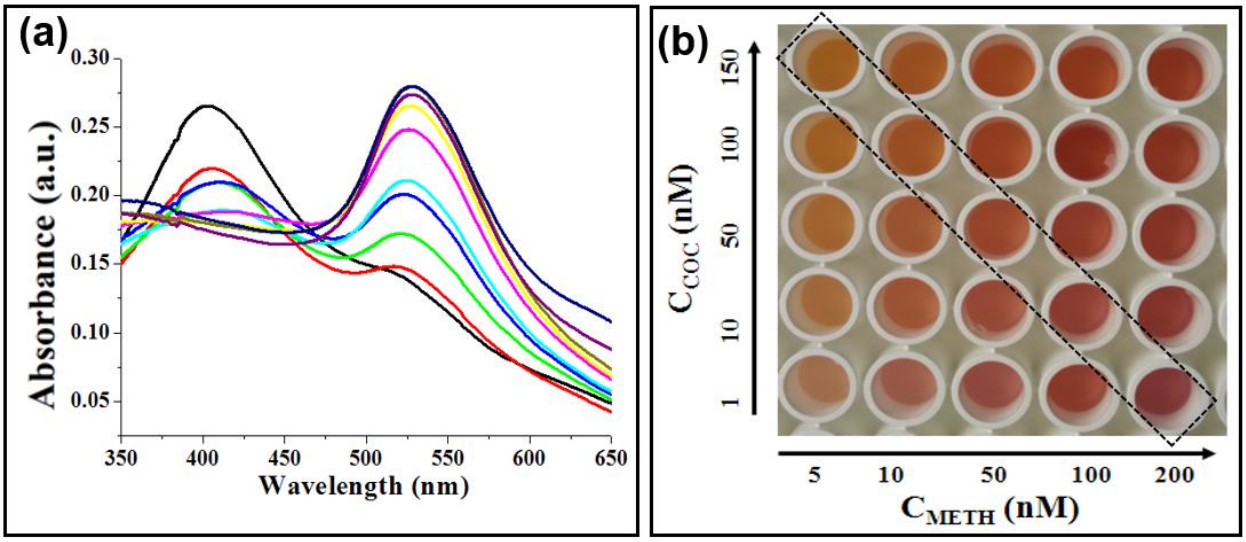

Figure 4. (a) UV-vis spectra of supernatant with the presence of different concentrations of METH and COC after incubation at 25 °C for 60 min (COC from bottom to top: 0, 1, 5, 10, 25, 50, 75, 100, 125, and 150 nM; METH from bottom to top: 0, 5, 10, 25, 50, 75, 100, 125, 150, and 200 nM.). (b) Photograph displaying the color change of the supernatant mixtures with different concentrations of targets (COC from bottom to top: 1, 10, 50, 100, and 150 nM; METH from left to right: 5, 10, 50, 100, and 200 nM).

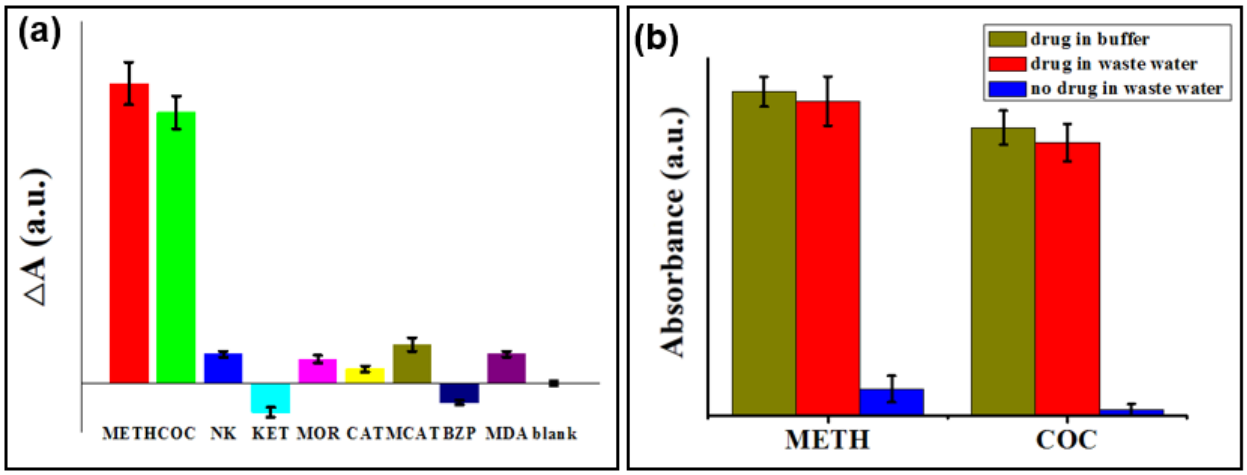

Figure 5. (a) Selectivity of the non-aggragation nanoparticles for METH and COC detection. The METH and COC concentration was 50 nM, while the concentrations of other illicit drugs were 1 μM, METH, COC, NK, KET, MOR, CAT, MCAT, BZP, MDA, and blank. (b) Histogram for SPR intensities of METH and COC detection in wastewater. The signal (ΔA) represents the relative absorbance with respect to the blank and error bars represent three independent measurements.