Highly sensitive fluorescent aptasensor based on MoS₂ nanosheets for one-step determination of methamphetamine

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Abstract
In this work, a simple and sensitive fluorescence aptasensor based on MoS₂ nanosheets (MoS₂-Ns) combined with a fluorophore-labeled aptamer (aptamer-FAM) for MTA determination in one step has been described. The aptamer-FAM can be spontaneously absorbed by the surface of MoS₂-Ns to form an aptamer-FAM/MoS₂-Ns’ sensing platform, resulting in quenching of the fluorescence of aptamer-FAM largely. However, after introducing the target MTA, the fluorescence will be restored depending on the levels of MTA added. Such an above reaction platform possesses a linear correlation of between 5 and 2400 nM, with a detection limit of 2.3 nM (S/N = 3). Moreover, the cross reactivity to ketamine, morphine and cocaine was only slightly significant. Simultaneously, the assay was also successfully applied to recognize MTA in spiked human blood and urine, as well as in the real forensic identification samples obtained from a forensic case about a MTA abuser.

Keywords Fluorescent aptasensor · MoS₂ nanosheets · Methamphetamine · Forensic toxicological identification

Introduction
Methamphetamine (MTA), as a novel excitatory drug, has been used frequently and illegally worldwide [1]. It can cause varying degrees of damage to the vital organs and mental system of humans [2–4], with plenty of forensic case reports of MTA poisoning occurring [5–8]. Therefore, the efficient determination of MTA is practically essential for forensic toxicological identification. Currently, multiple detection technologies have been established for MTA determination including high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), liquid chromatography–tandem mass spectrometry (LC–MS–MS), gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and gas chromatography/Fourier transform infrared spectroscopy (GC/FTIR) [9–14]. However, these methods involve some disadvantages, such as complicated operations, cumbersome sample pre-treatments, and professional staff. Therefore, proposing new assays for simple and sensitive determinations of MTA has become imperative.

Recently, aptasensor technology, which uses an aptamer as the superior capture probe, has been extensively applied in various toxic detections because of its simple fabrication, low cost, high specificity, and sensitivity. For example, Miao et al. designed an aptasensor-based assay for the sensitive detection of chloramphenicol in fish samples with good biocompatibility [15]. Oueslati et al. developed a cost-effective fluorescent aptasensor for the analysis of cocaine in serum samples [16]. Further, for monitoring malathion with excellent sensitivity, a detection platform based on an aptasensor was developed by Bala [17]. Thereby, as such a highly valuable analytical technology, using an aptasensor can be an attractive alternative method for target MTA determinations.

Notably, the synthesis and utilization of 2D materials in the aptasensor structure have increased enormously. As a type of emerging 2D materials, MoS₂-Ns have received tremendous research interests because of their good dispersion in water solution, large intrinsic bandgap, as well as optical and electronic properties [18–20]. More importantly, Zhu and co-workers first revealed that single-layered MoS₂-Ns can display strong affinity toward strand-single DNA, as well as high fluorescence quenching ability [21]. After that,
MoS2-Ns as an efficient fluorescence quencher have been employed to detect many biological analytes including proteins [22], pathogens [23], and other biomolecules [24]. However, using a MoS2-Ns based fluorescent aptasensor for drugs’ determination has not been explored.

Herein, a fluorometric aptasensing assay based on aptamer-FAM combined with MoS2-Ns has been established for MTA determination. In the absence of MTA, the aptamer-FAM can be absorbed to the surface of MoS2-Ns to cause a quenching of its fluorescence. Contrarily, in the presence of MTA, the aptamer-FAM will prefer to form a complex with the added targets instead of MoS2-Ns, which leads to increasing of the fluorescence of aptamer-FAM. Furthermore, the assay cannot only differentiate the target MTA from other drugs, but also directly recognize MTA in forensic identification samples.

**Experimental**

**Materials and reagents**

MoS2 powder (< 2 μm) was acquired from Sigma Aldrich (St. Louis, USA, https://www.sigmaaldrich.com/). FAM-labeled DNA sequence of 5′-/FAM/-ACG GTT GCA AGT GGG ACT CTG GTA GGC TGG GTT AAT TTG G-3′ was synthesized and purified by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China, http://www.sangon.com/). MTA, ketamine, morphine, and cocaine were supplied by Jiangsu Hengrui Medicine Co. Ltd. (Lianyungang, China, https://www.hrs.com.cn/), and then stored at −4 °C. Human blood and urine were provided by Xiangya Judicial Appraisal Center. Ultrapure water (18.25 MΩ cm) and phosphate buffer (PH 7.4, 20 mM) were used in the experiment.

**Apparatus**

The morphology of MoS2 was recorded on a Titan G2 60-300 TEM (FEI, USA). All the fluorescence spectra were obtained through a fluorescence spectrophotometer (Tokyo, F-4600, Japan), with scanning emission of 526 nm and the excitation of 497 nm. Excitation and emission slits were, respectively, at 2.5 and 5.0 nm. The PMT voltage was 700 V. The UV–visible absorption spectra were completed on a UV-2450 spectrophotometer (Shimadzu, Japan). The GC/MS system (an Agilent series 7890B GC interfaced with a model 5977A mass selective detector, USA) was applied to detect MTA in blood.

**The statistical processing of data**

The statistical processing of data was done using Microsoft office and the drawing software Origin. We used the standard deviations as the error bars, which were, respectively, calculated using the measurement data of three parallel samples (n = 3) through the STDEV function in the Excel software.

**Preparation of MoS2 nanosheets**

MoS2 nanosheets were synthesized according to the previous reported protocol with minor alteration [25]. Briefly, MoS2 powder (120 mg) was added to 60 mL of ethanol–water (45%, v/v) to form a solution. The mixture was then sonicated for 600 min, followed by centrifugation at 4000 rpm for 30 min to harvest the supernatant containing MoS2 nanosheets.

**Interaction of aptamer-FAM conjugates with MoS2-Ns**

To investigate the interaction of aptamer-FAM conjugates with MoS2-Ns, the MoS2-Ns’ solution was sonicated for a while for subsequent use. The concentrations of aptamer-FAM from 100 to 500 nM were selected through testing its fluorescence changes with the same amount of MoS2-Ns (50 μg/mL) before (F0) and after (F) addition of identical MTA (400 nM). Subsequently, MoS2-Ns with different concentrations (10–50 μg/mL) were, respectively, mixed with the optimized aptamer-FAM solution. After incubating for 20 min, the fluorescence spectra of the final mixture were recorded through a fluorescence spectrometer with λex/λem = 497/526 nm.

**Recognition of MTA**

For the determination of MTA, various concentrations of MTA were mixed with the aptamer-FAM/MoS2-Ns’ mixture (50 μg/mL of MoS2-Ns and 400 nM of aptamer-FAM) in phosphate buffer under mild conditions. The final reaction volume was at 100 μL for all of samples containing 70 μL of the aptasensor solution and 30 μL of test samples, with the incubation temperature at 25 °C for 30 min. Then, the fluorescence spectra were recorded with excitation at 497 nm to investigate the fluorescence recovery of aptamer-FAM. In addition, this assay was used to detect ketamine, morphine and cocaine (200 nM) under the same experimental conditions to explore its selectivity. Furthermore, the biological applicability of this method was verified by detecting MTA in spiked urine and blood samples, as well as real forensic blood identification samples obtained from forensic case about MTA abuser. All the thawed urine and blood samples were centrifuged at 4200×g for 10 min. Then, the supernatant solution was processed by phosphate buffer with × 10 gradient dilution. Subsequently, various concentrations of MTA were added into the blank urine and blood samples to provide some test samples for the aptasensor.
Results and discussion

Experimental principle

Scheme 1 presents the principle of this proposed aptasensor for MTA determination. In the absence of MTA, the aptamer-FAM can be absorbed on the MoS₂-Ns' surface, leading to fluorescence quenching due to electrons or energy transfer between aptamer-FAM and MoS₂-Ns [24]. After adding a certain amount of target MTA, the aptamer-FAM with high specific affinity will bind to the target and obtain the MTA/aptamer-FAM conjugates, in which the fluorescence of aptamer-FAM can be recovered owing to detachment of the aptamer-FAM/MoS₂-Ns' complex. Thus, we can obtain the level of MTA in samples via monitoring the changes of the reaction system fluorescence in one step.

Characterization of aptasensor

The UV–visible absorption spectrum of MoS₂-Ns is shown in Fig. 1a. From Fig. 1a, we can see that it has two absorption peaks at about 616 nm and 676 nm, which could be attributed to excitonic transitions (Fig. 1a) [26]. Moreover, the morphologies of synthesized MoS₂-Ns were characterized by TEM (Fig. 1b). As shown in Fig. 1b, the sizes of MoS₂-Ns were around 100 nm. Simultaneously, to test the feasibility of this method, the fluorescence changes of the reaction system under different conditions were detected (Fig. 1c). As illustrated in Fig. 1c, the fluorescence intensity of aptamer-FAM decreased drastically after the addition of MoS₂-Ns, which demonstrated that MoS₂-Ns can absorb aptamer-FAM and quench its fluorescence. However, when MTA was introduced into the aptamer-FAM/MoS₂-Ns' solution, a fairly significant fluorescence enhancement was observed. This above phenomenon suggests that the aptamer-FAM prefers to bind to the target drugs, resulting in destruction of aptamer-FAM/MoS₂-Ns' complex with changes of its fluorescence. Therefore, the level of MTA can be determined through monitoring the changes of the fluorescence intensity of the reaction platform.

Optimization of the reaction condition

The designed aptasensor is composed of MoS₂-Ns and aptamer-FAM, which means that the experimental concentration of the two components is a key factor affecting the sensitivity of the fluorescence aptasensor sensing system. Moreover, since the aptamer-FAM/MoS₂-Ns' fluorescence aptasensor is first applied to MTA determination, the optimal concentration of MoS₂-Ns and aptamer-FAM should be explored to obtain an aptasensor with the relatively best response performance for target MTA (Fig. 2). Figure 2a shows that different concentrations of the MoS₂-Ns (from 10 to 50 μg/mL) were mixed with an equal amount of aptamer-FAM (400 nM) in the phosphate buffer (Fig. 2a). From Fig. 2a, the fluorescence intensity of the reaction system dropped gradually with MoS₂-Ns' concentration rising from 10 to 50 μg/mL. Moreover, when the concentration of MoS₂-Ns reached 50 μg/mL, a quenching up to about 81% was observed (Fig. 2a). Thus, to avoid wasting materials, and to acquire more precise results, 50 μg/mL was chosen as the optimal MoS₂-Ns' concentration. Similarly, the effect of the aptamer-FAM concentration is shown in Fig. 2b. It shows that with the raising of the aptamer-FAM concentrations, the response of the sensing platform to the target MTA was gradually increased until the concentration of aptamer-FAM reached 400 nM (Fig. 2b). This might indicate that the excessive aptamer-FAM can inhibit the reaction between the nanoprobes and the target MTA in this sensing system. Thereby, considering the sensitivity of this method, 400 nM was used as the optimum aptamer-FAM concentration to construct the aptamer-FAM/MoS₂-Ns' aptasensor in following experiments.

Selectivity of strategy

For an excellent aptasensor, the high selectivity is a necessary condition to avoid interfering materials. Thus, we explored the selectivity of the method toward MTA relative to other drugs, including ketamine, morphine and cocaine, at the concentration of 200 nM. The relative fluorescence intensity of the sensing system was detected before (F₀) and
after \((F)\) the addition of each drugs, respectively (Fig. 3). As can be seen from Fig. 3, when adding equal amounts of MTA, ketamine, morphine and cocaine (200 nM) to the fluorescence sensing system under the same experimental conditions, the relative fluorescence intensity change of the aptasensor in the presence of MTA is the highest among the others, at almost 0.72, which is about four to seven times

![Figure 1](image1.png)

**Fig. 1** a UV–Vis spectra of MoS\(_2\)-N\(_s\). b TEM images of MoS\(_2\)-N\(_s\). c Fluorescence spectra of the MTA detection system under different conditions (Aptamer-FAM only, Aptamer-FAM/MoS\(_2\)-N\(_s\) + MTA, Aptamer-FAM/MoS\(_2\)-N\(_s\)). Concentrations of Aptamer-FAM, MoS\(_2\)-N\(_s\) and MTA were 400 nM, 50 μg/mL, and 400 nM, respectively

![Figure 2](image2.png)

**Fig. 2** Optimization of the reaction conditions \((n=3)\). a Optimization of the MoS\(_2\)-N\(_s\)' concentration \((0, 10, 20, 30, 40, \text{and } 50 \mu g/mL)\). Fluorescence intensity ratio of the Aptamer-FAM (400 nM) before \((F_0)\) and after \((F)\) addition of different concentrations of MoS\(_2\)-N\(_s\). b Optimization of the Aptamer-FAM concentration \((100, 200, 300, 400 \text{ and } 500 \text{ nM})\). Fluorescence intensity ratio of the different concentrations of Aptamer-FAM with MoS\(_2\)-N\(_s\) (50 μg/mL) before \((F_0)\) and after \((F)\) addition of identical MTA (400 nM)

![Figure 3](image3.png)

**Fig. 3** The selectivity test of the proposed aptasensor assay \((n=3)\). The fluorescence intensity of the MTA detection system before \((F_0)\) and after \((F)\), respectively, addition of each drugs (at 200 nM) including MTA, ketamine, morphine, and cocaine
as many as the others. Compared to the target MTA, the fluorescence signal caused by the interfering drugs is a little significant. Therefore, the results preliminarily indicate that the aptasensor has good specificity against the target MTA, which is contributed by the nature of the high selectivity of the aptamer.

**Fluorescence analysis of MTA in phosphate buffer**

To further demonstrate the application feasibility of the assay for MTA determination, the fluorescence spectra with different concentrations of MTA were performed under optimized conditions. As shown in Fig. 4a, with the increase of MTA level from 5 to 2400 nM, the fluorescence intensity was gradually intensified. Moreover, in Fig. 4b, a linear relationship was obtained between \((\frac{F - F_0}{F_0})\) and the concentration of MTA (5–2400 nM), with a linear regression equation of \(y = 0.0013x + 0.2799\) \((R^2 = 0.989)\). The limit of detection (LOD) was about 2.3 nM, calculated according to standard rules \((S/N = 3)\). Simultaneously, a comparison with different analytical assays for MTA determination is shown in Table 1 [9, 27, 28]. It can be seen from Table 1 that our method displays higher sensitivity and a lower detection limit than some of other assays reported. A method with detection linear range of 0.7 nM–6.7 μM was reported by Kimura [27] which has a wider detection range. However, the acquisition of anti-MTA in this assay involves complicated operations, and cross-reactions are more likely to occur. Thus, our method is more efficient and simpler.

**Identification of MTA in real samples**

The practicability of this assay was investigated via the recognition of MTA in spiked urine and blood samples, as well as in the real forensic blood identification samples. First, three spiked human blood and urine samples with different MTA concentrations (10, 50 and 120 nM) were, respectively, detected to obtain the recovery rates, under equivalent experimental conditions (Table 2). As shown in Table 2, the recovery rates are calculated as 94–113% in spiked blood samples, and 96–108% in spiked urine samples. Besides, we used this aptasensor to test the real forensic blood identification samples from an MTA abuser in a forensic case admitted to Xiangya Judicial Appraisal Center. As we can see from Table 3, the concentrations of the four forensic blood identification samples have been determined by GC–MS, an official standard detection method. These test results were further compared with that of our proposed method (Table 3). As shown in Table 3, the detection result of this

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**Table 1** Comparison of different analytical assays for MTA determination

| Method          | Detection range   | LOD    | References |
|-----------------|-------------------|--------|------------|
| Fluoroimmunoassay | 0.7 nM–6.7 μM     | Not given | [27]       |
| Fluorometry     | 8–62 and 62–110 nM | 3.2 nM | [28]       |
| HPLC            | 74 nM–7.4 μM      | 12.6 nM | [9]        |
| Fluorometry     | 5–2400 nM         | 2.3 nM  | This work |

**Table 2** Recoveries of MTA in spiked human blood and urine samples \((n = 3)\)

| Samples | Added (nM) | Aptasensor Detected (nM) | Recovery (%) |
|---------|------------|--------------------------|--------------|
| Blood   | 1          | 11.3                     | 113          |
|         | 2          | 51.6                     | 103          |
|         | 3          | 112.5                    | 94           |
| Urine   | 1          | 10.8                     | 108          |
|         | 2          | 50.5                     | 101          |
|         | 3          | 115                      | 96           |
Table 3  A comparison between the proposed aptasensor and official GC/MS for MTA determination in the real forensic blood identification samples (n = 3)

| Forensic blood identification samples | 1   | 2   | 3   | 4   |
|--------------------------------------|-----|-----|-----|-----|
| Aptasensor (nM)                      | 426.4 | 671.8 | 1050 | 1437.7 |
| GC/MS (nM)                           | 456.9 | 733.1 | 989.6 | 1352 |
| RE (%)                               | −6.7 | −8.4 | 6.1  | 6.3  |

The method is basically consistent with the reference value of the forensic toxicology analysis laboratory; also, the relative error (RE) of this method is less than 10%, with good accuracy. Therefore, the ability of this proposed method to correctly detect positive samples can further display its satisfactory high sensitivity, which initially indicates that it may be an alternative analytical platform for a practical identification of MTA.

Conclusions

A fluorescent aptasensor based on Aptamer-FAM/MoS2-Ns has been successfully developed for MTA monitoring. Under optimized experimental conditions, this proposed fluorescent assay obtained a good linear correlation of from 5 to 2400 nM with LOD of 2.3 nM, which is more sensitive and effective than that of some previous reported methods. More importantly, it can complete a sample test in just one step without any tedious sample pre-processing, which can greatly improve the efficiency of forensic workers in case detections. Besides, the good discrimination ability of this method is verified through identifying the MTA from other drugs. Simultaneously, it presents satisfactory results for MTA determination in the spiked urine and blood samples. Moreover, the test results of forensic blood identification samples indicated a good agreement with those obtained by GC–MS. Overall, the work is expected to provide a potential tool for drug detection, especially for forensic poisoning casework.

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Declarations

Conflict of interest  The authors declare that they have no conflict of interest.

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