

## An electrochemical codeine sensor based on CdS nanoparticle label

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**Abstract.** In the present study, we describe an electrochemical sensor for codeine detection by using the DNA aptamers against codeine. In the sensing protocol, a dually-labeled DNA Aptamer probe was designed to be labeled at one end with HS, and at its another end with an dabcyI as an electrochemical tag to produce electrochemical signal from recongization occurrence. One special electrochemical marker was prepared by modifying CdS nanoparticle with  $\beta$ -cyclodextrins (ab. CdS-CDs), which employed as electrochemical signal provider and would conjunct with the thrombin probe modified electrode through the host–guest recognition of CDs to dabcyI. With codeine adding, aptamer folding allows the CdS-CDs into soultion which caused a increased current signal. This sensor have the ability to detect 27pM codeine.

### Introduction

Codeine(3-methylmorphine) is an opiate that is widely used to treat mild to moderate pain and cough suppression. It is the secondmost predominant alkaloid in opium with a mild sedative effect [1]. Despite its extensive medical applications, codeine is often abused for its euphoric and depressant effects as well as to prevent opiate withdrawal [2]. It is very harmful to take codeine in large quantities and now it has been attributed to be controlled medicine in most countries. It is very meaningful to detect codeine in forensic analysis or clinic diagnostics. There are many methods, such as UV spectrophotometric techniques [3], gas chromatography–mass spectrometry[4], capillary electrophoresis[5] and high-performanceliquid chromatography [6], have been applied in the determination of codeine. Although these above assay methods have accuracy and sensitive detection limit, it also has the obvious disadvantages of valuable apparatus, complicated manipulation and unapproachable in applying in field-tests. Therefore, it is highly desired to set up a sensitive method for detection of codeine.

In this article, the nolve electrochemical sensor was constructed for codeine detction. As illustrated in Fig. 1, two key components were employed for our electrochemical sensing codeine strategy. One component is the CdS nanoparticle surface-modified  $\beta$ -cyclodextrins (CdS-CDs), which was used as both electrochemical signal provider and the host recognizer during the host–guest molecular recognition. The other one component is the 15-base codeine aptamer, which was terminal-labeled with dabcyI at its 5 end as  $\beta$ -CD's typical guest and labeled thiol at its 3 end for pre-immobilization onto Au electrode via the Au S bond. The aptasensor was completely fabricated after CdS-CDs were interacted with aptamer-modified electrode through the host–guest recognition between dabcyI labeled at aptamer and  $\beta$ -cyclodextrins-modified on CdS. During codeine detection, conformational change of the aptamer happened and forced CdS-CDs away from the electrode surface, thus the capture of target onto electrode was translated via the electrochemical current signal offered by released CdS-CDs. Compare to previous methods, CdS-CDs play the role of target recognition and the signal provider which make this present method display an simple-step detection and sensitivity. Therefore, this electrochemical aptasensor is expected to have wide applications in codeine monitoring.

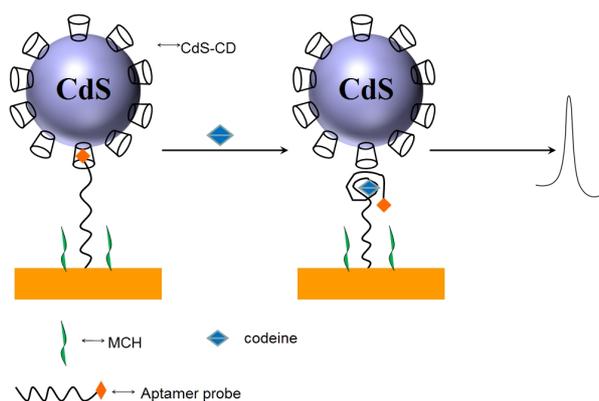


Fig.1 Schematic representation of the procedure to prepare electrochemical sensor for the determine of codeine.

## Experiment section

**Apparatus** Differential pulse voltammetry (DPV) measurements were performed using a CHI 660 Electrochemical Analyzer (CHI Instrument Inc., USA). The JB-1 stirring machine (Branson, Shanghai, China) and a TDL-16B centrifuge (Anting Science Instrument Inc., Shanghai, China) were used. The three-electrode electrochemical detection system consisted of a Au working electrode with sensing area of 3.14 mm<sup>2</sup>, a Ag/AgCl reference electrode (saturated KCl) and a platinum wire counter electrode. The detection was carried out in a 5 ml electrochemical cell containing a mercury-coated glassy carbon working electrode (2 mm diameter), an Ag/AgCl reference electrode, and a platinum wire counter electrode.

**Preparation of nano CdS** Cd(NO<sub>3</sub>)<sub>2</sub> and Na<sub>2</sub>S solutions were filtered through a 22 m microporous membrane filter prior to use. CdS nanoparticles were prepared according to the literature [7] by using mercaptoacetic acid as the stabilizer. In brief, 9.22 l mercaptoacetic acid was added to 50 ml 0.4 mM Cd(NO<sub>3</sub>)<sub>2</sub> solution, and then the pH was adjusted to 7 with 0.5 M NaOH. The solution was bubbled with nitrogen for 30 min, followed by the slow addition of 1.34 mM Na<sub>2</sub>S to the mixture solution. The molar ratio of Na<sub>2</sub>S to Cd(NO<sub>3</sub>)<sub>2</sub> was kept at 2.5. The reaction was carried out for 24 h under nitro-gen protection and then gradually a brown colloid which is the CdSnanoparticles covered with a carboxyl group was obtained. As TEM images show, the diameter of CdSnanoparticles was about 7 nm.

**Preparation of Per-6-thio-β-cyclodextrin** Per-6-thio-β-cyclodextrin (SH-β-CD) is synthesized according to the literature, but step slightly improved. The resulting precipitate was carefully filtered off and dried under vacuum to yield Per-6-thio-β-cyclodextrin (0.85g, 83%) as an off-white powder. mp 134-136 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ5.92 (d, *J* = 6.0 Hz, 7 H), 5.82 (s, 7 H), 4.93 (s, 7 H), 3.68 (t, *J* = 6 Hz, 7 H), 3.61 (t, *J* = 7.5 Hz, 7 H), 3.19 (br d, *J* = 15 Hz, 7 H), 2.77-2.74 (m, 7 H), 2.13 (t, *J* = 6.0 Hz, 7 H). MS (ESI) *m/z* [M + Na]<sup>+</sup>: 1269.1920 (see fig.2).

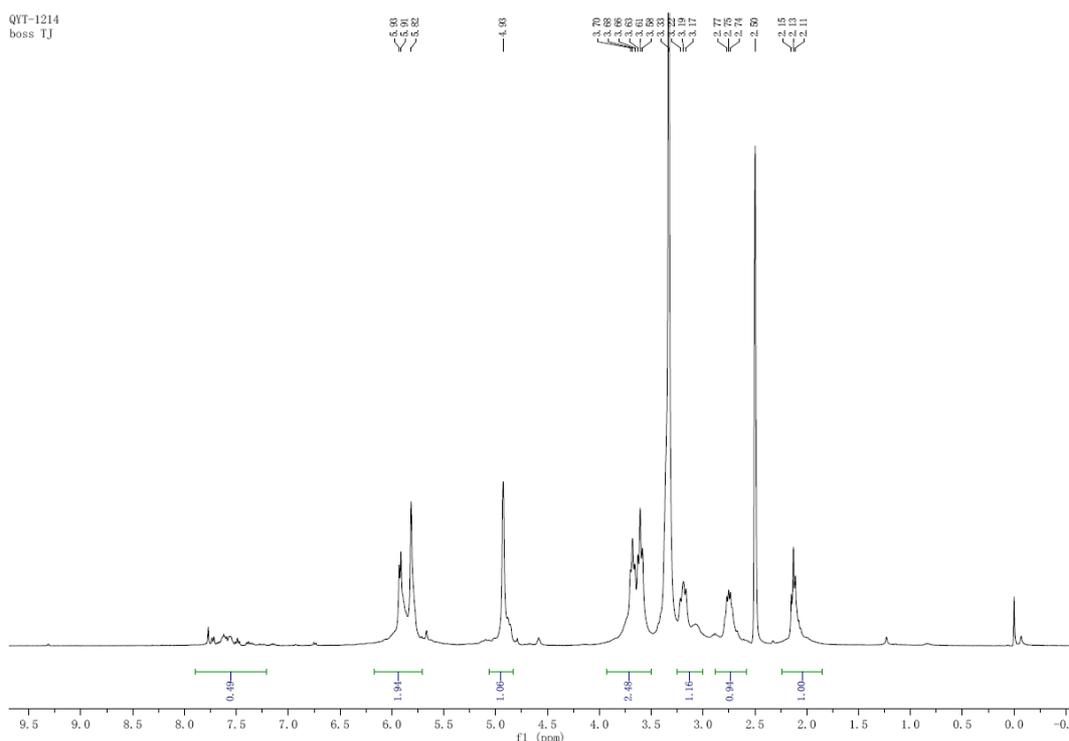


Fig.2  $^1\text{H}$  NMR of Per-6-thio- $\beta$ -cyclodextrin

## Results and discussion

### Principle of detection of codeine

The assay procedure was initiated by incubating the aptasensor with codeine in phosphate buffer solution at 37 °C for 40 min. During which, the aptamer would prefer to bind with codeine due to the strong combination between aptamer-to-protein and then the CdS-CDs which previously combined with aptamer were released away from the electrode surface (Fig. 1 ) into the buffer solution. These CdS-CDs were dissolved by adding 20 L of 1.0 M  $\text{HNO}_3$  into the buffer solution, and then 1.8 mL acetate buffer (0.1 M, pH 5.3) was added into it. Electrochemical detection of the dissolved  $\text{Cd}^{2+}$  was performed in the above acetate buffer solution by applying  $-1.0$  V for 5 min at one mercury-film electrode to reduce  $\text{Cd}^{2+}$  into Cd film. After which, DPV was immediately performed from  $-0.90$  to  $-0.54$  V (Incr E 0.004 V, amplitude 0.05 V, pulse width 0.05 s, pulse period 0.2 s), resulting in an electrochemical signal due to the oxidation of Cd film. The DPV peak height at the potential of  $-0.7$  V was used in all measurements, which was related to the released amount of the CdS nanoparticles from the electrode surface.

### Codeine determination

In the experiments, the aptasensor was incubated with the different concentration of codeine, the DPV signal owing to the released CdS-CDs was increased with the codeine concentration, which was logarithmically related to the target protein concentration from  $53 \times 10^{-12}$  to  $53 \times 10^{-9}$  M. The equation for the resulting calibration plot was calculated as  $y = 0.32 \log x - 0.072$  ( $x$  is the concentration of target codeine divided by pM,  $y$  is the DPV peak current value) with correlation coefficient of 0.9832 and detection limit of  $27 \times 10^{-12}$  M.

## Conclusions

The present study has introduced a novel electrochemical aptamer biosensor based on CdS-CDs as signal label had been proposed to determine codeine and a very low detection limit of 27 pM was achieved. Therefore, this electrochemical sensor is expected to have wide applications in medicine monitoring.

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