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Developing a Sensitive Method for the Electrochemical Determination of Tetracycline Using MB Tagged Aptamers on Gold Electrode Substrates†

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Abstract: An electrochemical aptasensor for the detection of tetracycline (TET) is prepared based on a methylene blue (MB) tagged DNA aptamer, with sequence 5′-MB-CCC CCG GCA GGC CAC GGC TTG GGTTGG TCC CAC TGC GCG-thiol-3′. The DNA aptamer is chemisorbed on a gold electrode and differential pulse voltammetry (DPV) is utilized for the detection. In particular, upon binding of the TET with the purpose designed aptamer, there is an increase in the current intensity, as a result of the increased proximity of the MB molecule to the gold surface. The sensor is tested using aqueous samples spiked with TET concentrations between 1–1000 nM and a limit of detection (LOD) of 1.2 nM is determined. Furthermore, the dissociation constant is estimated to be 1.4 nM using a Lineweaver-Burk plot.

Keywords: Biosensors; methylene blue; aptamer; Tetracycline; electrochemical detection

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1. Introduction

With the advent of antibiotics, human and veterinary medicine significantly advanced in fighting bacterial disease [1]. Nevertheless, the influx of drugs to the environment, can pose a significant threat to aquatic and terrestrial biodiversity as well as to the human food chain [2]. In this regard tetracycline (TET), a common drug used against intercellular bacteria, such as chlamydia, mycoplasmas, rickettsia, and protozoan parasites, has been often traced in water, food or milk samples [3,4]. Thus, it's qualitatively and quantitatively monitoring in various systems is important for public health.

Currently TET is often determined using high-performance or thin layer liquid chromatography [5,6]. Moreover, enzyme-linked Immunosorbent assay (ELISA) kits have also been used for this purpose [7]. Nevertheless, despite their ability to detect TET with both selectivity and sensitivity, they can be either expensive or also time consuming, requiring in many cases qualified staff. In this regard, biosensors [8] promise a fast, lower cost, technology for the detection of different pharmaceutical compounds. In particular, electrochemical aptasensors [9], provide a robust alternative based on synthetic DNA (or RNA) nucleotides, that are easily produced, are target specific and can be immobilized with ease on a number of surfaces. Moreover, they offer chemical stability and allow for the regeneration of the aptasensors, due to the electrostatic interactions between the aptamers and target.

A number of electrochemical aptasensors have been recently reported as a platform for robust, low cost and often multianalyte analysis, while being compatible with microfabrication technologies [8]. In particular, this work will focus on the detection of TET in an aqueous environment using an electrochemical aptasensors based on a gold working electrode where a methylene blue (MB) modified DNA synthetically produced oligonucleotide is immobilized via the Au-S bond. In this regard, the limit of detection (LOD) is investigated for our aptasensors, and the dissociation constant Kd is deduced.

2. Materials and Methods

2.1. Reagents and Aptamer Sequence

All reagents were purchased from Sigma-Aldrich. The tetracycline antibiotic (TET, Mw=444.43 Da), was made from bacteria of the genus Streptomyces. Tris(2-carboxyethyl) phosphine (TCEP) powder was used to break down any disulfide bonding while 6-Mercapto-1-hexanol (MCH) was utilized to avoid any non-specific binding. An MB tagged thiol modified DNA aptamer with the composition 5′-MB-CCC CCG GCA GGC CAC GGC TTG GGTTGG TCC CAC TGC GCG-thiol-3′ was used, as described by Niazi *et al.* [10]. The aptamer was purchased from GeneCust (Boynes, France). The stock solution for the aptamer was based on a TE buffer containing 10mM Tris-HCl and 1mM Ethylenediaminetetraacetic acid (EDTA) at pH=8. Finally, the working buffer was based on 10mM PBS containing Tris−HCl (20 mM), NaCl (100 mM), $MgCl_2$ (2 mM), KCl (5 mM), $CaCl_2$ (1 mM) at pH 7.6.

2.2. Preparation of the Aptasensor

The aptasensors used gold electrode disks with a 2 mm diameter, that were initially polished using alumina powder and then sonicated 15 min in ethanol. After the electrodes were rinsed in deionized water and dried under a nitrogen gas flow. In order the prepare the aptamers for immobilization, they were fist heated to 90 °C for 10 min and then cooled to room temperature, to provide proper aptamer folding. After that, 1 μM of aptamer was diluted in the TE buffer and incubated with 10 mM TCEP (1 h) for dissociation of disulfide bonds. For the immobilization, the aptamer was added on the clean Au electrode, until the 2 mm surface was completely covered and incubated for 1h at room temperature. After that MCH at a concentration of 2mM in deionized water was added on the surface overnight to prevent any non-specific binding. After rinsing the aptamer, the sensitized electrode was incubated for 30 min with various concentrations of TET between 1 nM to 1000 nM which were diluted in the above mentioned working buffer. The scheme of the sensing surface is presented in Figure 1. The TET detection was performed using Differential Pulse Voltammetry (DPV).

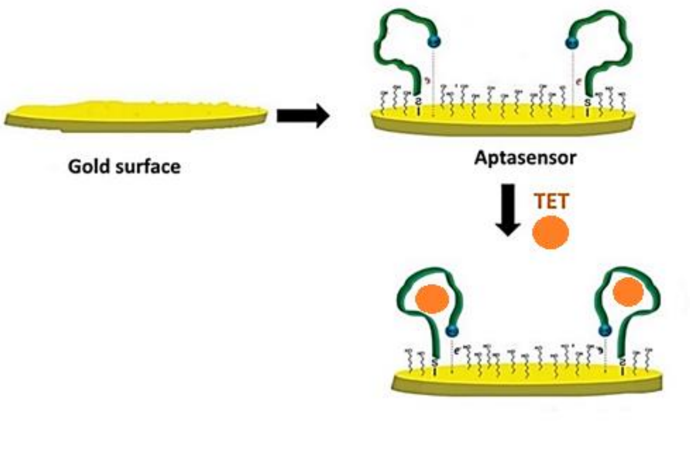

Figure 1. A schematical representation of the aptamer conformational changes upon binding to TET adapted from [11]. This results in the MB redox tag, coming in close proximity with the gold electrode surface.

2.3. Electrochemical Measurements

The electrical parameters were determined using a Zahner, Zennium Pro potentiostat, with a gold working electrode (2 mm), a Ag/AgCl reference and a Pt wire as a counter. All electrodes were purchased from CH Instruments, USA. The sensor response was studied using differential pulse voltammetry (DPV) with a potential range from from 0 V to –0.6 V and a resting time of 60 s.

3. Results and Discussion

3.1. Determination of Different Concentrations of TET Using Aptasensing

The determination of TET was investigated for different concentrations ranging between 1-1000nM in 3 independent experiments, with good reproducibility. The aptasensor showed selectivity to TET, using spiked samples in an aqueous solution. In particular, the following concentrations were looked at: 1 nM, 5 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1000 nM. The binding of TET on the aptasensor induced an increase in the oxidation peak, as can be seen in Figure 2 below.

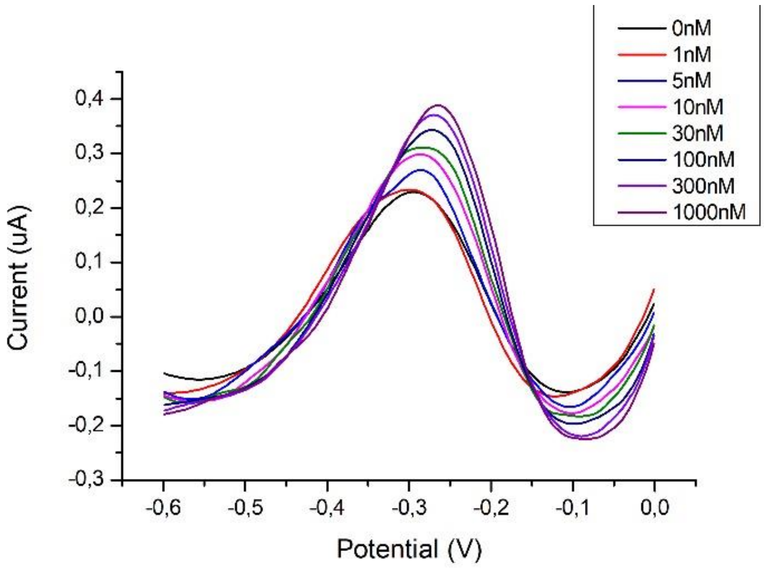

Figure 2. DPV of the aptasensor following with incubation of TET at various concentrations (see inset).

Furthermore, the calibration plot of (I-Io)/Io versus the logarithmic scale of the TET concentrations was examined (Figure 3) and regression was applied to identify the limit of detection (LOD) using the relationship LOD = $3\sigma/\alpha$, where σ denotes the standard deviation of the response and S denotes the slope of the linear calibration plot [12]. The limit of detection was then determined to be 1.2 nM

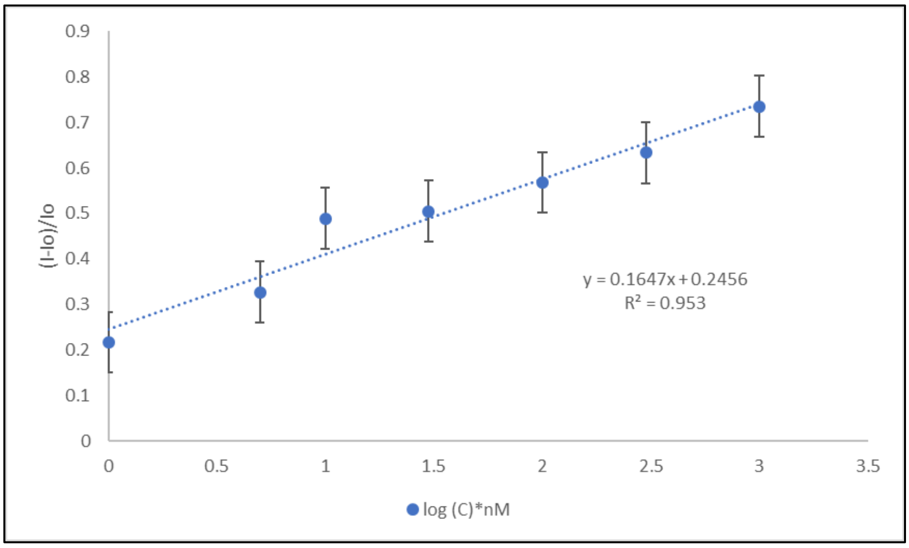

Figure 3. Calibration plot based on 3 independent measurements at each TET concentration.

Our results, are comparable with literature, considering that TET has been detected in aqueous solutions with an LOD varying from 7.8 × 10^{-11} M up to 5 × 10^{-9}M, depending on the method used, electrode architecture, aptamer sequence and immobilization [13].

3.2. Deduction of the Dissociation Constant Kd for Our Aptamer Sequence

In this investigation, we also deduced the dissociation constant (Kd) for our aptamer, under the specific buffer conditions using a Lineweaver-Burk plot [14], as seen in Figure 4 below:

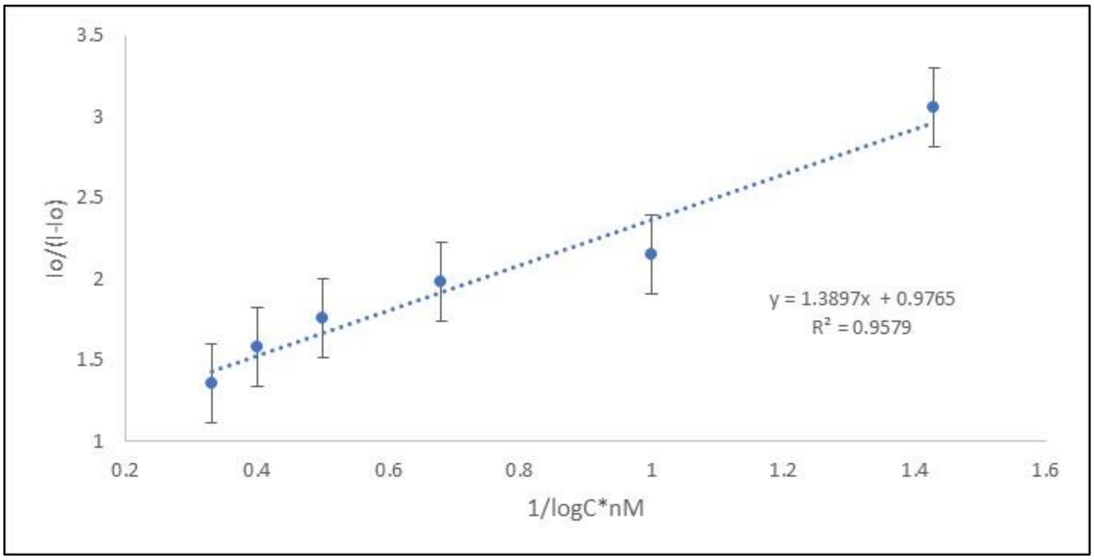

Figure 4. Lineweaver-Burk plot leading to a constant of dissociation, Kd=1.4 nM.

In particular Kd, shows the binding strength between the aptamer and target, with values ranging from micro to picomolar levels [15]. In general, a low value for the dissociation constant, relates to a high affinity between aptamer, indicating that a low aptamer concentration of aptamer and target are needed for binding. Linear regression yielded a linear fit, such that y = 1.3897x + 0.9765, thus leading to a value of Kd=1.3897/0.9579 nM or Kd= 1.4 nM, showing that aptasensors provide a robust structure for TET detection.

4. Conclusions

An electrochemical aptasensor was developed for the detection of TET using a single-stranded DNA aptamer, that selectively binds to TET in an aqueous solution. In particular it was immobilized on a gold electrode and the analysis was performed using DPV. Our results show that the electrochemical aptasensors is sensitive to TET where the limit of detection (LOD) was determined to be 1.2 nM, and the Kd was shown to be 1.4nM with the help of a Lineweaver-Burk plot. The aptasensor developed in this study can potentially be used for detection of tetracycline in pharmaceutical preparations, drinking water and contaminated food samples such as milk.

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Conflicts of Interest: The authors declare no conflicts of interest.

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