Iranian Journal of Analytical Chemistry
Volume 10, Issue 2, September 2023 (97-105) Original Research Article
مجله ایرانی شیمی تجزیه
دوره ۱۰، شماره ۲، مهر۱۴۰۲ (۱۰۵-۹۷)

The Role of a Semiconductor Chalcogenide in a Label-Free Aptasensor for Detection of Ultra-Trace amounts of Aflatoxin B1 in Wheat Flour

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Received: 1 December 2023 **Accepted:** 6 January 2024
DOI: 10.30473/ijac.2024.70101.1283

Abstract
Addressed herein, Bismuth sulfide (Bi_2S_3) as a synthetic semiconductor chalcogenide applied in fabrication process of an aptasensor as a signal promoter for ultra-trace detection of aflatoxin B1(AFB1). The analytical signal was improved by using optimized amounts of Bi_2S_3 for electrode modification. The AFB1 Aptamer single strand type(SSDNA) was simply immobilized on the cross section of a pencil lead through polydopamine layers. A wide concentration range of trace amounts of AFB1(0.3-630nM) was supported by the fabricated aptasensor (pencil/polydopamine@Bi_2S_3/aptamer) using differential pulse voltammetry. Simple fabrication and no needing to electrode refreshment were part of the advantages of the suggested aptasensor. Finally, very low resulted detection limit(0.04nM) with a great sensitivity (0.076µA/nM) and also appropriate stability and repeatability led to application of the aptasensor in real sample analysis such as wheat flour with brilliant recovery percentages.

Keywords
Aptasensor; Aflatoxin B1; Bismuth sulfide; Pencil electrode;Polydopamine.

1.INTRODUCTION

Aflatoxin B1 (AFB1) is a carcinogenic compound which causes serious damage to mammalian immune system, kidneys and liver [1]. AFB1 is a subcategory of mycotoxins which originated from Aspergillus fungi as the most harmful type of aflatoxins [2]. Iranian scientific committee for food (SCF) considers AFB1 as one of the most important factors of liver cancer in the Iranian society [3]. Many putable organizations reported the permitted level of AFB1 in foodstuffs. For example, the European commission has determined the safe level of AFB1 in pistachio less than 10µg/kg. As well, Iran's national standard recommended AFB1 contents less than 5 µg/kg in pistachio samples [4].

There are many suggested methods for detection of AFB1 in plants and food samples at low detection limits. High performance liquid chromatography [5,6], immunosensing based methods [7,8] and spectrophotometry [9,10] always has been known as the conventional methods for AFB1 detection. Also, some enzymatic strategies are suggested for sensitive determination of AFB1[11-13]. In the recent years, extensive studies on nucleic acid has led to the synthesis of aptamers. These high technical materials have significant ability to bind to compounds(targets) selectively [14]. In fact, aptamers are artificial antibodies from RNA/DNA that may possess many functional groups or ligands.

Generally, Aptamers synthesis by the "selection evolution of ligands by experimental enrichment" (SELEX) method [15]. These materials have good stability for tuning and also suitable reversible denaturation during binding with target molecules. All these features cause that aptamer based materials widely applied for detection of antibiotics, drugs and mycotoxins [16-18]. As previously mentioned, aptamers are synthetic oligonucleotides with single strand base pairs which are capable to link to target molecules to form a reversible double helix(duplex). There are three main forces between target and the aptamer: vander waals, dipole interactions and hydrogen bonding. Electrochemical aptasensors are highly sensitive and selective. Moreover, they can have developed as economic devices with easy handling.

Nanoparticles mostly can improve the electrocatalytic properties of electrochemical sensing methods. They often increase the effective surface area, sensitivity, conductivity and electrochemical reaction efficiency [19]. Nano materials such as fullerenes [20], carbon nanotubes [21, 22] graphene [18, 23] and metal chalcogenides

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[24,25] has been widely participated with aptasensors in order to determination of varied organic and inorganic species.
Labeled aptasensors may have some disadvantages such as disturbance in double helix formation beside time consuming. Thus, label-free aptasensors can be more attractive for biosensing[26].
Metal chalcogenides, such as MoS_2, $Nb_{21}S_8$, CdS_2 have graphene like morphologies where the layers stacked together via weak van der Waals forces with great exfoliation capability. Recently, the mentioned compounds have been considered in sensing fields due to their properties such as thin layered structure, high surface area, stability and facileness in heterogeneous electron transfer [27,28].
In the current work a pencil graphite lead was selected as the basis of the aptasensor for ultra-trace analysis of the AFB1 in real samples. The surface of the electrode was modified by P-type Bi_2S_3 nanoparticles, followed by polydopammine electropolymerization. The thin layeres of polydopamine formed on the cross section of the pencil electrode and acted as a coupling mediator for aptamer strands binding to the surface of the pencil electrode .The coated polydopamine played the role of conventional binders like NHS(N-Hydroxysuccinimide) and EDS(1-ethyl -3-(3-dimethyl aminopropyl) carbodimide[18].The developed aptasensor characterized by cyclic voltammetry and electrochemical impedance spectroscopy (EIS).Differential pulse voltammetry technique(DPV) was applied for real sample analysis by the fabricated AFB1 aptasensor.

2.EXPERIMENTAL

2.1 Chemicals and instrumentals

Bismuth nitrate pentahydrate, $Bi(NO_3)_3.5H_2O$, thiourea (NH_2CSNH_2), Dopamine hydrochloride and Aflatoxin B1 in 1:1 V/V of methanol/Chloroform were purchase from Sigma. Other needed reagents in their analytical grades were prepared from Sigma and Merck. Aflatoxin B1 binding single strand DNA (SSDNA aptamer) functionalized by C6-NH_2 groups was prepared through the SELEX method from Bioneer (south Korea) according to the following sequence and specifications:
5′ NH_2-C_6-
GTTGGGCACGTCTTGTCTCTCTGTGTCTCG
TGCCCTTCGCTACGCCCACA-3′
Optical Density (OD)=5, Length=50bases,
MW=15433.07g/mol

A stock solution of the aptamer (100µM) was prepared by diluting with sterilized deionized water and was put in frozen condition (-18ºC).

Phosphate buffer saline solutions (PBS) with the desired pHs were prepared by using certain amounts of phosphoric acid and NaOH solutions.
A "Steadleler HB graphite pencil lead" (Germany) with 0.7mm in diameter was used as the basis of the aptasensor. The body of the pencil lead was insulated by a teflon strip and silicon adhesive.
All of the electrochemical experiments were performed by a Metrohm Computrace Voltammetric Analyzer model 797 equipped with a three-electrode setup. The modified pencil electrode was acted as the working electrode. A platinum rod as the auxiliary and Ag/ AgCl electrode as the reference were used.

2.2 Preparing of the P-type Bi_2S_3

Bi_2S_3 nano particles was synthesized through a hydrothermal as follows: A 70 ml solution consist of 0.1 M of $Bi(NO_3)_3.5H_2O$ and 0.15 M of thiourea was prepared. Then 5 ml solution of a 65% of HNO_3 was added and stirred for 20 min. The mixture was transferred to a stainless steel to reactor and heated to 180 ºC for half of a day. The obtained black material was collected and rinsed with deionized water and ethanol and dried in oven 70 ºC for 24h. The as-prepared Bi_2S_3 was characterized by XRD patterns, Energy-dispersive X-ray spectroscopy (EDS) analysis and SEM images.
Also, the synthetic bismuth sulfide, Bi_2S_3, was identified as a p-type semiconductor by "Hall effect" analysis which confirmed the existence of positive charge carriers for electron capture.

2.3. preparation of the electropolymerized modified pencil electrode

The certain amounts of as prepared Bi_2S_3 was dispersed in 1 ml of tetrahydrofuran (THF). A few amounts of polyvinylchloride (PVC) granules was added to the mixture for more stickiness. After 20 min sonication, 5 µl volume of the suspension was loaded on the cross section of the pencil lead and prepared for electropolymerization. In order to creation active groups on the surface of the electrode, the pencil lead was immersed in 0.5M of H_2SO_4 and 10 scans were applied from -0.5 to 1.2 V (cyclic voltammetry, scan rate=100mV/s). Then, appropriate amounts of dopamine were dissolved in PBS (pH=5) and connected to the potensiostat /galvanostat system by a metallic clip. 10 cyclic voltammetry scans were applied to forming few layers of polydopammine on the surface of the electrode. Next, the electrode was washed carefully by sterilized/ deionized water.

2.4. Fabrication of the "pencil/polydopamine @ Bi_2S_3/aptamer"

5µl of the amino functionalized AFB1 aptamer (NH_2-SSDNA) was dropped on the cross section of

DOI: 10.30473/ijac.2024.70101.1283

the pencil electrode and incubated for 1h. For future analysis studies, the certain amounts of AFB_1 dissolved in PBS(PH=7) were loaded on the surface of the electrode and incubated for one hour to completing aptamer immobilization. The SSDNAs were bonded to polydopamine layers by Schiff base reaction and Michael addition through polydopamine functional quinones groups and NH_2 terminated aptamer[27, 28].

3.RESULTS AND DISCUSSION

3.1. Characterization of the synthetic Bi_2S_3

XRD spectrum of the as-prepared Bi_2S_3 has been shown in fig 1A. Herein, the main diffraction peaks were belonged to the orthorhombic phase. Also, no impurities were detected. The sharpness and narrowness of the peaks confirmed the high crystallinity of the product. The existence of the index peaks at the positions: 25, 29, 32, 40.5, 47.5 and 53.5 confirmed the structure of Bi2S3 according to JCPDS card No.17-0320[29].

EDS analysis diagram of the synthetic metal chalcogenide has been presented in fig 1B which confirms the existence of bismuth and sulfur in the structure of the as-prepared material. As well, FESEM image of Bi2S3 shows coralline shape of the prepared material in its nano dimensions(fig 1C).

3.2. Electrochemical studies of aflatoxin B1 aptasensing

3.2.1. Optimization of the loaded AFB1 aptamer on the surface of the pencil electrode

Undoubtedly, maximum response in an aptasensor will be achieved by immobilization of appropriate amounts of aptamer on the surface of the electrode. For this purpose, a typical pencil/ polydopamin electrode was incubated with different concentrations of NH_2 terminated AFB1 aptamer to form pencil/ polydopamin/aptamer electrode as described in the previous section.

In the following, the concentrations of 10, 30, 50, 70, 90 and 100µM of AFB1 aptamer was loaded on the surface of the pencil/ polydopamin. The current response was inspected by 0.01M of the mixture of $K_3[Fe(CN)6]$ /$K_4[Fe(CN)_6]$ (dissolved in phosphate buffer solution, pH=7) as the probe. As can be seen in fig 2, the current response remained almost constant after contact with 50µM of aptamer. Because, the highest amount of double helix formation between the aptamer and the target (AFB1) was occurred after applying 50µM of aptamer and above. So, incubation the pencil/polydopamin electrode with the mentioned concentration was performed for future electrochemical experiments.

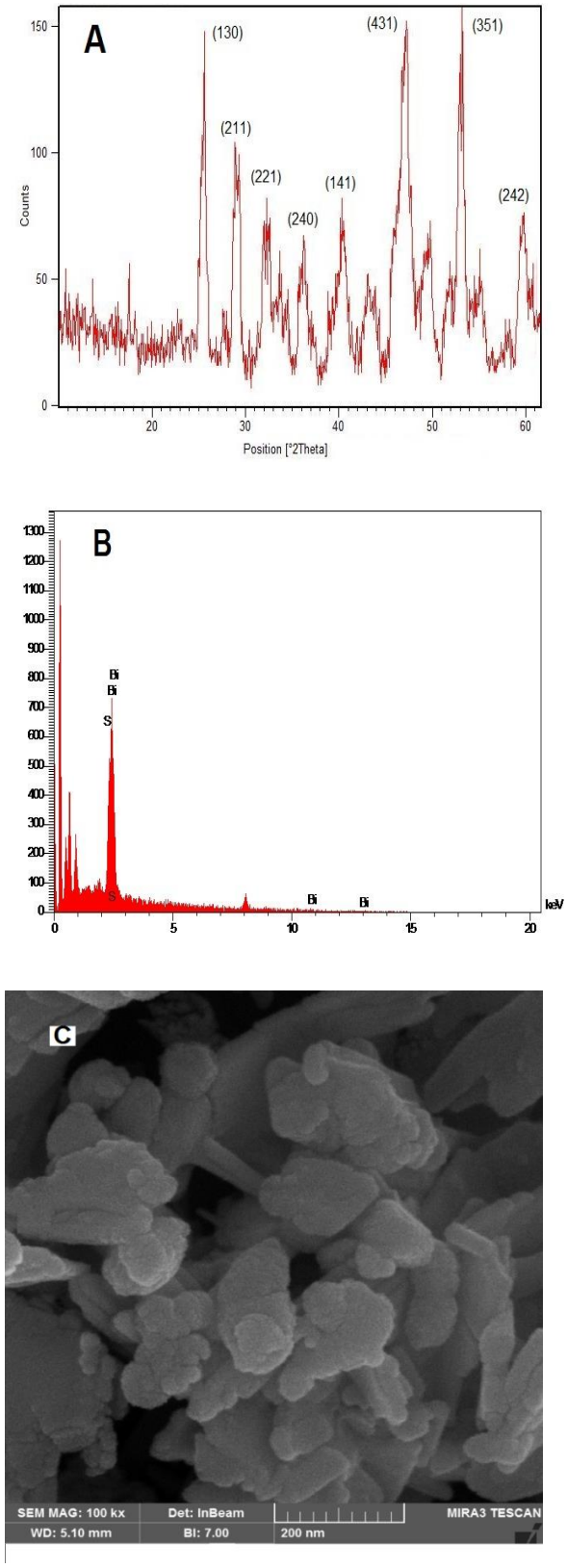

Fig.1. A: XRD pattern, B: EDS analysis and C: FESEM image of the synthetic Bi_2S_3

DOI: 10.30473/ijac.2024.70101.1283

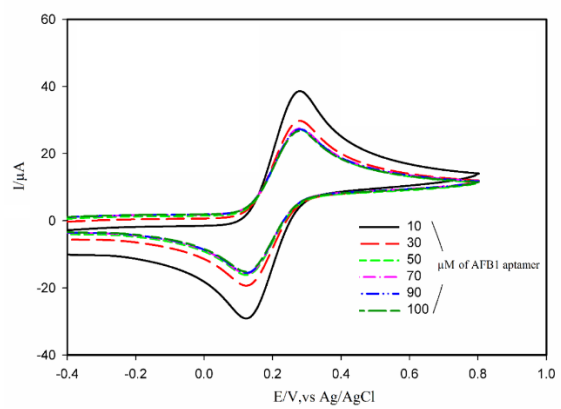

Fig 2. CVs corresponded to the various loading of AFB1 aptamer on the surface of the pencil/ polydoapmine electrode in presence of 0.01M of of $K_3/K_4[Fe(CN)_6]$-4/-3, dissolved in PBS(pH=7), scan rate=100mV/s.

3.2.2 Electrochemical characterization of the modified pencil electrodes

Cyclic voltammetry was applied to investigate the behavior of various pencil electrodes in presence of the 0.01 M of $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$. As can be found in fig 3A, the current responses corresponded to pencil/ polydopamine/aptamer electrode increased to maximum level due to the least hindrance against probe penetration to the surface of the electrode. The same electrode was incubated by 0.1M of aflatoxin B1 which caused noticeable decrease in current response. Herein, double helix formed due to presence of AFB1 molecules created serious space barrier against probe penetration. Following, adding appropriate amounts of the p-type Bi_2S_3 to the structure of the aptasensor (pencil/polydoamine@Bi_2S_3/ aptamer electrode) considerably led to improvement current response. In this case, the p-type Bi_2S_3 with its positive charge transfer holes can promote electron absorption for the probe redox process on the surface of the electrode. With increases in Bi_2S_3 content in the aptasensor, the current response decreased again and the conductivity drops drastically. So, a suspension containing 0.15g of prepared Bi_2S_3 in 0.5 ml of tetrahydrofuran was applied for modification the pencil electrode. By this way, an optimized aptasensor with the most sensitivity was developed for AFB1 analysis

Electrochemical impedance spectroscopy (EIS), as a powerful technique, was applied to affirm the cyclic voltammetry results. Nyquest plots were obtained in presence of 0.01M of probe for various pencil electrodes(fig 3B). As it was predicted, the lowest resistance against charge transfer(Rct), described as the diameter of semicircles in Nyquest pattern, was belonged to the pencil/polydopamine/aptamer electrode. By formation the duplexes of aptamers with the target molecule(AFB1), the Rct was significantly increased in the pencil/polydopamine/aptamer @ AFB1. Other results were in full compliance with the corresponded CVs.

The aptasensing process of the fabricated label-free aptasensor (pencil/polydopamine @ Bi_2S_3/aptamer @ AFB1) based on a signal-off manner, has been presented schematically in fig 4.

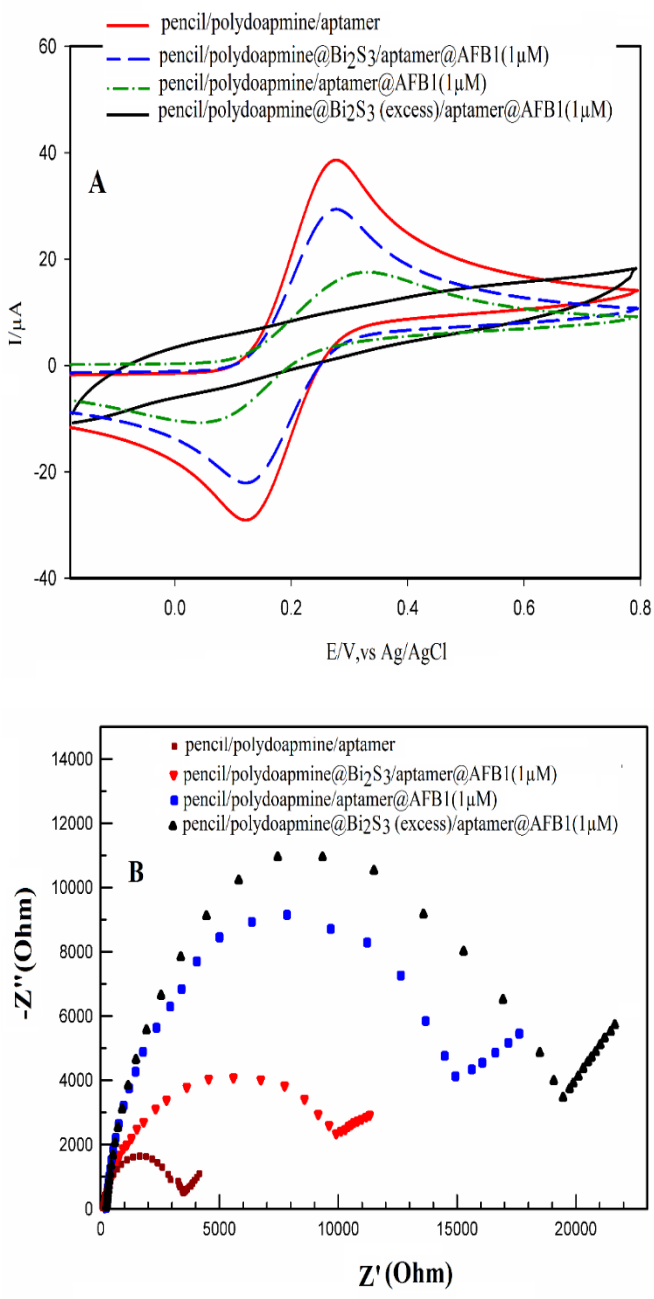

Fig. 3. A: CVs related to various modified pencil electrodes (scan rate=100mV/s), B: corresponded Nyquest plots that confirms the CVs at the frequencies range of 10kHz-40 mHz, All in the presence of 0.01 M of K_3/K_4Fe (CN)6-4/-3.

DOI: 10.30473/ijac.2024.70101.1283

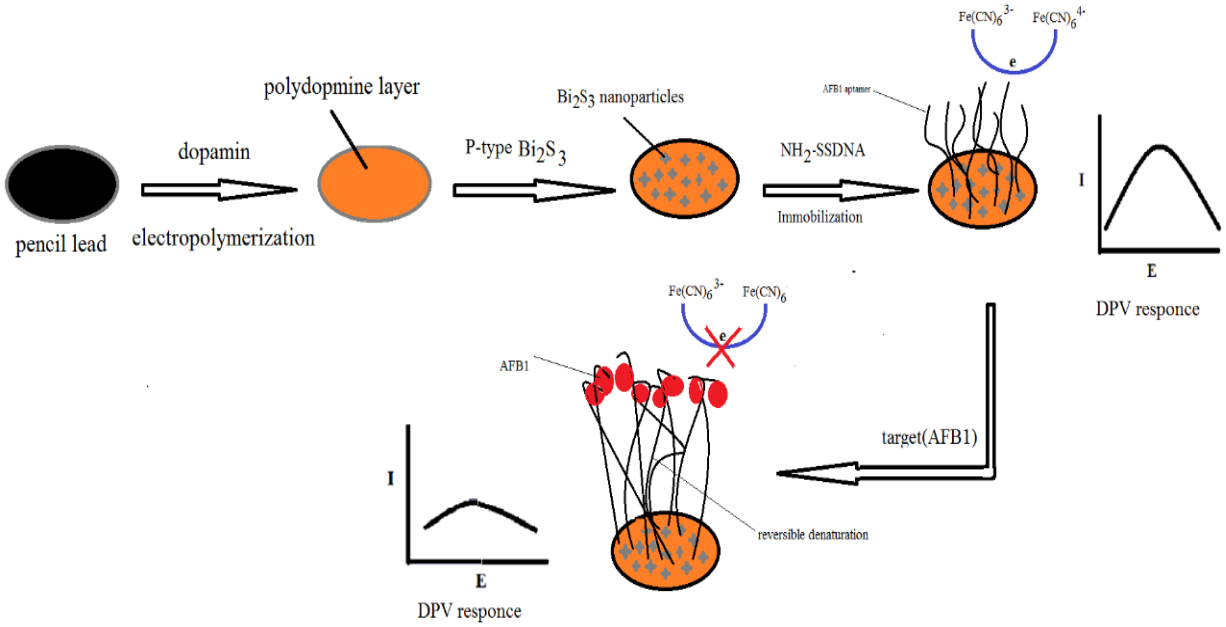

Fig. 4. Schematic performance of the signal–off manner of the fabricated aptasensor (the designed scheme may not be in agree with the real scales).

3.2.3, Quantitative analysis of AFB1 by the fabricated label-free aptasensor

A typical pencil/polydopamine@Bi_2S_3/aptamer was made in compliance with optimized conditions and evaluated by differential pulse voltammetry (DPV). The pencil/polydopamine @ Bi_2S_3/aptamer was incubated with various concentrations in the range of 0.3-630nM of AFB1(dissolved in PBS, pH=7) from down to up turn by turn with the same electrode. A solution containing 0.01M of $K_3[Fe(CN)_6]$ /$K_4[Fe(CN)_6]$-4/-3, dissolved in PBS(pH=7), propped the current response. As can be seen from DPVs (fig 5A), with increase in AFB1 concentration, the current response (Ip) dropped due to formation of more duplexes and more hindrance against probe infiltration. Hence, the quantitative analysis was done in a signal-off manner.

The calibration plot corresponded to DPVs (fig 5A) was obtained by plotting ΔIp (difference between current response of the blank and every desired concentration of AFB1) versus the different concentrations of AFB1.A straightforward line with an excellent linearity (0.998) was obtained in the range of 0.3-630nM.The limit of detection (LOD) was calculated by using 3Sb/B (where Sb is the standard deviation of seven replicated detections of the blank solution and b stands for the slope of the calibration plot). Hereby, the LOD was estimated 0.04nM which was a considerable value for detection of aflatoxin B1.

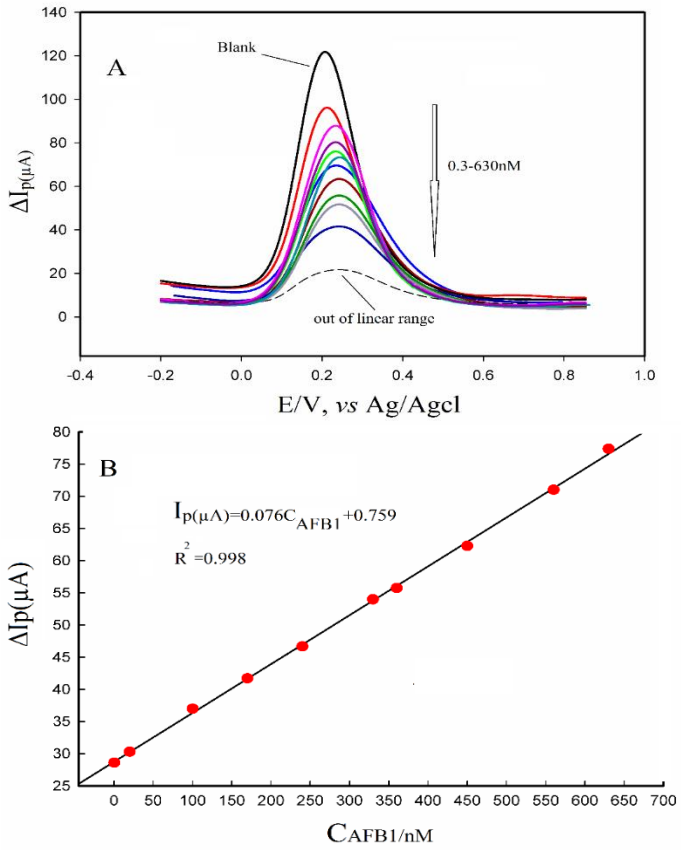

Fig. 5. A: DPvs related to various concentrations of AFB1(0.3,20,100, 170,330,360,450, 560, 240 and 630nM) in presence of 0.01 M of $K_3/K_4[Fe(CN)_6]$-4/-3(sweep rate=100mV/s, pulse amplitude=0.05V/s and pulse time=0.04s), B: The corresponded calibration plot.

DOI: 10.30473/ijac.2024.70101.1283

3.2.4. Repeatability and stability of the fabricated aptasensor

Relative standard deviation (RSD%) was used to guesstimate the repeatability of the electrode. Five replicated detection of 100nM of AFB1 was performed by a typical pencil/polydopamine @ Bi2S3/aptamer. Subsequently, the repeatability was estimated 2.53% as an acceptable precision for analysis.

The stability of the as-prepared aptasensor was tested by keeping a typical pencil/polydopamine @ Bi_2S_3/aptamer in PBS (pH=7) at 4ºC during two weeks. The electrode applied for analysis of AFB1(100nM). The observed current drop was less than 5% after the desired period.

3.2.5. Real sample analysis and comparison with other reports

For evaluation of the fabricated aptasensor, real sample analysis was done under optimized conditions by pencil/ polydoamine @ Bi_2S_3/aptamer. Three wheat flour samples were prepared from various local bakeries. Appropriate amounts of the samples transferred to volumetric flasks and 25 ml of methanol (60%) was added and shacked for 20 minutes. Then the mixture was filtered and the supernaut was collected and diluted with PBS (pH=7). Flowingly, adequate levels of AFB1 was spiked to the samples and the recovery percentages were calculated. As can be seen in table 1, satisfying performance has been obtained by the developed label- free aptasensor.

Table 1.: The results of real sample analysis performed by the suggested aptasensor

Sample (wheat flour)	AFB1 spiked (nM)	AFB1 detected (nM)	Recovery%	RSD
1	50	49.1	98.20	2.91
2	350	336.7	96.20	2.66
3	500	517.4	103.48	3.90

Table 2 represents the results of recent electrochemical and non-electrochemical reports for aflatoxin B1 analysis. As it is clear, the developed AFB1 apatasensor shows good performance in terms of LOD and dynamic linear range.

Three types of fibers (PDMS 100 µm, DVB/CAR/PDMS 2 Cm 50/30 µm , and CW/PEG 60 µm) were used to evaluate the effect of fiber types on the extraction of volatile compounds in *Ocimum sanctum L* . Fig 2 shows the total peak areas of the obtained compounds by the three types of fibers. As it is shown in Fig. 2, DVB/CAR/PDMS fiber achieved higher extraction of the analytes than the other fibers. This suggested that the retention ability of the DVB/CAR/PDMS fiber for the volatile compounds in the plant is much stronger than the rest two fibers. As shown in Fig. 2, the fibers with a medium polar coating appeared to be more efficient for the extraction of *Ocimum sanctum L* . compounds. It probably resulted from the fact that most of the analytes in the sample are of medium polarity. The polarity of the two fibers were supposed to be in the order of PDMS<DVB–CAR–PDMS. In this case, the fiber DVB–CAR–PDMS has higher extraction ability than the others. On the basis of the above results, the DVB–CAR–PDMS fiber was selected for the extraction of the volatile compounds in this medicinal plant.

Table 2. Comparison of the current work with recent studies for AFB1 determination

Method	Linear range of AFB1	LOD	RSD %	Ref.
fluorescence quenching method	3.2nM-320µM	1nM	------	[30]
Immunofiltration assay	---------	6.41nM	------	[31]
quantum dots adsorbed on Au nanoparticles aptasenor	10nM-400nM	3.4nM	-------	[33]
Enzymatic based conductometric biosensor	0.25mM - 1 mM	0.16µM	7.00	[26]
Graphene assisted aptasensor	0.5 nM- 4 µM	0.07nM	2.89	[18]
Current work	0.3-630nM	0.04nM	2.53	------

4.CONCLUSION

The main results of the current study have been summarized here:

Appropriate amounts of the p-type synthetic Bi_2S_3 was used as a signal promoter for development of an aptasensor for detection ultra-trace levels of aflatoxin B1. The p-type material in its optimum value encouraged the electron capture on the surface of the electrode.

Electropolymerized dopamine was acted as a simple binding mediator instead of expensive, complicated and sensitive reagents such as EDS and NHS.

A high concentration range of AFB1 with very low detection limit (0.04nM) was achieved by the fabricated aptasensor.

The pencil/polydoamine @ Bi_2S_3/aptamer applied for ultra-trace analysis of aflatoxin B1 in wheat flour.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

DOI: 10.30473/ijac.2024.70101.1283

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DOI: 10.30473/ijac.2024.70101.1283

نقش یک نیم هادی کلکوژنید در یک آپتاحسگر بدون برچسب، برای اندازه گیری مقادیر بسیار جزئی افلاتوکسین B1 در آرد گندم

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تاریخ دریافت: ۱۱ آذر ۱۴۰۲ **تاریخ پذیرش:** ۱۶ دی ماه ۱۴۰۲

چکیده

در این تحقیق سولفید بیسموت (Bi_2S_3) به عنوان یک کالکوژنید نیمه هادی مصنوعی در فرآیند ساخت یک آپتاحسگر به عنوان یک ارتقا دهنده سیگنال برای تشخیص مقادیر بسیار کم آفلاتوکسین B1 (AFB1) مورد استفاده قرار گرفت. سیگنال تجزیه ای به واسطه استفاده از مقادیر بهینه Bi_2S_3 برای اصلاح الکترود بهبود یافت. نوع تک رشته ای آپتامرAFB1 (SSDNA)به سادگی روی سطح مقطع الکترود مدادی از طریق لایه های پلی دوپامین تثبیت شد. گستره وسیعی از غلظت AFB1 (۰/۳- ۶۳۰ nM) توسط آپتاحسگر ساخته شده (مداد/پلی دوپامین /Bi_2S_3@آپتامر) با استفاده از ولتامتری پالسی تفاضلی پشتیبانی شد. ساخت ساده و عدم نیاز به تازه سازی الکترود بخشی از مزایای آپتاحسگر پیشنهادی است. در نهایت، حد تشخیص بسیار پایین (۰/۰۴ نانومولار) و حساسیت زیاد (۰/۰۷۶میکروآمپر بر نانومولار) و همچنین پایداری و تکرارپذیری مناسب ، منجر به استفاده از این آپتاحسگر در آنالیز نمونه‌های واقعی مانند آرد گندم با درصد بازیابی قابل قبول گردید.

کلید واژه ها

آپتاحسگر؛ افلاتوکسینB1 ؛ بیسموت سولفید؛ الکترود مداد؛ پلی دوپامین

DOI: 10.30473/ijac.2024.70101.1283