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1 **Development of a label-free electrochemical aptasensor based on diazonium**  
2 **electrodeposition: Application to cadmium detection in water**

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13 **Abstract:**

14 In this study we have developed a new aptasensor for cadmium ( $\text{Cd}^{2+}$ ) detection in water. Gold  
15 electrode surface has been chemically modified by electrochemical reduction of diazonium salt (CMA) with  
16 carboxylic acid outward from the surface. This was used for amino-modified cadmium aptamer immobilization  
17 through carbodiimide reaction. Chemical surface modification was characterized by cyclic voltammetry (CV)  
18 and electrochemical impedance spectroscopy (EIS). This latter was also used for  $\text{Cd}^{2+}$  detection. The aptasensor  
19 has exhibited a good linear relationship between the logarithm of the  $\text{Cd}^{2+}$  concentration and the impedance  
20 changes in the range from  $10^{-3}$  to  $10^{-9}$  M with a correlation  $R^2$  of 0.9954. A high sensitivity was obtained with a  
21 low limit of detection (LOD) of  $2.75 \times 10^{-10}$  M. Moreover, the developed aptasensor showed a high selectivity  
22 towards  $\text{Cd}^{2+}$  when compared to other interferences such as  $\text{Hg}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$ . The developed aptasensor  
23 presents a simple and sensitive approach for  $\text{Cd}^{2+}$  detection in aqueous solutions with application for trace  $\text{Cd}^{2+}$   
24 detection in spring water samples.

25 **Keywords:** Label-free detection, aptasensor, diazonium electrografting, gold electrode, cadmium detection.

## 26 **Introduction**

27           Recently, pollution caused by heavy metals is becoming more and more severe. Heavy metals are  
28 naturally occurring elements characterized by high atomic weight and density, which is at least five times greater  
29 than that of water [1]. The distribution of heavy metals; like Arsenic, Cadmium, Chromium, Lead, and Mercury  
30 in the environment is a result of their multiple applications, and raising concerns about their potential effects on  
31 human health and environment in most regions around the world, because of their high degree of toxicity [2].  
32 Cadmium ( $\text{Cd}^{2+}$ ) is a toxic heavy metal whose presence in the environment is mainly issued from human  
33 activities [3]. It is produced in the manufacture of batteries and pigments which can permeate, soil and water  
34 supply. Thereby, exposing the general population to low  $\text{Cd}^{2+}$  doses can affect a variety of cellular mechanisms  
35 such as proliferation, apoptosis, differentiation, cell signalling and gene expression. For these reasons,  
36 Cadmium has been classified as carcinogenic to humans by the World Health Organization (WHO) even at low  
37 doses, the maximum limit is  $0.003 \text{ mg}\cdot\text{L}^{-1}$ . Indeed, it has been reported that exposure to this ion plays a particular  
38 role in prostate cancer[4, 5].[4]. Numerous analytical methods traditionally used for detection of Cadmium (II)  
39 ions have been widely reviewed in the literature; like atomic absorption/emission spectroscopy (AAS/AES) [6,  
40 7], ultraviolet-visible spectrometry [8], Square-wave anodic stripping voltammetry [9] and atomic fluorescence  
41 spectrometry (AFS) [10]. Despite their performance, most of these methods are often tedious, time consuming  
42 and require great amounts of solvents, reagents and sophisticated apparatus. Therefore, inexpensive, sensitive  
43 and selective techniques are very desirable for cadmium monitoring.

44           Biosensors are considered as very promising tools for sensitive, rapid and low cost monitoring of wide  
45 range of analytes in different fields of applications. A wide range of biomolecules or chemical species have been  
46 used in the literature as sensitive layer for chemical or biosensors manufacturing[11]. Aptamers are considered  
47 as bio-inspired receptors which have gained great interest since their discovery in 1990 [12].

48           Aptamers are RNA or DNA molecules with specific 3D structures, selected by *in vitro* selection process  
49 called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [13]. Due to their unique  
50 conformations, aptamers can bind a wide range of targets such as small molecules, proteins, and even whole  
51 cells. Their advantageous characteristics, such as high stability, simple chemical synthesis, possibility of  
52 modification, made them strong competitors of antibodies in molecular analysis [14-16]. They constitute thus

53 good candidates as effective probes for chemical sensors. Several aptasensors have been developed in the  
54 literature for Cadmium ions detection. Hamid et al [17] have developed an electrochemical cadmium sensor  
55 using a DNA aptamer, where the sensing mechanism was based on target binding-induced conformational  
56 change. Here, magnetic beads were used as immobilization support for the aptamer. Despite the biosensor  
57 selectivity toward  $\text{Cd}^{2+}$ , the detection range [250-1000 nM] of this electrochemical sensor was still rather  
58 narrow. In another report, Guo et al. have developed a new colorimetric method for sensing  $\text{Cd}^{2+}$  by using  
59 unmodified AuNPs [18]. Here, the linear detection range [5-40  $\mu\text{M}$ ] of this method was narrow and could not be  
60 used for the detection of  $\text{Cd}^{2+}$  in various types of samples.

61 The critical point in aptasensor fabrication technology remains in the immobilization of active  
62 biomolecules onto the transducer surface. Several immobilization techniques can be adopted according to the  
63 biomolecules architecture and the desired application. For instance, direct attachment of bioreceptors to gold  
64 electrodes has been reported, by using 3' or 5' thiol-labelled aptamers self-assembly through gold-sulfur bond  
65 interaction by forming ordered single-carrier membrane [19, 20]. However, this technique suffers from non-  
66 specific adsorption (random adsorption) and cannot be applied on other materials. Attachment of aptamers onto  
67 sensor surfaces based on hybridization with partially complementary oligonucleotides has been also adopted  
68 [20]. Nevertheless, annealing and hybridization steps are required, enduring thus the experimental conditions.  
69 Covalent attachment to chemically-modified sensor surfaces is a promising method based on the interaction of a  
70 labelling group and a chemically functionalized surface resulting in a layer of ordered film of aptamers.  
71 Chemical covalent attachment may increase the specificity and selectivity of the aptasensing strategy [20].

72 In this study we have chosen an amino-modified aptamer for  $\text{Cd}^{2+}$  detection that we immobilized on  
73 screen printed gold electrode through diazonium salt chemistry. To the best of our knowledge, this is the first  
74 report describing an electrochemical aptasensor exploring diazonium-coupling reaction mechanism for  $\text{Cd}^{2+}$   
75 determination. To achieve that,  $\text{Cd}^{2+}$ aptamer was immobilized onto gold electrodes through 4-carboxymethyl  
76 aryl diazonium (CMA) molecules which are electrochemically reduced on the electrode surface. Unlike  
77 conventional grafting methods, diazonium salt-based electrochemical deposition involves simple reagents  
78 without isolation and purification steps [21-24]. Gold working electrodes (WE) have been characterized with  
79 cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) before and after WE chemical  
80 surface modification and after aptamer immobilization. EIS was then used to monitor the extent of binding  
81 between Cadmium and its specific capture aptamer.

## 82 **Experimental**

### 83 **Chemicals and Reagents**

84 Cadmium (II) nitrate [Cd (NO<sub>3</sub>)<sub>2</sub>], 4-aminophenylacetic acid (4-carboxymethylaniline CMA), Sodium nitrite  
85 (NaNO<sub>2</sub>), isopropyl alcohol (IPA) 96%, N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N'-  
86 ethyl-carbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich, France. Phosphate buffer  
87 (NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O ; Na<sub>2</sub>HPO<sub>4</sub>.2H<sub>2</sub>O) and KCl solution containing ferricyanide K<sub>3</sub> [Fe(CN)<sub>6</sub>] and ferrocyanide K<sub>4</sub>  
88 [Fe(CN)<sub>6</sub>] were used as supporting electrolytes. Cadmium aptamer was synthesized and purchased by  
89 Microsynth (Switzerland). The specific sequence of the aptamer was as follows: (5' amino-modified):5'-ACC  
90 GAC CGT GCT GGA CTC TGG ACT GTT GTG GTA TTA TTT TTG GTT GTG CAG TAT GAG CGA GCG  
91 TTG CG-3' [25].

### 92 **Electrochemical analysis**

93 The electrochemical experiments were carried out with a potentiostat (Biologic EC-Lab SP-300). The  
94 electrochemical measurements were performed in PBS with pH 7.4 at room temperature in a conventional  
95 electrochemical glass cell containing three-electrode system [26].

96 Cyclic Voltammetry (CV) measurements were used before and after chemical gold surface modifications. CV  
97 analysis were performed in 5 mM of [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup> solution within a potential range of (- 0.2V to + 0.6V) and a  
98 scan rate of 80 mV.s<sup>-1</sup>.

99 Electrochemical impedance spectroscopy (EIS) was used to study the barrier properties of the modified electrode  
100 [27, 28]. The EIS measurements were conducted at a potential of 128 mV on the Gold WEs over a frequency  
101 range between 100 mHz and 200 kHz. The data of EIS were fitted using Randles equivalent circuit model. The  
102 modelling of the obtained EIS data was made by the EC-Lab software using the Randomize + Simplex method.  
103 Here, randomize was stopped on 100 000 iterations and the fit stopped on 5000 iterations.

### 104 **Real sample analysis**

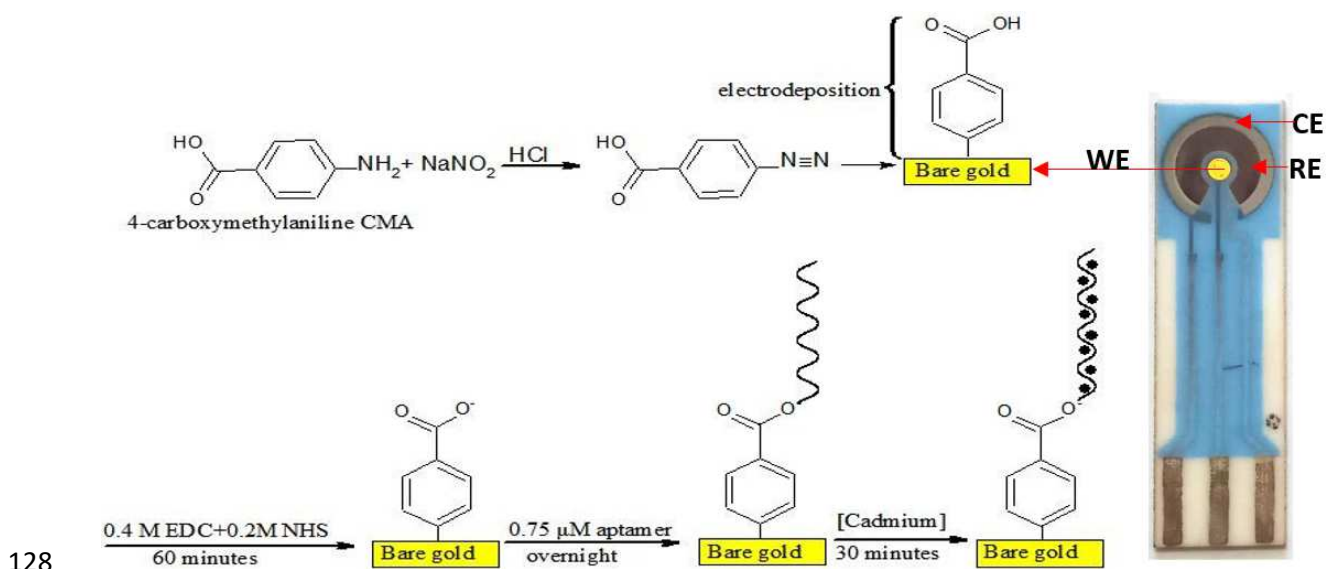
105 In order to validate and evaluate the accuracy and applicability of the our aptasensor, analyses were carried out  
106 on a real river water which is polluted by public work wastes and containing nitrate and cadmium [29, 30]. This  
107 river is crossed by a volcanic water source from Hammam Essalihine valley (khenchela, Algeria) which is rich in  
108 minerals, such as bicarbonates, sulphates, calcium and magnesium. We have made sampling from the river

109 which contains cadmium and other potential interferences. Real samples were tested by EIS combined with  
110 standard addition method [31]. This method consists of adding known amounts of a standard solution to a  
111 sample of interest with unknown concentrations. Then, the analytical signals of standard solutions are recorded  
112 in response to each addition [31]. In brief, a constant volume (2.5mL) of water real sample was added to each  
113 flask of 25mL. Then in the first flask, a 22.5mL of PBS were added to the 2.5mL of real samples in order to  
114 reach a final volume of 25 mL and this flask was called **Level 1**. The standard solution containing the analyte  
115 was then added in increasing volumes: 0.25, 2.5 and 5 mL, implying the addition of 10, 100 and 200 nM of Cd<sup>2+</sup>  
116 to the subsequent flasks 2, 3 and 4. Finally these flasks were made up to the volume of 25 mL with PBS giving  
117 thus **Level 2, Level 3 and Level 4**, respectively.

## 118 **Results and discussion**

### 119 *Electrochemical aptasensor preparation*

120 Fig.1 illustrates the detection principle of Cd<sup>2+</sup> by using the electrochemical aptasensor. Cd<sup>2+</sup>-aptamer  
121 immobilization was achieved through the covalent linkage between amino groups of the aptamer and the  
122 abundant reactive carboxylic groups on the gold surface. The electrode surface was first modified by deposition  
123 of a diazonium salt film, and activation of the terminal carboxylic groups with EDC/NHS. Then, the aptamer  
124 was linked to the gold surface via its activated carboxylic groups. In the presence of Cd<sup>2+</sup>, a variation in the  
125 aptamer conformation takes place from a random coil structure to an aptamer–Cd<sup>2+</sup> complex [32, 33]. This  
126 aptamer-target interaction hampers the electron transfer, enhancing thus the surface resistance which is  
127 proportional to cadmium concentration in the sample.

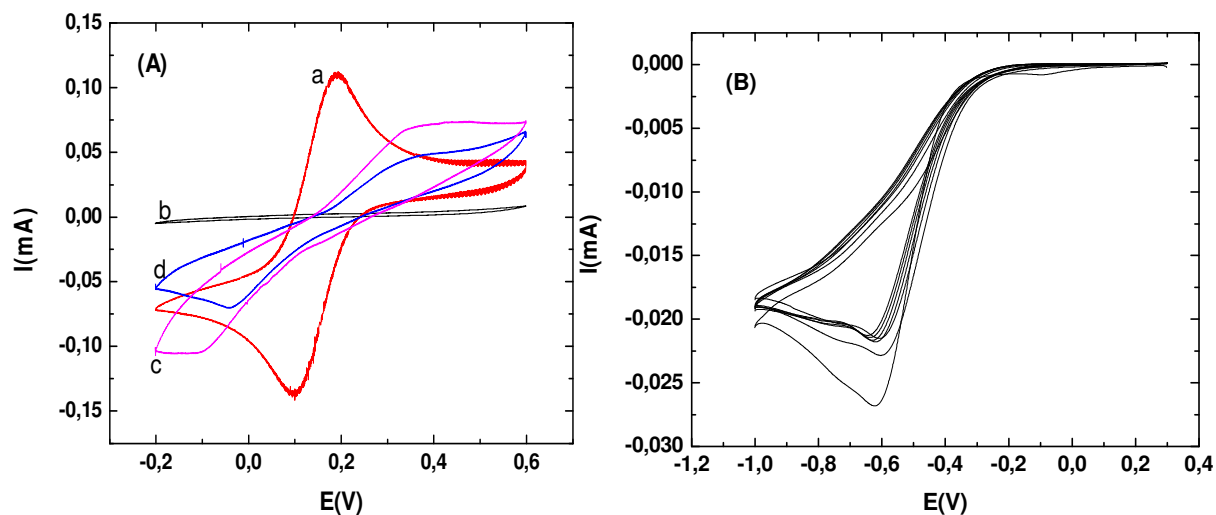


129 **Fig. 1** Schematic illustration of gold WE chemical surface modification by CMA followed by aptamer and  
130 immobilization for Cadmium detection[32, 33]using integrated RE and CE.

### 131 **Characterization of the functionalized gold surface**

#### 132 *Cyclic voltammetry studies*

133 For the Cyclic Voltammetry studies, **Fig.2A** shows the different cyclic voltammograms of the construction steps  
134 of this aptasensor. The oxidation-reduction peaks of the redox couple are visible for the bare Gold WE. **Fig.2A**  
135 **(a)** showing a pair of quasi-reversible peaks characteristic of the oxidation-reduction with cathodic and anodic  
136 current peak ratio of approximately one and peak-to-peak separation of 0,1V. Then, a significant decrease in the  
137 current **Fig.2A (b)** has been observed after the electrochemical grafting of diazonium salt **Fig.2B**. The  
138 electrochemical reduction of the diazonium salt was generated *in situ* by a reaction of CMA with NaNO<sub>2</sub> in a  
139 solution of HCl, with a scanning speed of 80 mV.s<sup>-1</sup>, and within a potential range of (-0.2 to -1V). In the first  
140 cycle, the current variation showed a reduction wave localized at -0.6V. This current corresponds to the  
141 reduction of the diazonium salt in solution which leads to the formation of a radical amine. Afterwards, we  
142 noticed from the second scan cycle that the reduction current decreases remarkably. This can be attributed to the  
143 decrease of the electron transfer rate that was created by the CMA blocking layer. This was confirmed by a  
144 significant decrease in the current **Fig.2A (b)**. After aptamer immobilization, CV peaks at the electrode have  
145 significantly increased compared to CMA modified gold WE electrode **Fig.2A(c)**. This implies that most of the  
146 surface of the electrode was covered with aptamer and demonstrates successful aptamer immobilization. Then,  
147 the modified gold WE was incubated in a PBS solution containing 1nM cadmium Cd<sup>2+</sup> during 30mn followed by  
148 CV measurement **Fig.2A (d)**. Here we have again a decrease of CV peaks which means that the Cd<sup>2+</sup> have been  
149 successfully adsorbed onto the gold WE surface. The Cd<sup>2+</sup> adsorption prevents or decreases the transfer of  
150 electrons between gold WE surface and the redox couples in solution due to the opening of the rod portion of  
151 aptamer and formation of the complex Cd<sup>2+</sup>- aptamer



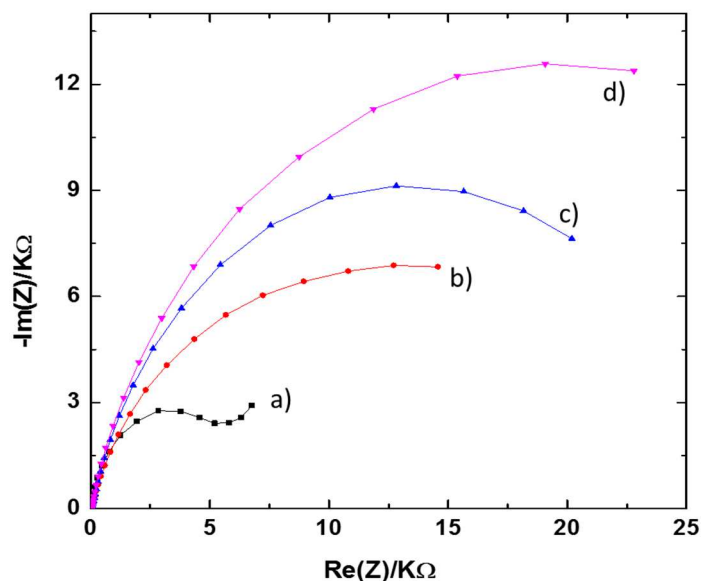
152

153 **Fig. 2** (A) Cyclic voltammograms of (a) bare gold WE (b) after CMA deposition (c) after aptamer immobilization  
 154 and (d) after the detection for 10<sup>-9</sup>M of Cd<sup>2+</sup> solution. (B) Cyclic voltammograms for electrodeposition of CMA  
 155 for repetitive potential cycles from -0.2V to -1V with a scan rate of 0.8V.s<sup>-1</sup>  
 156

157 **Electrochemical impedance spectroscopy studies**

158 Impedance spectra were employed to assess the electrochemical behaviour of the aptasensor fabrication and  
 159 realized in ferric potassium ferrocyanide solution. The Nyquist plot semi-circles allow to assess the transfer  
 160 charge resistance  $R_{ct}$  generated by any modification on the gold WEs surface. The more the surface is passivated,  
 161 the higher is the resistance  $R_{ct}$  value **Fig.3**. Here, the first Nyquist plot semi-circle **Fig.3 (a)** corresponds to the  
 162 bare gold which presents negligible resistance  $R_{ct}$ . Then, the formation of an organic layer of CMA onto the gold  
 163 WE surface has increased the  $R_{ct}$  to 25 290  $\Omega$  **Fig.3 (b)**. This was due to the CMA layer which decreases the  
 164 electron transfer. Subsequently, the surface carboxylic groups were activated using carbodiimide chemistry to  
 165 allow the Cd<sup>2+</sup>-aptamer immobilization through the covalent linkage between amino groups of aptamer and the  
 166 abundant reactive carboxylic groups of CMA. Aptamers immobilization has induced a strong increase in the  
 167 semi-circular diameter, with an  $R_{ct}$  of 28 096  $\Omega$  **Fig. 3(c)**. Finally, the developed aptasensor was tested for  
 168 Cadmium detection by incubating the aptasensor in Cd<sup>2+</sup> solution at 10<sup>-9</sup>M for 30 min. Here we observed an  
 169 increase of  $R_{ct}$  up to 42 537  $\Omega$  **Fig.3(d)** due to the conformational changes induced by aptamer switching from a  
 170 random coil structure to an aptamer–target complex[32, 33].

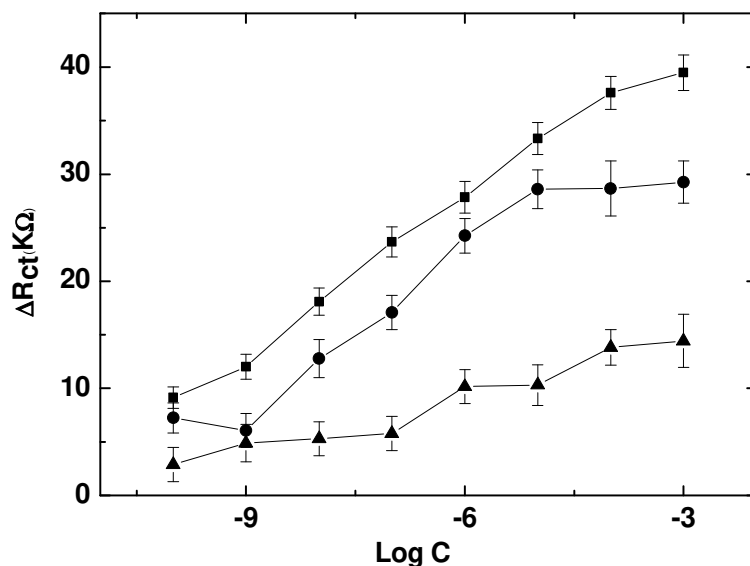




171 **Fig. 3:** Nyquist diagrams of the bare gold electrode (■), the electrochemically grafted CMA (●), after deposition  
 172 of the aptamer (▲) and after incubation of Aptamer/CMA/AU in  $10^{-9}$ M  $\text{Cd}^{2+}$  (▼) in the presence of 5 mM of  
 173  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  solution in a frequency range of 100 mHz - 200 kHz. The potential polarization used for these  
 174 measurements was optimized at 128 mV.  
 175

176 **Optimization of aptamer concentration**

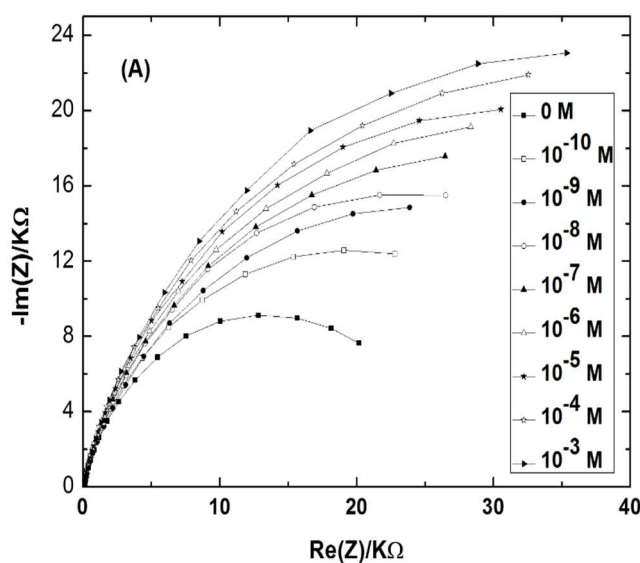
177 The aptamer concentration determines its final density onto the gold WE surface [34]. The optimization of  
 178 aptamer concentration is a crucial step which affects the  $\text{Cd}^{2+}$  ions detection and the analytical performance of the  
 179 aptasensor. Therefore, three different concentrations of aptamer (0.25, 0.5 and 0.75  $\mu\text{M}$ ) were tested within the  
 180 range of  $10^{-10}$  to  $10^{-3}$ M of cadmium by using EIS. Fig. 4 shows the variation of  $R_{ct}$  as function of  $\text{Cd}^{2+}$   
 181 concentration for the three aptamer concentrations. As it can be observed, when using the aptamer concentration  
 182 of 0.25  $\mu\text{M}$ , the  $R_{ct}$  did not change significantly, to the weak complexation of the aptamer with its target. This  
 183 aptamer concentration generated a low signal with a slope of 1.744  $\text{k}\Omega$  ( $R^2= 0.927$ ). The aptamer concentration  
 184 of 0,5 $\mu\text{M}$  resulted a narrow range of linearity with a slope of 3.891  $\text{k}\Omega$  ( $R^2=0.911$ ). Finally, by using the aptamer  
 185 concentration of 0.75  $\mu\text{M}$ , the signal was improved and the analytical response of the aptasensor was linear  
 186 within the range of  $10^{-9}$  to  $10^{-4}$  M with a LOD of  $2.75 \cdot 10^{-10}$ M. In addition, the sensitivity increased to 4.683  $\text{k}\Omega$   
 187 when compared to that obtained with the lower concentrations.



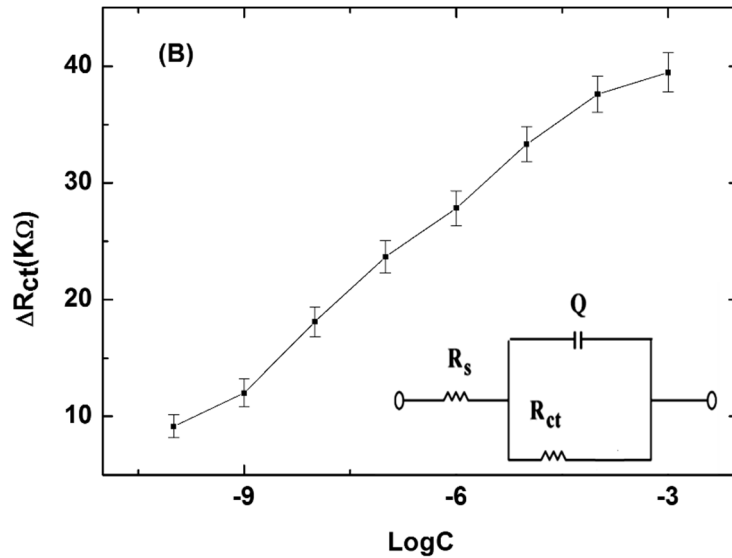
188 **Fig. 4** Optimization of Cd<sup>2+</sup>-aptamer concentration by EIS; (▲) 0.25 μM, (●) 0.5 μM and (■) 0.75 μM of  
 189 aptamer.  
 190

191 **Analytical performance of the aptasensor**

192 Under the optimal conditions, the aptasensor was used for cadmium detection in PBS buffer using EIS. To  
 193 achieve that, the modified aptamer gold WE surface was incubated with different concentrations of cadmium for  
 194 30 minutes. Experimental results showed a significant increase in the diameter of Nyquist plot semi-circles by  
 195 increasing Cd<sup>2+</sup> concentration **Fig. 5A**. The first Nyquist plot semi-circle corresponds to the aptamer-modified  
 196 gold WE before the addition of cadmium. After incubation of aptasensor with increasing concentrations of the  
 197 target, the Nyquist plot semi-circles continue to increase indicating the interaction between cadmium and its  
 198 specific aptamer.



199



200

201 **Fig.5:** (A) Nyquist plots of Cd<sup>2+</sup>-aptamer-Gold WE surface incubated with increasing Cd<sup>2+</sup> concentrations  
 202 ranging from 10<sup>-3</sup> to 10<sup>-10</sup> M, (B) sensitivity of the aptasensor by normalization of the data towards the varying  
 203 Cadmium concentrations. Inset: equivalent circuit used for the EIS Nyquist plot semi-circles fitting.

204

205 The transfer charge resistance R<sub>ct</sub> was extracted from Nyquist plot semi-circles by using the equivalent  
 206 circuit in Figure 5B Inset [21]. Where R<sub>s</sub> represents the solution resistance, R<sub>ct</sub>; the charge-transfer resistance  
 207 and Q1; the constant phase element (an equivalent model of double-layer capacitance). The real part of  
 208 impedance (Re(Z)) increased as function of cadmium concentration. The linear regression equation was  
 209 normalized to  $\Delta R_{ct} = R_{ct}(\text{aptamer-cadmium}) - R_{ct}(\text{aptamer})$  as a function logarithm of Cadmium concentration.  
 210 R<sub>ct</sub> (aptamer-cadmium) is the value of the electron transfer resistance after Cd<sup>2+</sup> binding to the immobilized  
 211 aptamer on the modified electrode surface. R<sub>ct</sub> (aptamer) represents the value of the electron transfer resistance  
 212 after the aptamer immobilization on the CMA-modified electrode surface. The resistance of the aptasensor  
 213 exhibited a linear correlation to Cadmium concentration through the range of (10<sup>-10</sup>-10<sup>-4</sup>M), with a good  
 214 correlation coefficient of (R<sup>2</sup> = 0.9954) and a sensitivity of 4.683kΩ per cadmium concentration decade. The  
 215 limit of detection (LOD) of the aptasensor is the concentration of cadmium corresponding to the 3 times “s/m”  
 216 ratio, where “s” is the standard deviation of the blank impedance signal (three replicates) and “m” is the slope of  
 217 the related calibration curve [35]. Here, the calculated LOD was 2.75\*10<sup>-10</sup>M. The detection limit obtained in this  
 work was lower the detection limits of Cd<sup>2+</sup>aptamer strategies and other methods (**Table 1**).

218  
 219

Table 1: Comparison of other sensors with different detection methods for the determination of Cd<sup>2+</sup>.

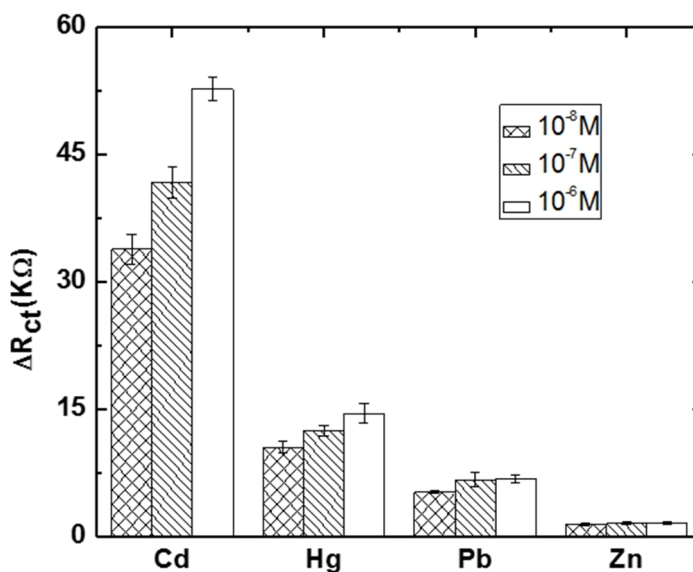
Detection methods	Linear range (nM)	Limit of detection (LOD) (nM)	Refs.
Fluorescent aptasensor	0 to 1000	40	[36]
Fluorescent aptasensor	7.19 to 200	2.15	[37]
Colorimetric aptasensor	10 to 400	4.6	[25]

Electrochemical aptasensor	1to1000	0.337	[38]
Electrochemical aptasensor	250 to 1000	92	[17]
Electrochemical aptasensor	0.1to10 <sup>6</sup>	0.27	This work

220

221 The reproducibility of the developed aptasensor was tested with interassay precision. The interassay  
 222 precision was evaluated by incubating three electrodes, independently prepared under the same experimental  
 223 conditions with a same cadmium concentration of 10<sup>-6</sup> M. A relative standard deviation from of 4.49% was  
 224 calculated, indicating acceptable aptasensor reproducibility.

225 For the specificity study, the **Fig.6** depicts the impedance changes after incubation with Cd<sup>2+</sup> and other  
 226 heavy metals such as Hg<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>during 30 minutes. Three concentrations have been tested for each  
 227 analyte; 10<sup>-8</sup>, 10<sup>-7</sup> and 10<sup>-6</sup>M. At this stage, the interactivity of the aptasensor and cadmium detection was  
 228 performed only in PBS buffer. As it can be observed in (Fig.6); the impedance responses show clearly that the  
 229 aptasensor was highly selective to cadmium as compared to the other tested heavy metals. No significant signals  
 230 have been recorded, even in the presence of Pb<sup>2+</sup> and Zn<sup>2+</sup>. However, a slight cross-reactivity was noted with  
 231 Hg<sup>2+</sup>,it may be attributed to the special T–Hg–T mismatched base pair between Hg<sup>2+</sup> and Cd<sup>2+</sup>- aptamer[39-  
 232 41].These results show that the selected aptamer immobilized on the electrode surface maintains its affinity and  
 233 specificity in reaction with the cadmium, giving significant  $\Delta R_{ct}$  increase. This may be considered as a  
 234 validation of the proposed aptasensor selectivity, because it allows the discrimination between the tested heavy  
 235 metals into the entire selected range.



236  
 237  
 238

Fig. 6 Selectivity studies of the aptasensor with Hg, Pb and Zn.

239 **Application in water samples by the standard addition method**

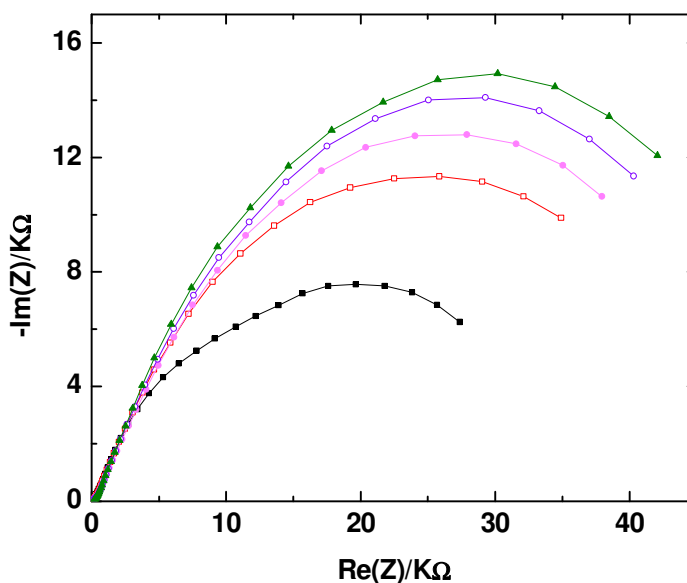
240 The developed aptasensor was applied for the detection of Cadmium in water samples of Hammam Essalihine  
 241 valley using standard addition method as described previously. Four flasks containing the real water samples  
 242 were prepared as mentioned in experimental, and then analysed by EIS measurements **Fig.7** depicts the first  
 243 Nyquist plot corresponding to the aptamer-modified gold surface before cadmium addition, while the second one  
 244 is attributed to the aptasensor after being incubated in the first flask (**level 1**) for 30 min. A shift in the semi-  
 245 circle from the first one was observed, it was explained by the increase of resistance provided from aptamer  
 246 reaction with cadmium. The second flask, corresponding to **level 2**, showed the same behaviour as the first flask.  
 247 The analysis of the other flasks was performed in the same parameters. An increase in the semi-circular diameter  
 248 was noticed and was absolutely explained by the increase of cadmium amount in the flasks. In order to determine  
 249 the concentration of Cd<sup>2+</sup> in the real sample, slope (m) and y-intercept (b) of the resulting calibration curve (R<sup>2</sup> =  
 250 0.987) were used, and the unknown Cd<sup>2+</sup> concentration of real sample was estimated to be 4.29.10<sup>-8</sup>M.

251 Table 2: Determination of Cd<sup>2+</sup> by proposed aptasensors and atomic absorption spectroscopy (AAS) in water  
 252 samples and recovery study of the aptasensor for determining cadmium

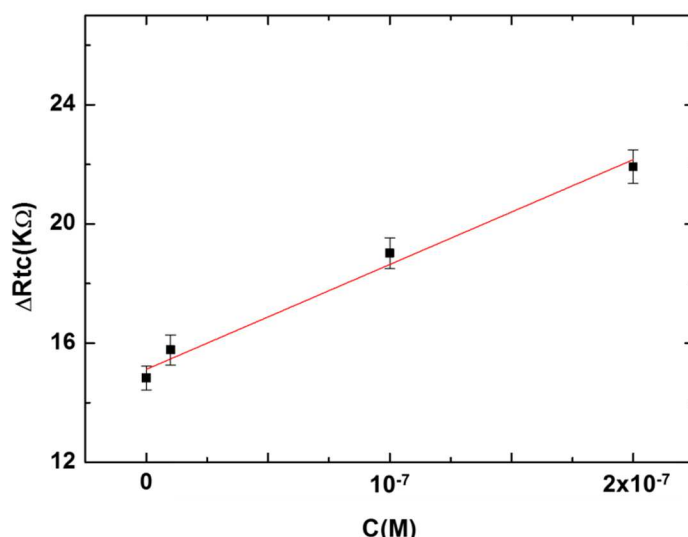
Ion in sample	AAS(C)	The aptasensor(Cs)	Recovery (%)
Cd <sup>2+</sup>	4.76*10 <sup>-8</sup> M	4.29*10 <sup>-8</sup> M	90.12

253  $Recovery\% = C_s/C \times 100\%$ [42]

254 Aiming to validate our aptasensor, the tested sample was also analyzed by atomic adsorption  
 255 spectroscopy (AAS) which is a reference method usually used for Cadmium detection[6, 7] . The obtained  
 256 results are presented in (Table2). They are in a good agreement with those obtained by the standard addition  
 257 method.



258



259

260 Fig. 7 (A) Nyquist impedance plots obtained from the performance application on a real sample. (■) PBS; (□ )  
 261 Level 1 (corresponding to an addition of 0 M); (●) Level 2 (addition of 10<sup>-8</sup>M); (○) Level 3 (addition of 10<sup>-7</sup>M)  
 262 and (▲) Level 4 (addition of 2\*10<sup>-7</sup>M). (B) Sensitivity curve used to calculate the concentration of analyte in the  
 263 unknown sample by the standard addition method. Error bars represent the relative standard deviation on the  
 264 measurements performed in triplicate.

265

## 266 Conclusion

267 In summary, we have developed an electrochemical aptasensor for Cadmium detection. The aptamer was  
 268 covalently immobilized onto gold WE through CMA molecules. The studied aptasensor showed a linear  
 269 relationship between the impedance changes and the logarithm of Cd<sup>2+</sup> concentration in a broad range from 10<sup>-3</sup>  
 270 to 10<sup>-10</sup> M, with a LOD about 2.75\*10<sup>-10</sup>M. Indeed, the present aptasensor displays excellent sensitivity and  
 271 selectivity, in addition to a good reproducibility. Moreover, the aptasensor was highly selective to cadmium in  
 272 the presence of other interferences, such as Hg<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>. In order to prove the method applicability, the  
 273 constructed aptamer sensor has been employed to detect cadmium in real spring water samples with high  
 274 accuracy. These promising results show that the developed method could be an excellent alternative for  
 275 traditional techniques because of its accuracy, reliability, simplicity and low cost. This miniaturized sensor could  
 276 be used wide wearable devices in rivers or directly in spring water sources. In addition, the working electrode  
 277 could be functionalized by other aptamers to allow the concomitant determination of several heavy metals.

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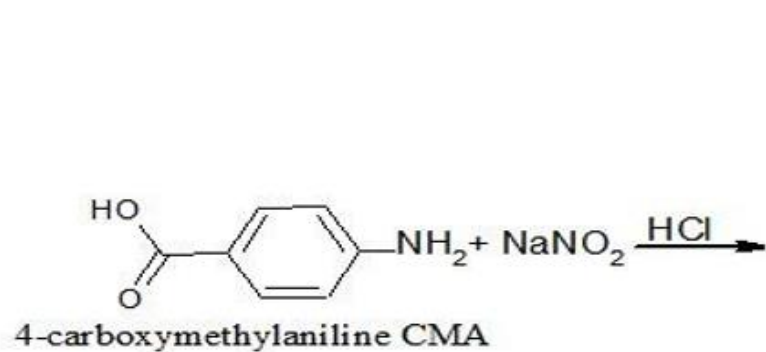
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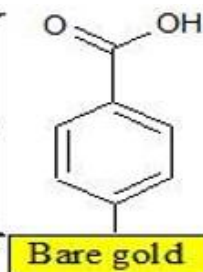
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electrodeposition



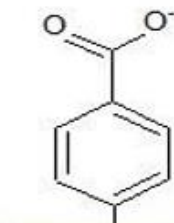
Bare gold

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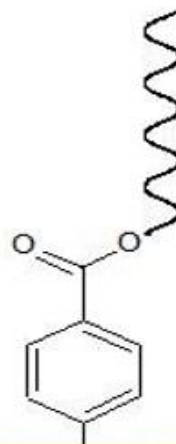
RE

$0.4 \text{ M EDC} + 0.2 \text{ M NHS}$   
60 minutes



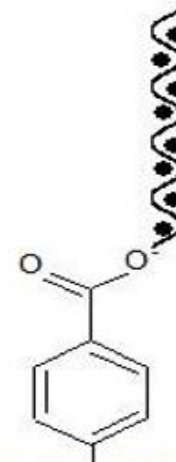
Bare gold

$0.75 \mu\text{M aptamer}$   
overnight



Bare gold

[Cadmium]  
30 minutes



Bare gold

