Materials Science and Engineering C 52 (2015) 151–154

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Short communication

Non-Faradaic electrochemical impedance spectroscopy as a reliable and facile method: Determination of the potassium ion concentration using a guanine rich aptasensor

Sayed Habib Kazemi [a,*], Maryam Shanehsaz [b], Mostafa Ghaemmaghami [a]

[a] Department of Chemistry, Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan 45137-66731, Iran
[b] Analytical Chemistry Research group, Research Institute of Petroleum Industry (RIPI), Tehran, Iran

ARTICLE INFO

Article history:
Received 6 December 2014
Received in revised form 19 March 2015
Accepted 24 March 2015
Available online 26 March 2015

Keywords:
Aptasensor
Aptamer
Impedance
Biosensor

ABSTRACT

In this article we report the application of non-Faradaic mode of electrochemical impedance spectroscopy (EIS) for determination of potassium ion (K^+) concentration using a guanine rich K^+-selective aptasensor (K^+-aptasensor). This is a simple, electroactive probe free, sensitive and reproducible method allowing determination of K^+ ion concentration without any disturbance from electroactive probes used in similar works based on the Faradaic EIS method. Herein, a wide linear range of K^+ ion concentrations (1 μM–0.1 mM) with a 200 nM limit of detection was achieved which is better than most of the previously reported Faradaic biosensing methods. The proposed method maintains valuable applications when it is used for K^+ determination in the presence of potentially important interferences in biological media. Thus, application of the non-Faradaic EIS method for sensing the concentration of K^+ ion with the presented K^+-aptasensor can find an important role in clinical assay.

1. Introduction

Determination of metal ion concentration in environmental and physiological media has attracted enormous attention in the fields of chemical and electrochemical sensing [1,2]. Among them, potassium ion (K^+) plays an essential role in biological functions, including blood pressure regulation, transmission of nerve impulses, proper digestion, and enzyme activation [3–5]. The anomalous K^+ concentrations are side effects of or caused some diseases such as kidney diseases, alcoholism, anorexia, bulimia, heart disease, and diabetes. Development of sensors for detection of K^+ ion in physiological environment is one of the most important issues in biomedical diagnosis. The presence of sodium ion (Na^+) in excess concentrations along with K^+ in physiological environment makes it necessary to develop new strategies for detection of K^+ ions. In previous studies, aptamer based sensors, which are artificial single-stranded RNA or DNA molecules that can specifically tie to certain target molecules such as metal ions, small molecules, proteins, cells, and even tissues, were utilized to actuate their target molecules with high affinity and specificity. Modification of the electrode surface using thiolated molecules, self-assembled monolayers or SAMs, is a well-known method to design nano-reactor sites and sensing interfaces [6–9]. Among them, biological elements such as DNA and RNA molecules play a critical role for fabrication of the sensing and diagnostic interfaces [4,10–12]. It is reported that, in the presence of metal ions, a single-stranded DNA with guanine (G)-rich sequences can fold into specific four-stranded helical conformations which are known as the G-quadruplex structure [3]. There is a channel at G-quadruplex center which its size is equal to the size of the K^+ ion. Among all alkali cations, only K^+ can be located in this cavity by binding to eight oxygen atoms from the G-tetrads because of its highest efficiency. A number of aptasensors have been constructed based on the G-quadruplex. Among these aptasensors, some have been reported for K^+ detection based on different strategies, including ion chromatography, fluorometric, colorimetric, flame atomic absorption spectrometry and electrochemical methods [3,5,11]. Electrochemistry has been widely employed and has been considered to play a very important role in future research due to its high sensitivity, fast response, simple application, low operation cost, and portability.

Electrochemical impedance spectroscopy (EIS) is a powerful technique to monitor the events occurring on the electrode–electrolyte interfaces; therefore it can be advantageous in analytical methods for analyzing the species concentrations [7,12]. In brief, EIS measurements can be performed in two ways: Faradaic EIS and non-Faradaic method. Faradaic impedance measurements were usually carried out by using a reversible redox probe while non-Faradaic impedance measurements are done without using any redox probe [13]. In a non-Faradaic sensor, the capacitance of the electrode–electrolyte interface can be considered as the main indicator of interaction between aptamer and K^+ ions [14].

Herein, we presented an easy and facile way to determine the K^+ ion concentration. Conversely to other previously reported works for

* Corresponding author.
E-mail address: habibkazemi@iasbs.ac.ir (S.H. Kazemi).

http://dx.doi.org/10.1016/j.msec.2015.03.058
0928-4931/

determination of K^+ by using Faradaic electrochemical impedance spectroscopy, we for the first time employ the non-Faradaic method to probe the K^+ concentration changes. In this simple, sensitive and noninvasive method, a selective K^+-aptamer was utilized as a biological sensing element and as a detection tool. To seek this thought, a thiolated aptamer was immobilized on the electrode surface by a self assembly method. In fact, adding K^+ ion to the solution makes changes in the structure of aptamer self-assembled monolayers (SAMs) from random coil into a G-quadruplex to form a cavity with the ionic radius size of K^+. In fact, upon adding K^+, the K^+-aptamer complex was formed leading to a change in the interfacial capacitance of electrode surface which can be estimated by the EIS method. Our results confirmed a good linear range within 0.1 μM to 0.1 mM for detection of K^+ with a detection limit of 200 nM.

2. Experimental

2.1. Chemicals and apparatus

The sequence of the single strand oligonucleotide employed (5′-TTTGGTTGGTGTGGTTGGTTT-3′-$(CH_2)_6$–SH) was purchased from Bioron company. Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from Sigma-Aldrich to prepare Tris buffer solution (TBS). All other reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water.

A Zahner/Zennium Potentiostat–Galvanostat was used for all electrochemical measurements. All experiments were performed using a conventional three electrode-system. A 0.0314 cm^2 gold electrode modified with the thiolated aptamer, a platinum wire and an Ag/AgCl sat'd KCl were used as working, counter and reference electrodes, respectively.

2.2. Aptasensor fabrication

Firstly, the gold electrode was carefully polished with 0.3 and 0.05 μm Alumina to have a mirror like finish. Then, the electrode was sonicated in ethanol–water solution at least for 10 min to remove any physically adsorbed particles. The cleansed electrode was introduced in a three electrode cell containing 0.5 M of H_2SO_4 and subjected to successive cyclic voltammetry within −0.5 to 1.5 V vs reference electrode to obtain a reproducible voltammogram.

In the next step, the gold electrode was washed thoroughly with doubly distilled water and immersed in a 0.1 M TBS containing thiolated-aptamer for at least 2 days. Also, to ensure the full blocking of the electrode surface by SAMs, the aptamer modified electrode was immersed in a solution of 2-mercaptohexanol for 10 min, in which mercaptohexanol chains cover the unmodified regions between aptamer chains at the electrode surface.

3. Results and discussion

3.1. Performance of the aptasensor

Sensing mechanism of the proposed K^+-aptasensor has been reported in the literature [3,4]. In the absence of potassium, the K^+-aptamer SAMs with multiple guanine-rich segments exhibit a random-coil structure. Upon introducing the modified electrode into a solution containing K^+ ions, the K^+-aptamer SAMs will fold into the G-quadruplex form and a cavity with an equivalent size to K^+ ion is created. During the conformational changes, interfacial capacitance was changed and non-Faradaic EIS was applied to determine the concentration of K^+ ion based on the equivalent circuit analysis and complex nonlinear least square method. Thus, all the EIS experiments were performed by applying a bias potential selected in the non-Faradaic or double layer region to avoid any Faradaic current in the EIS responses. The interfacial capacitance depends on the nature of the modified layer on the electrode surface; its thickness and electrolyte concentration. When K^+ is added, conformational changes result in the change of the thickness of the interface in addition to the changes in the dielectric of the modified layer depending on the amount of incorporated K^+ ions.

In this report, we introduce the ability of monitoring the capacitance changes as sensing signal in the non-Faradaic EIS method. The possibility of using no redox probe such as potassium Ferro/Ferricyanide in high concentration is a very important advantage of the non-Faradaic EIS approach, since the complexity of the electrochemical system will be reduced. Firstly, to find the optimum potential range in which the EIS results can be gathered; cyclic voltammograms of the electrochemical system were recorded in the different potential windows. It should be mentioned that the EIS bias potential was chosen in a current region in which no Faradaic processes can be observed. It was found that there is insignificant Faradaic current in the potential range of −0.25 V to +0.25 V vs Ag/AgCl for the modified electrode (shown as Fig. S-1, supplementary information).

It is well-known that by immersing the K^+-aptasensor electrode in an electrolyte solution containing K^+ ions; opposite charges will accumulate near the electrode surface to form the double layer capacitor. After conditioning the aptasensor with a specific concentration of K^+ ion, it was washed thoroughly with distilled water to remove any physically adsorbed ions; then it was introduced to electrochemical cell containing only TBS. Also, to check the next concentration of K^+ ion it is necessary to regenerate the modified electrode after interaction with a specified concentration of K^+ ion. Fig. 1A and B depicts the EIS signal changes by conditioning the aptasensor in K^+ ion solution with different concentrations and the corresponding calibration curve obtained by plotting relative change in interfacial capacitance against concentration. Table 1 shows the electrical parameter obtained by equivalent circuit analysis of EIS experimental results. A simple parallel RC loop in series with solution resistance was used to fit the EIS results. A constant phase element (CPE) was used instead of ideal capacitor in equivalent circuit analysis. A wide dynamic linear range of K^+ ion concentrations (1 μM–0.1 mM) with a 200 nM limit of detection was achieved from Fig. 1B, according to the following equation:

$$\mathrm{LOD} = \frac{3\sigma_b}{m}$$

where σ_b refers to the standard deviation of blank sample and m is the analytical sensitivity of the calibration curve plotted.

To examine the practical ability of the aptasensor for successive complexation–regeneration (through breaking down the complex of the aptamer-K^+ in a hydrochloric acid media), corresponding EIS signal was monitored for at least 10 times of aptamer-K^+ formation and regeneration of K^+ free aptamer. To achieve this purpose, the modified electrode was immersed in HCl solution (0.1 M) and was kept under stirring for 1 h to regenerate the biosensor. After 10 times of regeneration and analysis cycles of K^+, the electrochemical signal was changed less than 5%. Thus, it can be concluded that the transition between the random coil and G-quadruplex forms is relatively dynamic.

3.2. Selectivity, stability and practical application of K^+-aptasensor

In order to investigate the selectivity and stability of the K^+-aptasensor, the electrochemical responses of the biosensor were determined in the presence of some of the interfering metal ions including Mg^{2+}, Ca^{2+}, and Na^+ at concentrations 1000 times higher than the upper limit of the linear concentration range recorded for K^+. As seen in Fig. 2A, in the presence of the aforementioned cations, there is no severe problem for determination of potassium concentration within the concentration range of 1 μM to 0.1 mM (less than 7% interference observed by fitting to the proposed equivalent circuit). Additionally, the present aptasensor shows good selectivity for K^+ determination even in the presence of high concentration of Na^+ (up to 0.5 M), one of the most serious interferences in biological samples. It should be considered

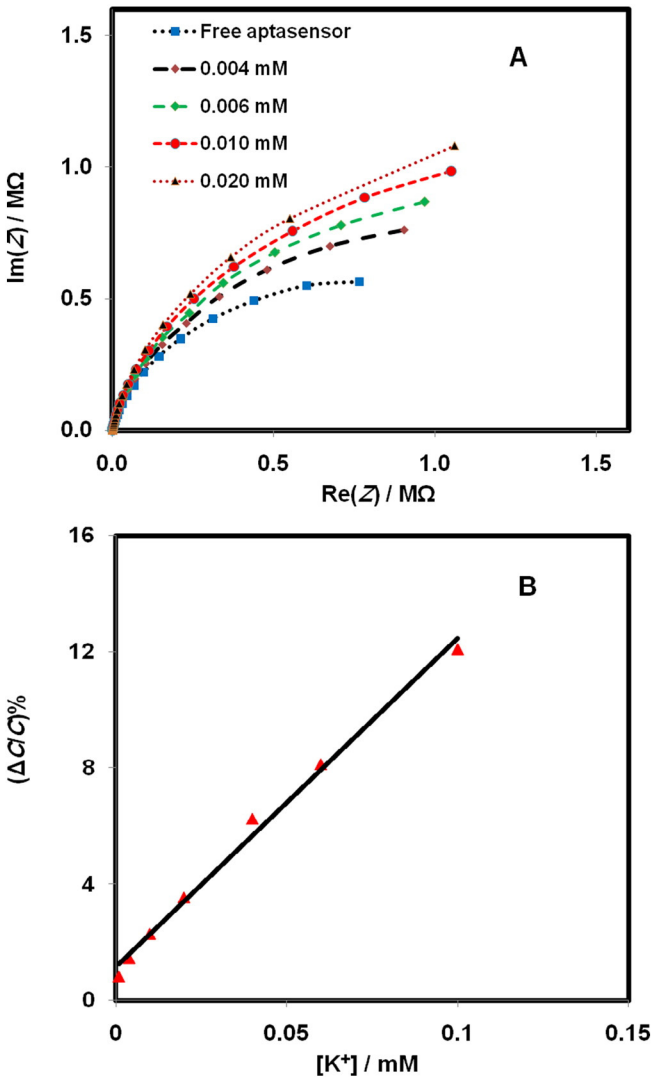

Fig. 1. A) Nyquist diagrams representing the corresponding signal changes by conditioning the aptasensor in K^+ ion solution with different concentrations, and B) typical calibration curve obtained by plotting relative change in interfacial capacitance against concentration for K^+-aptasensor.

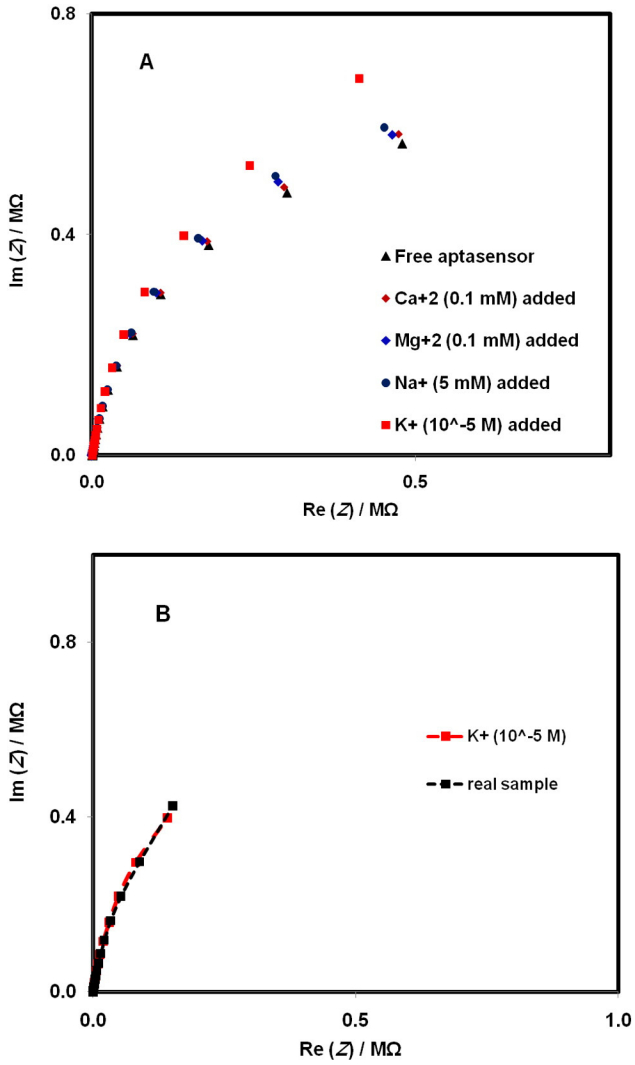

Fig. 2. A) Nyquist plots recorded for K^+-aptasensor in the absence and presence of interfering metal ions (Mg^{2+}, Ca^{2+}, and Na^+) at concentrations 1000 times higher than the K^+ ion concentration, and B) Nyquist plots comparing the aptasensor response to a pure K^+ solution and a simulated physiological solution (real sample) including the same K^+ concentration by applying the K^+-aptasensor.

that the amount of K^+ ions is one of the important indicators of some kidney diseases. To check the ability of the proposed K^+-aptasensor in physiological conditions; a simulated physiological solution was prepared and the K^+ ion assay was carried out by applying the aptasensor. The physiological solution consists of Na^+ (1 mM), Mg (2×10^{-5} M) and Ca (2×10^{-5} M) and in this case, reasonable results were achieved (Fig. 2B). We also examined the K^+-aptasensor in a real physiological sample, serum plasma, after carful pretreatment of the sample. Our observation revealed that the level of error by the matrix of real sample was less than 10%.

To check the stability of the aptasensor, electrochemical results were collected after one month of application–regeneration of the biosensor. It was found that the aptasensor preserves its ability to determine the potassium concentration very close to the initial responses, even in the presence of high concentration of interferences. Also, as mentioned above, it maintains its initial analytical merits with a RSD of less than 5.0%. Thus, the non-Faradaic EIS method used for determination of K^+ concentration can be considered as a low price and facile method in clinical applications.

Table 1
Electrical parameters for K+-aptasensor obtained by fitting EIS experimental data to a simple series of solution resistance to a parallel RC equivalent circuit (CPE employed instead of ideal capacitor).

Conc./mM	C_{dl}[a]/μF	n	%ΔC/C
0.001	0.475	0.98	0.84
0.004	0.472	0.98	1.46
0.010	0.468	0.98	2.30
0.020	0.462	0.98	3.55
0.040	0.449	0.98	6.26
0.060	0.440	0.98	8.14
0.100	0.421	0.98	12.11

[a] All EIS plots were modeled by CPE elements instead of ideal C_{dl}. However, deviations from ideal capacitor were insignificant and all CPE exponents were nearly 0.98 in all cases.

4. Conclusions

A G-rich DNA aptamer was used as a recognition element for potassium ions. A simple, sensitive and reproducible aptasensor was developed for the K^+ ion assay by applying non-Faradaic EIS. Using the non-Faradaic EIS method a linear range of K^+ ion concentrations (1 μM to 0.1 m) with a 200 nM limit of detection was achieved. Non-Faradaic EIS can be performed without adding any redox probes which is one of the important and beneficial features of this aptasensor which offers some superiority over the limited similar works using the

Faradaic EIS method. The proposed non-Faradaic EIS method is still being developed to extend its potential application in clinical assay and improve its sensitivity and precision.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2015.03.058.

Acknowledgment

The financial supports of the work by the Institute for Advanced Studies in Basic Sciences (1392 annual grant) are acknowledged.

References

[1] M.K. Bojdi, M. Behbahani, A. Sahragard, B.G. Amin, A. Fakhari, A. Bagheri, A palladium imprinted polymer for highly selective and sensitive electrochemical determination of ultra-trace of palladium ions, Electrochim. Acta 149 (2014) 108–116.
[2] M.K. Bojdi, M.H. Mashhadizadeh, M. Behbahani, A. Farahani, S.S.H. Davarani, A. Bagheri, Synthesis, characterization and application of novel lead imprinted polymer nanoparticles as a high selective electrochemical sensor for ultra-trace determination of lead ions in complex matrixes, Electrochim. Acta 136 (2014) 59–65.
[3] Z. Chen, L. Chen, H. Ma, T. Zhou, X. Li, Aptamer biosensor for label-free impedance spectroscopy detection of potassium ion based on DNA G-quadruplex conformation, Biosens. Bioelectron. 48 (2013) 108–112.
[4] A.-E. Radi, C.K. O'Sullivan, Aptamer conformational switch as sensitive electrochemical biosensor for potassium ion recognition, Chem. Commun. (2006) 3432–3434.
[5] X.-J. Xing, Y. Zhou, X.-G. Liu, H.-W. Tang, D.-W. Pang, Amplified fluorescent assay of potassium ions using graphene oxide and a conjugated cationic polymer, Analyst 138 (2013) 6301–6304.
[6] S.H. Kazemi, A. Alizadeh, R. Mohamadi, M.M. Khodaei, D. Kordestani, pH-regulated release of dopamine from well-ordered self-assembled monolayers: electrochemical studies, Mater. Sci. Eng. C 33 (2013) 5095–5099.
[7] S.H. Kazemi, M. Shamsipur, A. Alizadeh, M.F. Mousavi, Electrochemical behaviors of novel electroactive Au nanoparticles protected by self-assembled monolayers, J. Iran. Chem. Soc. 10 (2013) 333–338.
[8] M. Shamsipur, S.H. Kazemi, A. Alizadeh, M.F. Mousavi, M.S. Workentin, Self-assembled monolayers of a hydroquinone-terminated alkanethiol onto gold surface. Interfacial electrochemistry and Michael-addition reaction with glutathione, J. Electroanal. Chem. 610 (2007) 218–226.
[9] M. Shamsipur, S.H. Kazemi, A. Mehdinia, M.F. Mousavi, H. Sharghi, Electron transfer behavior through densely packed self-assembled monolayers of a novel heteroaromatic thiol derivative onto the gold surface, Electroanalysis 20 (2008) 513–519.
[10] J.-W. Park, S.S. Kallempudi, J.H. Niazi, Y. Gurbuz, B.-S. Youn, M.B. Gu, Rapid and sensitive detection of Nampt (PBEF/visfatin) in human serum using an ssDNA aptamer-based capacitive biosensor, Biosens. Bioelectron. 38 (2012) 233–238.
[11] A.E. Radi, J.L.A. Sanchez, E. Baldrich, C.K. O'Sullivan, Reusable impedimetric aptasensor, Anal. Chem. 77 (2005) 6320–6323.
[12] H. Xu, K. Gorgy, C. Gondran, A. Le Goff, N. Spinelli, C. Lopez, E. Defrancq, S. Cosnier, Label-free impedimetric thrombin sensor based on poly(pyrrole-nitrilotriacetic acid)-aptamer film, Biosens. Bioelectron. 41 (2013) 90–95.
[13] C. Gautier, C. Esnault, C. Cougnon, J.-F. Pilard, N. Casse, B. Chénais, Hybridization-induced interfacial changes detected by non-Faradaic impedimetric measurements compared to Faradaic approach, J. Electroanal. Chem. 610 (2007) 227–233.
[14] V.M. Mirsky, M. Riepl, O.S. Wolfbeis, Capacitive monitoring of protein immobilization and antigen–antibody reactions on monomolecular alkylthiol films on gold electrodes, Biosens. Bioelectron. 12 (1997) 977–989.