Biosensors and Bioelectronics 182 (2021) 113125

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: http://www.elsevier.com/locate/bios

Chemogenetic biocompatibly of mercury as specific hypercalcemia actuator in neuronal spinal cord cell manipulation: Zeta bio-sensing analysis

Sajedeh Karami , Mohammad Mahdi Doroodmand *

Department of Chemistry, College of Sciences, Shiraz University, Shiraz, 71454, Iran

ARTICLE INFO

Keywords:
Chemogenetic
Spinal cord
Mercury
Hypercalcemia
Zeta potential
Bio-sensor

ABSTRACT

Chemogenetic property of mercuric ion (Hg^{2+}) was investigated as a specific hypercalcemia actuator in the neuronal spinal cord cell manipulation by Zeta-based potentiometric bio-sensing analysis via introducing a novel array-based Hg^{2+} bio-sensor. For this purpose, the array of a two-electrode system including Ag/AgCl (sat'd Cl^-) as reference electrode and a paste nano-composite as the indicator electrode was utilized. The indicator electrode was made of activated multi-walled carbon nanotubes as conductive support, a grounded slice of sheep's spinal cord as natural neuron stem cells (ionophore), and oxalate ion as both the dispersed phase and cationic site. Under optimum conditions by one-at-a-time method, a two-linear range between 1.3×10^{-4}- 6.5×10^{-12} and 2.7×10^{-14}- 1.4×10^{-21} mol L^{-1} with correlation coefficients (R^2) of 0.96 and 0.99, respectively, and response time (t_{90}) of maximum 5.0 min were approximated. The percentages of relative standard deviation were estimated to be 4.05 (repeatability, n = 10) and 6.14 (reproducibility, n = 12). The detection limit was estimated to be sub 5.3×10^{-22} mol L^{-1} based on the $X^-_b+3S_b$. The reliability of this phenomenon was evidenced by different analytical techniques. The Zeta-based electrical response was therefore attributed to highly Ca^{2+} pumping from the stem cells ionic channel gates as the proposed mechanistic behavior of the spinal cord. Actuating (triggering) the stem cells by Hg^{2+} consequently led to generate significant Zeta potential as the proposed mechanism. The results pointed to the potentiometric responsibility of a protein with gram molecular weight of 66.2 ± 0.3 KCU in the stem cell matrix as a specific hypercalcemia actuator.

1. Introduction

Mercuric ion (Hg^{2+}) is considered as one of the most important toxic reagents; due to its especially toxic characteristics such as volatility, and perturbation in the neuron electrical signals (Xu et al., 2012). These challenges exhibit the intrinsic interaction between the nerve systems with Hg^{2+} even at the ultra-trace levels. About the neuron systems, these trials are often appeared in different forms, especially in the electrical perturbations of the Zeta potential (Chen et al., 2020). Control of these problems looks to be important during dealing with the medical curing processes for prevention from some neurological diseases, for instance, multiple sclerosis, etc. (Kahrizi et al., 2016). As a result, the introduction of simple, rapid, portable, and ultra-high selective and sensitive detection systems appears to be essential for the mercury detection purpose in different types of real samples; such as water, neuronal liquids, and inter/intra-cellular fluids and air atmosphere. Furthermore, following the chemogenetic property of Hg^{2+} on the neuron matrices would provide light viewpoint during treating some neuronal disorders (Saleh et al., 2006). Accordingly, in this study, a novel Zeta-based potentiometric bio-sensor has been fabricated based on the Zeta bio-sensing analysis using a two-electrode system including multi-walled carbon nanotube (*MWCNTs*), sheep's spinal cord as naturally differentiated stem cells, and oxalate ion nano-composite as indicator electrode and Ag/AgCl (Sat'd Cl^-) as the reference electrode. It is expected that, the chemogenetic manipulation of the natural stem cells in the spinal neuron cord system by specific reagents can consolably be adopted as a specific ionophore.

2. Material and methods

2.1. Spinal cord

Detail of the reagent materials, instrumentation system, stem cell sample preparation was reported in detail in Sections 1-7.SI (See Supporting Information, Sections: 1 to 7. SI). This part includes detail description about the adopted material and reagents, besides the source

* Corresponding author.
E-mail addresses: Sajedeh.karami1395@gmail.com (S. Karami), Doroodmand@shirazu.ac.ir (M.M. Doroodmand).

https://doi.org/10.1016/j.bios.2021.113125
Received 12 October 2020; Received in revised form 13 February 2021; Accepted 26 February 2021
Available online 20 March 2021

of the spinal cord tissue as the stem cell ionophore. The procedure related to the matrix of the indicator electrode has also been reported in detail. Scheme. 1.SI exhibits the sequence of the fabrication of spinal cord-based nanocomposite (See Supporting Information, Section: 7. SI). Packing this nanocomposite inside a plastic syringe therefore led to have homogeny, smooth (flat) surface, and renewable indicator paste electrode for the Hg^{2+} detection and determination.

2.2. Array system preparation

The introduced array-based potentiometric bio-sensors (Scheme. 2. SI, See Supporting Information, Section: 8. SI) consisted of fifteen independent potentiometric indicator electrodes, introduced to fifteen plastic (*PTFE*) containers (20.0 mL, 16–9522, Iran, Tehran). Each container (10.0 mL) was then half-filled with electrolyte, containing Hg^{2+} solutions at the selected molar concentrations at pH 5.6 ± 0.1 (n = 3) using CO_3^{2-}/HCO_3^- (0.1 mol L^{-1}, 2.0 mL) buffer solution. All the containers were connected through glass tubes (diameter: 3.0 mm, length (2.0 cm), packed with porous disks (GSA-234, pore size of 150 μm, China) to the main PTFE container (50.0 mL). This main bottle contained the electrolyte solution (25.0 mL) at the optimum pH condition for the introducing the Ag/AgCl (sat'd Cl^-, MetrohmAG Company) as the reference electrode. All parts of the electrode system were connected to the potentiometer through an electronic multiplexer (EmStatMUX8-R2, Palmsens BV, Randhoeve 221, 3995 GA Houten, Nederland) via a software program written in Visual Basic 6 (*VB_6*) software. To protect the system, from any environmental noise(s), the array-based bio-sensors were located inside a cubic Faraday's cage (50 × 50 × 60 cm, stainless steel, pore diameter: 1.0 mm). Also, coaxial cables (RG_6, CIMPLE CO - 30′ Feet, USA, BNC connector) were used to eliminate the Zeta-based potentiometric signals from any high impedance noise(s). The Zeta-based potentiometric data related to each indicator electrode were then independently requisitioned versus the reference electrode at different time scales; sequentially, with time interrupts of maximum 0.5 s; and stored in a PC for further analyses. Detail of this system has been shown in detail in the Supplementary Manuscript (See Supporting Information, Section: 11.SI). As shown, all the Zeta-based potentiometric responses were measured, independently and sequentially, vs. the reference electrode at the selected time scale.

2.3. Zeta-based electromotive force (Emf) measurements of the bio-sensing system

The optimum weight percentage was based on the measurement of the Zeta-based potentiometric gradient (*ΔE*, mV, vs. the reference electrode), defined based on the potentiometric response gradient between initial time and that estimated as t_{90} of the maximum Zeta-based potentiometric response (at maximum 20 min, See Supporting Information, Section: 9. SI). The introduced bio-sensing system is represented as follows (Scheme. 3):

Ag| AgCl(s), KCl (3.0 mol L^{-1}) | Electrolyte| MWCNTs/spinal cord/ $C_2O_4^{2-}$| Cu wire (Schem. 3).

The optimization process by the one-at-a-time method was achieved during individual introducing the fresh standard Hg^{2+} solutions with 1.0×10^{-5} and 1.0×10^{-15} M concentrations as the selective probes. The selection of these concentrations was based on the estimation of their partial middle linear ranges, during preliminary rough optimization by the selected method. It should be noted that, along different stages such as optimization, assessment of the figures of merit, mechanism's evaluation, and the real sample analysis process, the Hg^{2+} standard solutions were standardized by elemental determination using neutron activation analysis (*NAA*) as the standard method (See Supporting *Information*, Section: 13. SI).

2.4. Procedure

After conditioning the indicator electrodes by the recommended procedure, they were introduced to the array-based bio-sensing system. After that, the electrodes were introduced and delayed for 10.0 min to have full confidence about the dampness of the porous disks during reaching minimum electrical resistance and accessing to the best electrical connectivity. Then, the potentiometer was turned on and the Zeta-based potentiometric responses were recorded versus the reference electrode at different time scales, sequentially. For having full confidence about the reliability of the data acquisition, each Zeta-based potentiometric datum was considered as the average of at least 10 sequential data. Besides, *a Q-test* (Degrees of freedom: 9, confidence level: 95.0%) was applied to each datum through the VB_6 software to have accurate data processing.

2.5. Real sample preparation and analytical analyses

Procedures related to the real sample preparation and different analytical analysis processes were reported in detail (See Supporting Information, Sections: 10, 11. SI). Also, NAA was adopted to standardize the Hg^{2+} stranded solution and evaluate the reliability of the real sample analysis (See Supporting Information, Section: 12. SI).

3. Results and discussion

In this report, due to the significant characteristics of the spinal cord for interaction with the Hg^{2+} at different molar concentrations, ranged from sub aM to serval mM levels, Zeta potential analytical process was selected as the Hg^{2+} detection system. This technique was regarded as one of the classical fields in the electrochemical analysis (Andrade et al., 2006). This process has often been employed for detecting determining most of the inorganic electroactive species, almost in the aqueous real samples (Ismaiel and Aroua, 2013). However, this method is widely used in biological, environmental, and industrial analyses (Faridbod et al., 2007). The Zeta-based potentiometric analysis possesses various advantages such as high enough fast, cost-effective, and authentic technique (Bratovčić et al., 2009). Though, this technique demands frequent recalibration (Bratovčić et al., 2009).

When the performance and operational features of the Zeta-based potentiometry are combined with the novel reagents with special properties, various applications of the techniques can be adapted to detect different analyses at very low concentrations such as sub nanomolar scales (Düzgün et al., 2011). For example, based on the literature, different analytical methods have been described for rapid and direct detection/determination of dissimilar analytes such as inorganic ions like Ag^+, Pb^{2+}, Ca^{2+} and K^+ ions (Chumbimuni-Torres et al., 2006) as well as some different organic reagents like neurotransmitters and proteins (Düzgün et al., 2013) based on special types of direct/indirect Zeta potential analyses (Wu et al., 2017).

However, the selectivity and sensitivity of these bio-sensing systems should be promoted during the modification with suitable transducers (Mehrvar and Abdi, 2004). For instance, one of the most enduring sensing probes is the cell-based bio-sensor, which can detect some bio-chemical effects directly using natural tissues such as proteins, etc. (Pancrazio et al., 1999). Among these living tissues, focusing on the spinal cord as natural nervous stem cells would open novel fields in bio-sensing technology. Strong physicochemical interaction between the spinal cord and metal/non-metal impurities is found as the main reason. To evaluate this phenomenon, the iontophoretic effect of spinal cord-based stem cells is studied in detail in the following sections.

3.1. Spinal cord as Hg^{2+}-sensitive ionophore

The selectivity and sensitivity of a certain ion-selective sensor are greatly related to the properties of the ionophore used. About the stem

cells, due to the presence of lots of ionic channels such as Na^+, K^+, Cl^-, and Ca^{2+} (Schmidt et al., 2018), ionic binding equilibrium constants of different ionic species as the electroactive reagents are noticeable (Carocci et al., 2014). These channels have also interacted with Hg^{2+} based on different mechanisms using various ionic channel blockers (Pohl et al., 2011). It seems that this interaction is so strong that even an ultra-trace of Hg^{2+} would be recognized. To test this hypothesis, direct Zeta-based potentiometric trace (i.e., the diagram of potential vs. time) as the Hg^{2+}-sensitive detection system during the introduction of the Hg^{2+} standard solution with 1.0×10^{-10} mol L^{-1} concentration has been shown in Fig. 1.

As shown, a sensitive response was detected despite the very small concentration of Hg^{2+}, solution. Also, compared to the blank solution, the significant potentiometric gradient was observed at the time interval between 0.0 and 20.0 min that pointed to the significant interaction between the indicator electrode and Hg^{2+} species. However, this small concentration was not correlated to the potentiometric reduction of Hg^{2+} as electroactive species according to the "*Nernst*" equation. Besides, the zero current potentiometric response canceled other electrochemical phenomena such as Tensammetry. Consequently, the measured potential was attributed to the surface electrical potential gradients, named as "*Zeta Potential*" (Bratovčić et al., 2009) during the ionic channel activities of the spinal cold as natural stem cells (Schmidt et al., 2018) as the probable mechanism of this system. In another word, interaction between ultra-trace of Hg^{2+} with other ionic species as the intermediate reagent has probably resulted in generation of the significant Zeta potential at the surface of the indicator electrode at the open current circuit condition; that caused to the Hg^{2+} recognition, indirectly.

The results (Fig. 2.SI.A) also pointed to the insignificance of the spinal cord of male and female animals during the analysis of Hg^{2+} standard solution (1.0×10^{-10} mol L^{-1}) under similar conditions. This consequence therefore exhibited to the appearance of global interaction between the Hg^{2+} and spinal cord, partially independent from the strain and the sexuality of the vertebrate. Accordingly, the effects of different sources of spinal cords of animals such as sheep, cows, and camels were evaluated (Fig. 2.SI.B) at 10–12 months years old partially under the same conditions. Also, similar tests on male sheep with different ages between at least 2 months to 1.5 years (Fig. 2.SI.C) showed maximum sensitivity (the highest potential gradient) during applying the Hg^{2+} biosensing process (See Supporting Information, Section: 14. SI). These results were probably attributed to the some factors such as i) growth of the stem cells in the spinal cord, ii) the amount (share) of phospholipids inside the spinal cord matrix, and finally iii) mechanical stability and flexibility of the spinal cord when selecting an nominal with 10–12 months years old, relative to other ages.

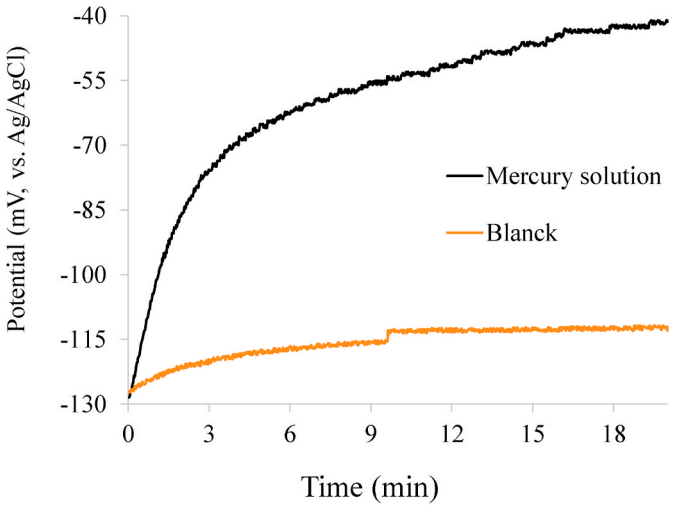


Fig. 1. Electrical potential versus time (trace) during the introduction of Hg^{2+} standard solution with 1.0×10^{-10} mol L^{-1} concentration. Conditions: MWCNTs: $C_2O_4^{2-}$: spinal cord ratio of 2: 2: 1, pH: 5.6 ± 0.1 using CO_3^{2-}/HCO_3^- (0.1 mol L^{-1}) solution, at room temperature.

3.2. Significance of spinal cord as Hg^{2+} ionophore

To evaluate the effective role of the spinal cord as Hg^{2+} binding reagent, it was compared to other nanocomposites made of MWCNTs, oxalate ion, and different forms of binding reagents such as nujol oil, fat, phospholipid, and spinal cord of the lamb's brain tissue under similar conditions (See Supporting Information, Section: 15. SI). Based on the results (Fig. 3.SI), no significant responses were detected when using lipids and nujol oil as a binder, owing to their less improved detection limit of Hg^{2+} as electroactive species. Whereas, noticeable Zeta-based potentiometric response was detected with the sequence of the spinal cord and brain tissue, respectively. This observation pointed to the significance as well as the reusability of the interaction between the spinal cord, as natural neuronal stem cells, and Hg^{2+} (especially at the ultra-trace levels) as the selective probe. In addition, appearance of maximum sensitivity between the stem cells of the spinal cord and the Hg^{2+} strongly revealed maximum susceptibility of the spinal cord (vs. other neuronal tissues) to be considered as natural and available stem cell source. All the results pointed to the effective role of the spinal cord for Hg^{2+} detection purposes during optimization of the related factors by the one-at-a-time method.

3.3. Optimization process

3.3.1. Paste electrode nano-composite

To obtain bio-sensing deviance for sensitive Hg^{2+} recognition, activated MWCNTs were considered as conductive support. In addition, as discussed in detail in the previous sections, spinal cord tissue was adopted as both ionophore and binder during the preparation of the paste electrode. However, based on the direct observations, to promote the homogeneity of this mixture, it was recommended to grind the spinal cord tissue using as a food grinder (Amazon, China), along with using oxalate ion as a homogenizer. To optimize the weight ratios of each species, paste electrodes with different weight percentages were prepared. In addition, the potential gradients were followed using 1.0×10^{-10} mol L^{-1} Hg^{2+} standard solution as the selected probe.

To optimize the weight ratio of each MWCNTs, spinal cord, and calcium oxalate components during the formation of the nanocomposite, different masses of these reagents were precisely weighted and physically (handy) mixed. About some weight ratios such as from around three-fold excess of the spinal cord to MWCNTs, nanocomposites with small electrical conductivity were received. This was almost attributed to the share of some nonpolar compounds in the spinal cord tissue especially phospholipid, resulted in lowering the electrical conductivity of the fabricated nanocomposite. Whereas, other weight ratios showed a significant potentiometric response (See Supporting Information, Section: 16. SI). According to the results (Fig. 4.SI), maximum sensitivity, and reproducibility (n = 3) were observed, when using MWCNTs: spinal cord: oxalate with a weight percentage of 2.0:2.0:1.0. Maximum flexibility, homogeneity, electrical conductivity and adhesion property were detected when using this weight ratio. Scanning microscopic images have been shown in Fig. 5.SI (See Supporting Information, Section: 17. SI). Thus, this ratio was selected to fabricate ideal nanocomposite as the indicator electrode for bio-sensing of Hg^{2+}.

3.3.2. Effects of pH, ionic strength, and temperature

To test the effect of pH (acidity/basicity) on the sensitivity of the introduced bio-sensor to Hg^{2+}, different pH conditions, ranged between 2 and 12 were provided using HCl and NaOH standard solutions with 0.01 mol L^{-1} concentrations (Fig. 6.A.SI). Based on the results, the highest sensitivity was observed at pH 5.6 ± 0.1 during the analysis of Hg^{2+} with 1.0×10^{-10} mol L^{-1} concentration. About this system,

however, we deal with an open circuit (zero current condition) during Hg^{2+} detection and recognition, nevertheless to have fixed pH conditions all over the experiment, buffer condition was selected (Detailed study is reported in the Supporting Information, Section: 18. SI). The results pointed to the suitability of CO_3^{2-}/HCO_3^- as buffer species, based on appearance of maximum potentiometric response. According to the results, maximum potentiometric sensitivity was observed at room temperature (Fig. 6.B.SI, See Supporting Information) with independency from the ionic strength gradients, analyzed using NaCl (0.1 mol L^{-1}) solution. Fortunately, these two features were considered as the ideal behavior of the fabricated Hg^{2+} bio-sensing probe during utilizing spinal cord as natural tissue for long time at room temperature conditions; besides the unnecessity of the real samples for controlling and adjusting the ionic strength, prior the potentiometric analysis. However, independence of the potentiometric response furthermore from the ionic strength majorly reduced the probability of the presence of any electrochemical interaction(s) between the spinal cord and the Hg^{2+} as electroactive species, especially at sub nano-molar levels.

3.3.3. Figures of merit

Trace diagram of the Zeta-based potentiometric analytical response versus the Ag/AgCl as a reference electrode at different time scales is shown in Fig. 2A). These results are based on simultaneous analyses of different Hg^{2+} standard solutions, ranged between 1.0×10^{-20} to 1.0×10^{-2} mol L^{-1} concentrations under optimum conditions. Besides, the correlation between the Zeta-based potentiometric analytical gradient versus –log [Hg^{2+}] has been shown in Fig. 2B.

As shown, wide linear range (Fig. 8.SI, See Supporting Information, Section: 19. SI), acceptable calibration sensitivity (Fig. 2), and high enough specificity (Fig. 9.SI, See Supporting Information) were recognized for the fabricated bio-sensor during the Hg^{2+} detection process. This result is considered as the main benefit of the introduced bio-sensor for reliable Hg^{2+} detection and determination in different real samples with advantages, especially dependency from the ionic strength gradient, and specificity from external and foreign species.

3.4. Analytical application

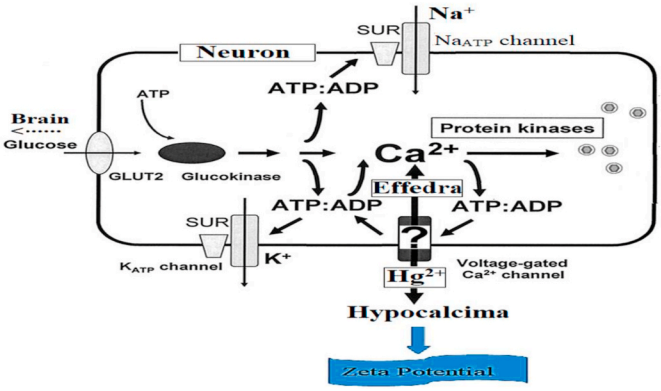
3.4.1. Hg^{2+} detection and determination

Specific detection of Hg^{2+} at the sub-nano and the atto-molar level was applicable for Hg^{2+} detection and determination in different real samples, especially the natural plasma fluidic samples. This system was therefore adopted for reliable evaluation of the quality of the intercellular medium with high enough accuracy, during comparison with NAA as standard reference method (Table 1.SI, See Supporting Information, Section: 20. SI).

As shown, acceptable recovery percentage and relative error percentages were detected when adopting this method for reliable real sample analysts at wide concentration range. Hence, this process showed the suitability of the introduced array-based bio-sensor based direct Zeta-based potentiometry analysis with acceptable relative error percentages. Also, randomly, positive and negative values of the relative error percentages as well as random tolerance versus 100.0% recover percentage, exhibited to the lack of any significant error(s) during the real sample analysis.

3.4.2. Proposed behavior

Regarding the neuron cells, Hg^{2+} is considered an important toxic reagent. This reagent has been recognized as the most toxic heavy metal that readily is able to penetrate the live neuron cell and disturbs its construction (Carocci et al., 2014). However, a distinct molecular mechanism for mercury toxicity in neuronal cells is not well established, but some reports have been present in the literature review. For instance, at a cellular level, mercury causes inhibition of neurotransmitter receptors and calcium ion channels. This process often damages mitochondria and results in different challenges such as oxidative stress, inhibition of protein synthesis, microtubule assembly, and upregulation of programmed cell death genes (Jaishankar et al., 2014). Nevertheless, in this experiment for the first time, novel bio-sensing detection of Hg^{2+} pointed to the importance of Zeta potential as well as the significant roles of the Ca^{2+} ionic channels (See Supporting Information, Section: 21. SI). This was based on different pieces of evidence as reported in Table 2.SI (See Supporting Information) according to different analyses like inductively coupled plasma (Fig. 10.SI.A), liquid-/solid-state ultraviolet spectroscopy (Fig. 10.SI, B, C), electrophoresis (Fig. 10.SI. D), and SDS-page electrophoresis (Fig. 10.SI.E and F, See Supporting Information, Section: 22. SI). These results pointed to the potentiometric responsibility of a protein with gram molecular weight of 66.2 ± 0.3 KCU in the stem cell matrix as a specific hypercalcemia actuator. Based on above reported analytical techniques, all these evidenced majorly differentiated this report from all the previously reported electrochemical-based potentiometric systems as well as the specific neuron cell manipulation by Hg^{2+}. According to these pieces of



Scheme. 1. | Recommended mechanism during the interaction between stem cells and Hg^{2+} during hypercalcemia and Zeta potential.

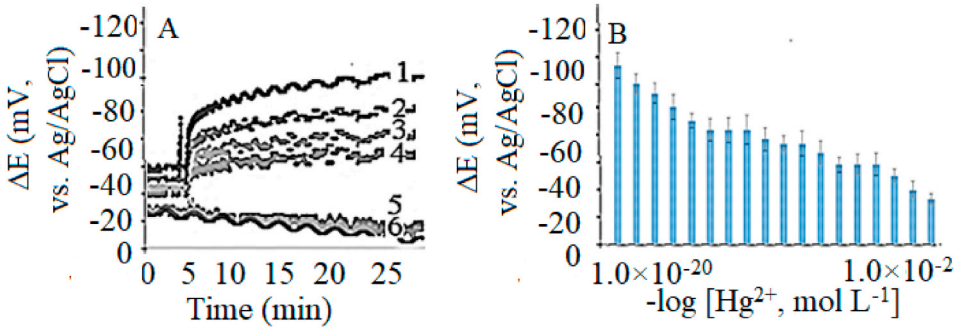


Fig. 2. A) Diagram of Zeta-based potentiometric analytical response versus the Ag/AgCl as reference electrode at different time scales during analysis of different Hg^{2+} standard solutions ranged between 1.0×10^{-20} to 1.0×10^{-2} mol L^{-1} concentrations under optimum conditions: 1) 1.0×10^{-20}, 2) $1 \times 10\text{-}^{16}$, 3) 1.0×10^{-14}, 4) 1.0×10^{-12}, 5) 1.0×10^{-4}, and 6) $1.0 \times 10\text{-}^{2}$ mol L^{-1}, B) correlation between the Zeta-based potentiometric analytical gradient versus –log [Hg^{2+}]. Conditions: MWCNTs:$C_2O_4^{2-}$: spinal cord ratio of 2: 2: 1 under similar conditions, pH = 5.6 ± 0.1 using CO_3^{2-}/HCO_3^- (0.1 mol L^{-1}). Data are the average of three replicate analyses, error bars: ±Standard deviation.

evidence, the recommended mechanism has been graphically shown in Scheme 1.

The suggested proposal behavior was therefore based on the triggering behavior f Hg^{2+} for stimulating the Ca^{2+} ionic channels during the hypercalcemia process.

3.5. Comparison

Typical analytical methods used for the determination of Hg^{2+} including atomic fluorescence spectroscopy (Sanchez-Rodas et al., 2010), atomic absorption/emission spectroscopy (Erxleben and Ruzicka, 2005); inductively coupled plasma mass spectrometry (Yamasaki, 2006); and inductively coupled plasma-optical emission spectrometry (Han et al., 2006) are often expensive and possessed sophisticated instruments with anticipate consuming (Wang et al., 2018). Whereas, the introduced bio-sensor enjoys a wide range of advantages, which to the best of knowledge, is not comparable with a previously introduced detection system for Hg^{2+} recognition (Wang et al.,2020 and Shunru et al., 2020).

Similarly, portability, miniaturization, and easy conversion of the related physio/chemical properties to electrical signal domain are considered as other advantages of this novel detection system. The detection limit of this bio-sensing device is not comparable even with the previously introduced electrochemical detection systems such as potentiometry, and voltammetry (Han et al., 2006).

Likewise, low cost, green structure of spinal cord as specific the ionophore, and availability majorly differentiate this bio-sensing devices (See Supporting information, Section: 19. SI) from other previously reported sensing devises for instance lab-on-a-chip, optical beam-affecting field effect transistors (Sanchez-Rodas et al., 2010), Elisa, Fret, MosFET, etc. (Wagner et al., 2014). Additionally, as proteomics study on the protein Hg^{2+} interactions especially at sub nano-molar concentrations are sensitive, further reliable analyses about this specific interaction need to access analytical instruments such as precise calibrated “liquid chromatography/mass spectrometry/mass spectrometry” with high enough resolution, which is considered as the main limitation of the present study at the same time. Nevertheless, to the best of our knowledge, this study is considered as the first report that reports the bio-sensing mechanistic interaction (Chemogenetic) between spinal cord neuron cell’s manipulation and mercuric ions as specific hypercalcemia actuator.

However, it should be noted that further analysis about the structure of the protein structure and morphology needed i) controllable hydrolysis of the responsible, and ii) well-behaved *N*-terminal labeling of the hydrolyzed amino acids for *N*-terminal amino acid sequence determination, which pointed to the frontier topic for future researches in this field.

4. Conclusions

The fabricated bio-sensor is considered as simple, available, low cost, and reliable Hg^{2+} recognition probe for Zeta-based potentiometric approaching towards single Hg^{2+} ion recognition. This effective capability is related to the physiologic property or even spinal cord slice when opening the Ca^{2+} channels during interaction with Hg^{2+} ions and spontaneous cleavage of the Ca^{2+}. This process, therefore, results in the direct detection of Ca^{2+} at around sub-nano-molar scale and indirect detection of Hg^{2+} as the Ca^{2+} channel-interacting probe at sub atto-molar concentrations. Perspective to the biological application of this study has been reported in detail in the Supporting Information manuscript (See Section 22. SI). However, in spite the dependency of the morphology of the spinal cord tissue to the different blind and in-blind factors, but the spinal cord seems to be considered as natural stem cells. This is attributed to the noticeable characteristics such as mechanical stability, ling neuronal tissue length, availability, low cost, etc. That can be adopted for the fabrication of different types of the stem cell-based bio-sensing devices. Specific rectifying the neuron cells by Hg^{2+} as biocompatible reagent would open light viewpoint to efficiently treat different hypercalcemia induced disorders. We finally hope the present study would be able to provide the future possibility for efficient curing the tumor by focusing on the positive aspect of the Hg^{2+} on the neurolysis process.

CRediT authorship contribution statement

Sajedeh Karami: Formal analysis. **Mohammad Mahdi Doroodmand:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors wish to acknowledge the support of this work by the Shiraz University Research Council.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113125.

References

Andrade, F.J., Blondeau, P., Macho, S., Riu, J., Rius, F.X., 2006. Encycl. Anal. Chem. Appl. Theory Instrum. 1–17.
Bratovčić, A., Odobašić, A., Ćatić, S., 2009. Agric. Conspectus Sci. 74, 139–142.
Carocci, A., Rovito, N., Sinicropi, M.S., Genchi, G., 2014. Springer, pp. 1–18.
Chen, S., Dong, H., Yang, J., 2020. Sensors 20, 1690.
Chumbimuni-Torres, K.Y., Rubinova, N., Radu, A., Kubota, L.T., Bakker, E., 2006. Anal. Chem. 78, 1318–1322.
Düzgün, A., Zelada-Guillén, G.A., Crespo, G.A., Macho, S., Riu, J., Rius, F.X., 2011. Anal. Bioanal. Chem. 399, 171–181.
Düzgün, A., Imran, H., Levon, K., Rius, F.X., 2013. Sci. World J. 2013.
Erxleben, H., Ruzicka, J., 2005. Anal. Chem. 77, 5124–5128.
Faridbod, F., Ganjali, M.R., Dinarvand, R., Norouzi, P., 2007. Afr. J. Biotechnol. 6.
Han, F.X., Patterson, W.D., Xia, Y., Sridhar, B.B.M., Su, Y., 2006. Water Air Soil Pollut. 170, 161–171.
Ismaiel, A.A., Aroua, M.K.R. Yusoff., 2013. Chem. Eng. J. 225, 306–314.
Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Interdiscipl. Toxicol. 7, 60–72.
Kahrizi, F., Salimi, A., Noorbakhsh, F., Faizi, M., Mehri, F., Naserzadeh, P., Naderi, N., Pourahmad, J., 2016. Iran. J. Pharm. Res. IJPR 15, 834.
Mehrvar, M., Abdi, M., 2004. Anal. Sci. 20, 1113–1126.
Pancrazio, J.J., Whelan, J.P., Borkholder, D.A., Ma, W., Stenger, D.A., 1999. Ann. Biomed. Eng. 27, 697–711.
Pohl, H.R., Roney, N., Abadin, H.G., 2011. Met Ions Life Sci 8, 62.
Saleh, M.B., Soliman, E.M., Abdel Gaber, A.A., Ahmed, S.A., 2006. Anal. Lett. 39, 659–673.
Sanchez-Rodas, D., Corns, W.T., Chen, B., Stockwell, P.B., 2010. J. Anal. At. Spectrom. 25, 933–946.
Schmidt, A., Steinritz, D., Rothmiller, S., Thiermann, H., Scherer, A.M., 2018. Toxicol. Lett. 293, 98–104.
Shunru Jin, Sh, Wu, C., Ying, Y., Zunzhong Ye, Z., 2020. Anal. Bioanal. Chem. 412, 3779–3786.
Wagner, M.J., Li, J.Z., Gong, Y., Schnitzer, M.J., 2014. Nat. Commun. 5, 1–11.
Wang, Y., Gao, M., Chen, Q., Yu, F., Jiang, G., Chen, L., 2018. Anal. Chem. 90, 9769–9778.
Wang, Y., Zhang, L., Chen, L., 2020. Anal. Chem. 92, 1997–2004.
Wu, F., Campos, I., Zhang, D.-W., Krause, S., 2017. Proc. R. Soc. A Math. Phys. Eng. Sci. 473, 20170130.
Xu, F., Farkas, S., Kortbeek, S., Zhang, F.-X., Chen, L., Zamponi, G.W., Syed, N.I., 2012. Mol. Brain 5, 30.
Yamasaki, S., 2006. Encycl. Anal. Chem. Appl. Theory Instrum.