Original Article

Designing an Electrochemical Biosensor Based on Voltammetry for Measurement of Human Chorionic Gonadotropin

Mohammad Samare-Najaf[1,2], Amirreza Dehghanian[3,4], Gholamreza Asadikaram[5], Maryam Mohamadi[6], Morteza Jafarinia[7], Amir Savardashtaki[8,9], Afrooz Afshari[10], Sina Vakili[9]

[1]Blood Transfusion Research Center, Kerman Regional Blood Transfusion Center, High Institute for Research and Education in Transfusion Medicine, Tehran, [2]Department of Biochemistry, School of Medicine, [3]Trauma Research Center, [4]Department of Pathology, Molecular Pathology and Cytogenetics Division, Shiraz University of Medical Sciences, Shiraz, [5]Applied Cellular and Molecular Research Center, Kerman University of Medical Sciences, Kerman, [6]Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, [7]Shiraz Neuroscience Research Center, [8]Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, [9]Infertility Research Centre, [10]Department of Pathology, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Human chorionic gonadotropin (hCG) is a polypeptide hormone synthesized during pregnancy and is also upregulated in some pathologic conditions such as certain tumors. Its measurement is essential for diagnosing pregnancy and malignancies. Despite numerous attempts to introduce an accurate method capable of detecting hCG levels, several limitations are found in previous techniques. This study aimed to address the limitations of current hCG assay methods by designing an electrochemical biosensor based on voltammetry for the rapid, selective, inexpensive, and sensitive measurement of hCG levels. **Methods:** A carbon paste electrode was prepared and functionalized by para-aminobenzoic acid. The primary anti-β-hCG monoclonal antibody was immobilized on the electrode surface by activating the carboxyl groups with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide solutions. The study also involved optimizing parameters such as the time for primary antibody fixation, the time for hCG attachment, and the pH of the hydrogen peroxide solution to maximize the biosensor response. Different concentrations of hCG hormone were prepared and loaded on the electrode surface, the secondary antibody labeled with HRP enzyme was applied, thionine in phosphate-buffered saline solution was placed on the electrode surface, and the differential pulse electrical signal was recorded. **Results:** The linear range ranged from 5 to 100 mIU/ml, and the limit of detection was calculated as 0.11 mIU. The relative standard deviation was 3% and 2% for five repeated measurements of commercial standard samples with concentrations of 2 and 20 mIU/mL, respectively. The percent recovery was obtained from 98.3% to 101.5%. **Conclusion:** The sensor represents a promising advancement in hCG level measurement, offering a potential solution to overcome the existing limitations in current diagnostic strategies. Simple and inexpensive design, detecting hCG in its important clinical range during early pregnancy, and successful measurement of hCG in real serum samples are the advantages of this sensor.

Keywords: *Biosensor, electrochemistry, voltammetry, β-human chorionic gonadotropin*

Submitted: 15-Dec-2023 **Revised:** 19-Feb-2024 **Accepted:** 12-Apr-2024 **Published:** 25-Jul-2024

Introduction

Human chorionic gonadotropin (hCG) polypeptide hormone is synthesized by placental development trophoblast cells during pregnancy; however, its levels are upregulated in trophoblast and nontrophoblast tumors.[1,2] Indeed, it is widely suggested that the level of hCG was found elevated in some cancerous states such as prostate cancer, testicular tumors, trophoblastic tumors, and gestational choriocarcinoma.[3,4] Hence, this 37-kDa multi-functional heterodimeric hormone, which consists of two subunits including a-hCG and b-hCG, has been considered a valuable molecular biomarker for the diagnosis of pregnancy and a number of malignancies.[5,6]

The measurement of hCG levels is frequently performed by either quantitative or qualitative approaches to determine the state of pregnancy or the presence of malignant cells.[7] Although the qualitative analysis of urine or serum samples at a point-of-care testing center provides a prompt strategy to determine hCG levels, serum quantitative hCG testing is mostly preferred in a clinical setting.[7] The lateral-flow immunoassay, known as the hCG diagnostic kit, is a facilitated-to-use method representing a qualitative outcome with a detection limit of nearly 20 mIU/mL in urine samples.[8] Nevertheless, this approach

How to cite this article: Samare-Najaf M, Dehghanian A, Asadikaram G, Mohamadi M, Jafarinia M, Savardashtaki A, *et al.* Designing an electrochemical biosensor based on voltammetry for measurement of human chorionic gonadotropin. J Med Sign Sens 2024;14:21.

Address for correspondence:
Dr. Sina Vakili,
Infertility Research Center,
Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail: sinavakili68@gmail.com

Access this article online

Website: www.jmssjournal.net

DOI: 10.4103/jmss.jmss_64_23

Quick Response Code:

is limited as it is difficult to quantify low levels of hCG in the samples of patients with cancer.[8] Thereby, several immunoassays have been designed for quantitative analysis of hCG such as fluorescent immunoassays, radioimmunoassays, enzyme-linked immunosorbent assays,[9] and electrochemiluminescence.[10,11] Despite the relatively sensitive and reliable function of the aforementioned techniques, their application is faced with major challenges such as being expensive, time-consuming, the use of hazardous radioactive materials, restricted specificity, and measurement limitations arising from the nature of techniques.[10,11] As a result, it is widely desirable to develop novel detection strategies with the merits of reasonably priced, convenient operation, and appropriate sensitivity and specificity.

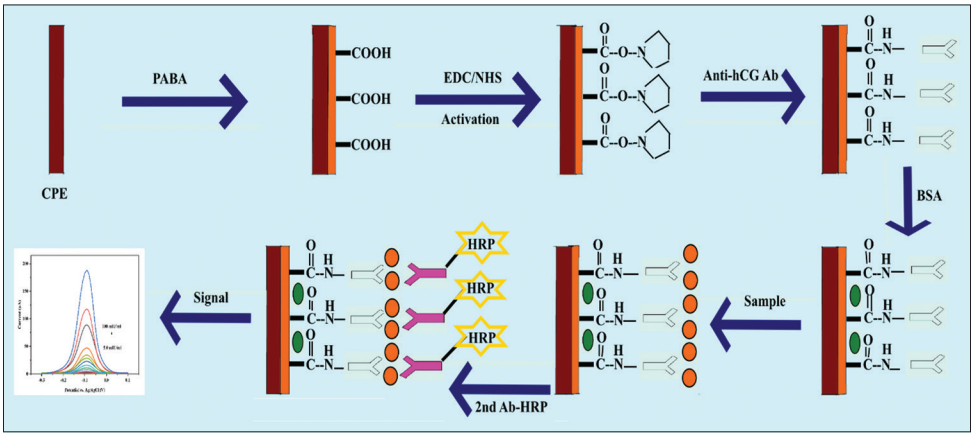
Biosensors represent a promptly expanding field of devices to assess the concentration of substances of biological interest.[12] These biodevices are widely applicable in clinical diagnostics, environmental monitoring, and bioprocess monitoring. Electrochemistry, a branch of science that encompasses physicochemical processes involving the transfer of charge, represents an important contribution to the development of biosensors.[13] The electrochemical biosensors convert biological information such as analyte concentration, a biological recognition element, or biochemical receptor, into voltage or current.[14] This class of biosensors depicts a propitious diagnostic strategy capable of biomarker detection in body fluids.[14] An efficient biosensor could have resulted from the combination of effective transducers with suitable immobilization techniques. Electrochemical biosensors benefit from biological molecules' properties along with physicochemical transducers to convert a biological signal into an electrochemical signal. In fact, electrochemical detection is based on monitoring electrical signal changes caused by an electrochemical reaction at an electrode surface, usually originating from an imposed current/potential.[15] Voltammetry, which utilizes a three-electrode electrochemical cell, is described as the evaluation of current resulting from the application of potential.[16,17] It is evidenced that electrochemical biosensors based on voltammetry represent a much higher sensitivity than optical biosensors and also provide the possibility of determining several analytes simultaneously.[18]

Several studies have utilized electrochemical biosensors to detect and quantify hCG levels in human samples. Despite some significant merits, the lack of quantification ability, the poor limit of detection, the ambiguity of ease of use, and the use of expensive metals (which in turn makes the final product expensive and consequently restricts the possibility of being widely used in medical diagnostic laboratories, especially in underdeveloped territories) are some of the most important limitations of the previously designed techniques.[19-23] Therefore, it was necessary to design a novel method to address the drawbacks while strengthening the merits of previous strategies. Hence, the current study aimed to design and validate an electrochemical biosensor based on voltammetry capable of measuring the lowest hCG levels that provide merits such as being inexpensive, rapid, and high sensitivity and specificity, with an appropriate limit of detection and affordability. Scheme 1 illustrates the fabrication and sensing approach of the developed electrochemical biosensor.

Experimental

Chemicals and reagents

Graphite powder, paraffin oil, para-aminobenzoic acid (PABA), and hCG were purchased from Sigma-Aldrich (Missouri, United States). Lithium perchlorate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-Hydroxysuccinimide (NHS), phosphate-buffered saline (PBS), Tween 20, bovine serum albumin (BSA), thionine, and hydrogen peroxide (H_2O_2) solution were provided by Merck (Darmstadt, Germany). Primary anti-hCG antibody and secondary antibody labeled with HRP were purchased from Abcam (Cambridge, United Kingdom).



Scheme 1: Fabrication and sensing approach of the developed electrochemical biosensor

Apparatus

An Autolab potentiostat/galvanostat was used for the voltammetry measurements to be taken within a routine three-electrode cell. The cell was composed of a reference electrode containing Ag | AgCl | KCl (3.0 M), a working electrode, and a platinum wire serving as an auxiliary electrode. Employing a pH meter (model 780) manufactured by Metrohm Herisau, Switzerland, pH levels were measured concurrently.

Fabrication of the carbon paste electrode

To fabricate the carbon paste electrode, a uniform mixture of graphite powder and paraffin oil was produced by hand-mixing 14.0 mg of graphite powder and 6.0 mg of paraffin oil in an agate mortar for 30 min until a consistent composition was achieved. The resulting mixture had a weight ratio of 70 wt% graphite powder to 30 wt% paraffin oil. Subsequently, the paste was packed into a one-end glass tube with an inner diameter of 3.0 mm. The electrical connection was provided by a copper wire. The surface of the electrode was thoroughly smoothed by rubbing it on a piece of filter paper until it became perfectly uniform.

Functionalization of the CPE surface with carboxyl groups

PABA was used for functionalization of the CPE surface with carboxyl groups. This molecule contains both NH_2 and carboxyl groups. To attach PABA on the surface of the electrode, the prepared electrode was placed in a solution containing lithium perchlorate ($LiClO_4$) and PABA, and three potential scans using cyclic voltammetry techniques were applied to the electrode ranging from 0.0 to 1.0 V with a scan rate of 10 mV/s. Thereby, PABA was connected to the electrode surface through its NH_2 functional group, and its carboxyl groups will remain freely on the electrode surface. Finally, the electrode surface was rinsed with 0.1 M PBS buffer (pH 7.0), followed by thorough washing in distilled water, and then dried.

Immobilization of primary monoclonal antibody on the electrode surface

For antibody-electrode attachment, the free carboxyl group of PABA was activated by EDC and NHS solutions. 10 µL of EDC and NHS solution in acetate buffer was cast on the electrode surface for 10 min at 4°C. EDC converts the carboxyl group of PABA into an amine-reactive intermediate O-acylisourea. This intermediate is susceptible to attack by lysine amino groups on the antibody and creates an amide bond between the antibody and the carboxyl groups. In addition, the formed intermediate is prone to rapid hydrolysis and is unstable in aqueous medium. The addition of NHS by converting this reactive amine intermediate to NHS ester stabilizes it and improves the efficiency of EDC-mediated antibody binding. After activation, the electrode surface was placed in 10 mM PBS buffer containing Tween 20 and then washed three times with stirring distilled water and then dried by nitrogen gas flow.

To connect the primary antibody to the activated surface, 10 µl of primary antibody (50 µg/ml) in 0.1 M PBS buffer with pH = 7 was cast on the electrode surface at room temperature. After attaching the antibody, washing was done according to the previous step, and the electrode surface was dried with nitrogen gas.

Blocking the electrode surface

BSA, as a blocker, was used to fill exposed parts of the electrode surface where the antibody was not attached. In this regard, 10 ml of 10 mg/ml BSA solution was placed on the surface of the electrode at 4°C and then washed as described earlier. Afterward, the electrode was ready to measure the hCG levels.

Antigen binding (human chorionic gonadotropin hormone)

Different concentrations of hCG hormone (from 1.0 to 10 IU/mL) were prepared in 0.10 M PBS buffer (pH 7.40). Next, 10 µL of each concentration of hCG hormone solution was placed on the electrode surface at room temperature. The electrode surface was then washed and dried according to the previously described method. The differential pulse voltammetry (DPV) responses of each electrode were measured in 5.0 mM ferro/ferricyanide redox solution, and the resulting data were used to determine the optimal antigen concentration, which was found to be 1 IU/mL [Figure 1].

Secondary antibody binding

The secondary antibody labeled with HRP enzyme (10 µl with a concentration of 50 µg/ml in 0.1 M PBS buffer with pH = 7) was placed on the electrode surface and then the electrode surface was washed and dried.

Signal recording

A 10 mM thionine solution in 0.1 M PBS (pH 7.4) was applied to the electrode surface. After washing and drying, the electrode was placed inside an electrochemical cell containing a degassed H_2O_2 solution in acetate buffer (pH 6.8). DPV was used to record the signals. To ensure accurate measurements, the H_2O_2 solution was first degassed by passing a stream of inert nitrogen gas through it because oxygen can interfere with the measurement process.

Optimization of experimental parameters

To achieve the highest response of the designed electrochemical biosensor, we investigated and optimized several key parameters that influence its performance. These parameters included the incubation time for the primary antibody to bind to the electrode surface, the binding time for the hCG molecule to attach to the primary antibody,

and the pH of the H_2O_2 solution. Optimization was performed using the one-factor-at-a-time method, whereby one parameter was varied while maintaining the others constant. Specifically, we assessed different incubation times and pH values across a range of concentrations to determine their effects on the sensor response. Once the parameters were optimized, the response of the sensor was measured to validate our findings.

Optimizing the required time for primary antibody fixation

The duration of the applying voltage for binding the primary antibody is a critical factor in the fabrication of an electrochemical sensor. Insufficient time for binding results in a limited number of connections between the primary antibody and electrode surface, subsequently reducing the sensitivity of the sensor. Conversely, excessive accumulation of the primary antibody on the electrode surface leads to an increase in the thickness of the layer, which leads to a decrease in the charge transfer and compromises the performance of the sensor. Various time intervals were investigated to optimize this parameter.

Optimizing the time required for the attachment of human chorionic gonadotropin on the primary antibody

By increasing the contact time of the electrode with the hCG-containing solution, more hCG molecules accumulate on the surface, resulting in a higher current output. However, beyond a certain point, further increasing the contact time does not significantly enhance the current production; instead, it prolongs the analysis time. Thus, optimizing this parameter is essential to achieve the highest current in the shortest time. To determine the optimal contact time for maximum hCG absorption onto the electrode surface, we investigated the effect of accumulation time on the anodic peak current within various time ranges.

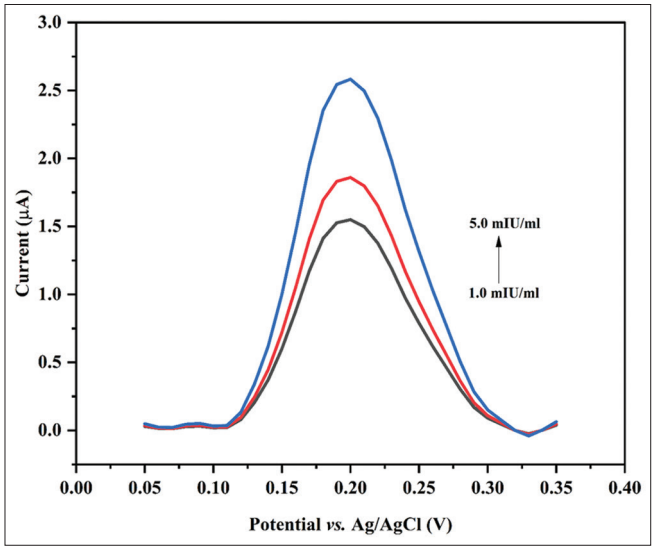


Figure 1: Primary antibody concentration optimization (The experiments were conducted in a 5.0 mM ferro/ferricyanide redox solution in phosphate-buffered solution buffer, with a pH of 7.4.)

Optimizing the pH of the hydrogen peroxide solution

The pH of the H_2O_2 solution plays a significant role in determining the electrochemical signal. To optimize this parameter, we examined the impact of varying the pH levels across different ranges (4.0–9.0) at 5.0 µM H_2O_2. As shown in Figure 2, the highest current was observed between pH 6.8 and 7.0, which is close to the biological pH value. Therefore, we considered pH 7.0 as the optimum pH for our experiment.

Results

Primary antibody attachment time optimization

In the present study, the attachment time of the primary antibody to the electrode surface was optimized. At 30 s, the surface of the electrode was saturated with the antibody; hence, this time was considered the optimized binding time [Figure 3].

Optimized time for antigen attachment

A fixed number of primary antibodies are allowed to bind to the electrode surface, which limits the maximum number of antigens that can bind to the antibodies. Extending the time spent binding the antigen to the antibody did not alter the electric current generated. The goal of this study was to determine the optimal time required for antigen binding to antibodies. The findings revealed that the antibodies became saturated after 50 s; thus, this time was considered the optimal time for antigen binding [Figure 4].

Differential pulse voltammetry measurements

Under optimal experimental conditions, DPV calibration curve was obtained for hGC using PBS solution (PBS, 0.1 M, pH 7.4) that spanned a concentration range of 5.0–100 mIU/ml (n = 3) [Figure 5]. As shown in Figure 6, a linear relationship (R^2 = 0.989) exists between the peak current response[24] and the hGC concentration (hGC),

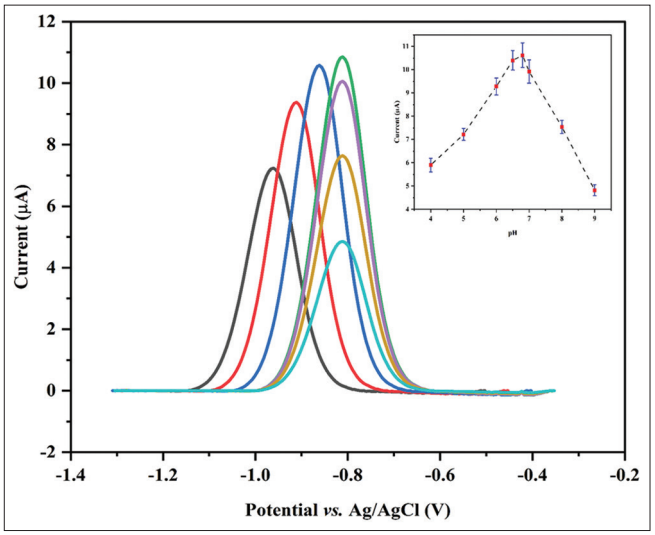


Figure 2: Optimization of H_2O_2 pH Value by differential pulse voltammetry technique (at 5.0 µM hydrogen peroxide)

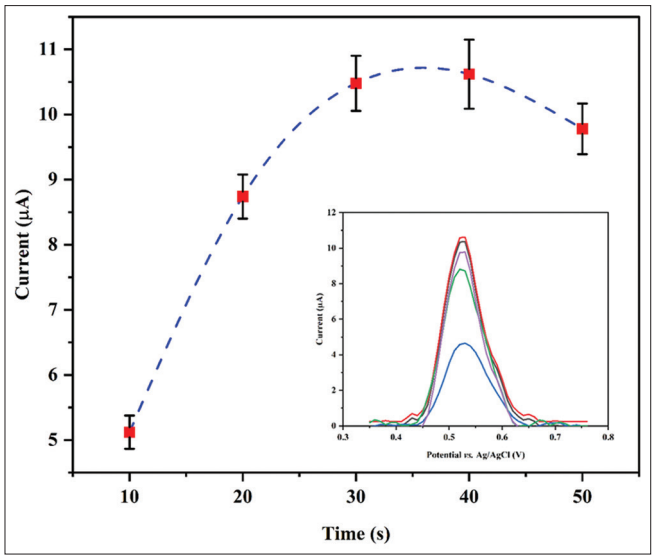


Figure 3: Primary antibody attachment optimization

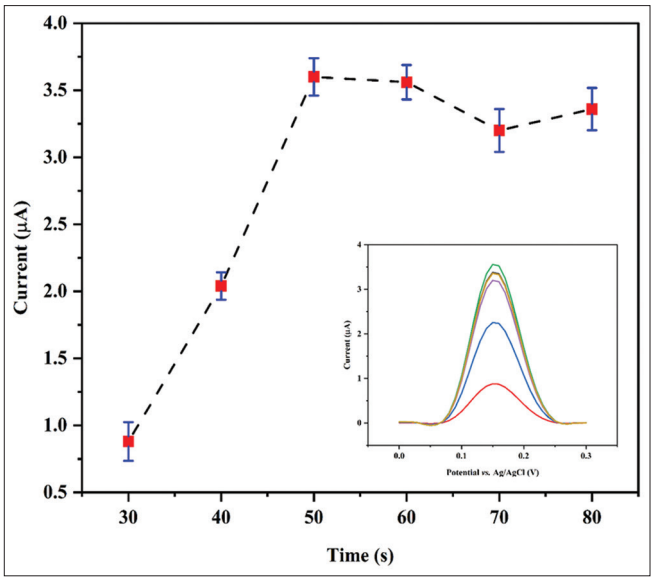


Figure 4: Antigen attachment time optimization

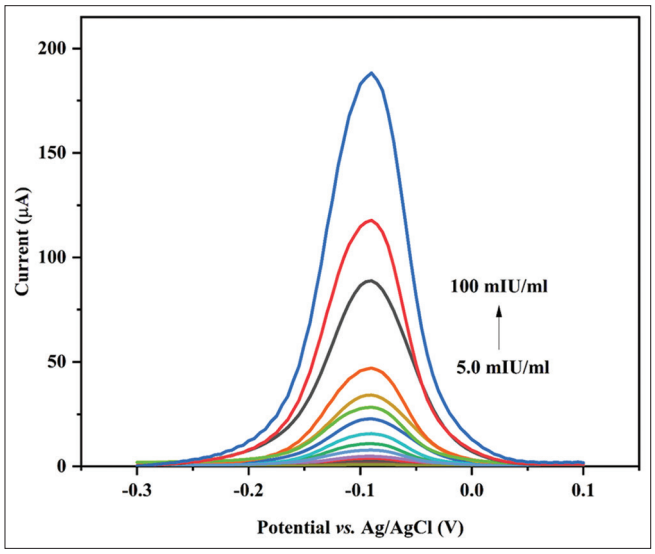


Figure 5: Differential pulse voltammograms for different concentrations of human chorionic gonadotropin (5.0–100 mIU) in the presence of fabricated electrochemical biosensor (phosphate-buffered solution of 0.10 M, pH 7.4)

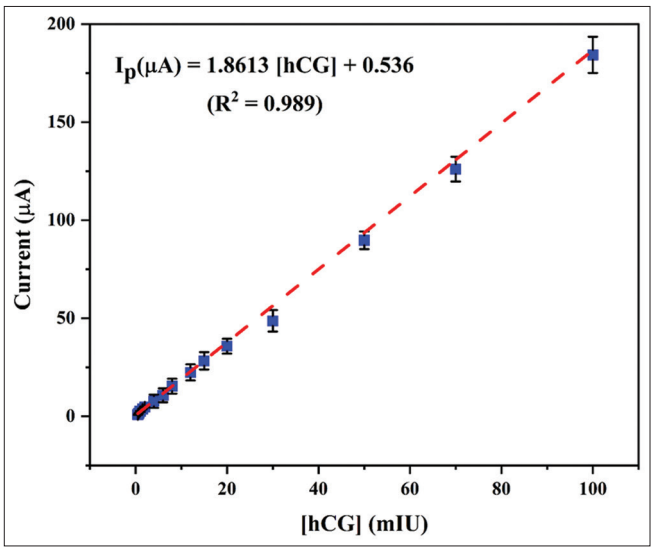


Figure 6: Differential pulse voltammetry calibration plot of I_p (μA) versus (human chorionic gonadotropin). The error bars depict the standard deviations of three measurements (*n* = 3)

which can be expressed as follows: Ip (μA) = 1.8613 (hGC) (mIU/ml) +0.536.

Assessing the repeatability and limit of detection of the method

The method's detection limit (LOD) was calculated and was found to be 0.11 mIU [Table 1]. To evaluate the method's repeatability, we analyzed commercial standard samples with concentrations of 2 and 20 mIU/mL five times each. The relative standard deviations were 3% and 2%, respectively, which are acceptable for this method.

Real samples analysis

To assess the capability of the proposed method to detect various concentrations of hCG in human samples, we compared four distinct samples, each with a known concentration determined through quantitative luminescence, a highly sensitive approach. As shown in Table 2, the results obtained by the proposed method were satisfactory.

Discussion

An increased level of hCG is considered a reliable diagnostic biomarker of physiological (such as pregnancy) and pathological (e.g., prostate cancer and gestational trophoblastic neoplasia) states.[25-27] However, the common strategies of measuring hCG are faced with major limitations such as being time-consuming and expensive, the usage of hazardous materials, and undesired specificity with false results,[28,29] which has caused researchers to make a significant effort in designing an approach that overcomes

these concerns. Hence, the present study aimed to design an inexpensive, prompt, and affordable method with high specificity and appropriate LOD to overcome the common limitations of current diagnostic strategies [Table 3].

The findings of the present study determined that the designed electrochemical biosensor has a LOD equal to 0.11 mIU/ml. There is a plethora of evidence that hCG levels in the blood of pregnant women begin to increase from the 4th week (ranging from 0 to 0.75 mIU/ml) and its highest level is found in the 8th to 16th weeks (ranging from 9 to 210 mIU/ml) of pregnancy. Subsequently, hCG levels tend to decrease during the second trimester (ranging from 1.4 to 53 mIU/ml) and the third trimester (ranging from 0.94 to 60 mIU/ml).[34,35] Therefore, the method designed by the present study is able to determine the level of hCG from the 1st weeks of pregnancy. Moreover, a nondetectable level of hCG is considered the gold standard to rule out the diagnosis of ectopic pregnancy, whereas appropriately rising hCG levels are thought to exclude it as well. In addition, increased levels of hCG have been reported in pathological conditions such as gestational trophoblastic diseases and nontrophoblastic neoplasms. Considering the determined range of hCG during gestational trophoblastic diseases, which is between 50,000 and 100,000 mIU/ml, and nontrophoblastic neoplasms, which is between 1900 and 160,000 mIU/ml,[36] it could be expected that the current electrochemical technique can detect hCG levels during these pathological conditions. Furthermore, it is widely documented that in normal males, there is no presence of hCG, while its detection in men has always been considered a biomarker of malignancies such as prostate cancer and testicular tumor.[37,38] Accordingly, based on the LOD obtained by the present electrochemical-based method, this technique is expected to be able to detect male malignancies as well. Moreover, the application of the present technique on commercial standard samples with concentrations of 2 and 20 mIU/mL revealed that relative standard deviations were 3% and 2%, respectively, which are acceptable for this method. Previously, it was documented that the fabricated electrochemical biosensors for hCG detection exhibited different detection limit ranges [Table 3]; hence, the current designed method is expected to detect the appropriate limited levels of hCG.

Table 1: The repeatability and limit of detection of the method

Repeatability (mIU/mL)				LOD ($3s_b$/m)	
2.0		20.0		Sample	Current (amps)
Sample	Current (amps)	Sample	Current (amps)		
1	4.83	1	35.81	1	0.13
2	4.50	2	34.25	2	0.18
3	4.91	3	36.39	3	0.21
4	4.88	4	35.62	4	0.27
5	4.64	5	34.93	5	0.25
S	0±0.18	S	0±0.83	6	0.10
Mean	4.75	Mean	35.40	s_b	0±0.67
RSD%	3.0±0.70	RSD%	2.0±0.30	m	1.815
				LOD (mIU/mL)	0.11

LOD – Limit of detection; RSD – Relative standard deviation

Table 2: Evaluation of biosensor performance in four real samples

Sample	Designed method (mIU/mL)	Standard method (mIU/mL)	Recovery percentage
1	8185	8326	98.30
2	2956	2911	101.50
3	2580	2596	99.40
4	0.65	<1	-

Table 3: A comparison of previously designed electrochemical biosensors with the results of the present method

Study	Sensor	Type of detection	Electrode	LOD	Linear range	Reference
Damiati *et al*.	Microelectrochemical biosensors	Point-of-care	Carbon	1 pg/mL	N/A	[19]
Koterwa *et al*.	Electrochemical biosensors	Quantitative	Gold	0.0095 mIU/mL	0.5–50 000 mIU/mL	[21]
Yuksel *et al*.	Electrochemical biosensors	Point-of-care	Gold and silver	2.17 mIU/mL	N/A	[23]
Xia *et al*.	Peptide aptamer-based electrochemical biosensors	Quantitative	Silver	0.4 mIU/mL	1 mIU/mL–0.2 IU/mL	[22]
Shen *et al*.	Molecularly imprinted electrochemical sensor	Quantitative	Carbon	0.035 pg/mL	0.5 pg/mL–250 ng/mL	[30]
Rizwan *et al*.	Electrochemical immunosensor	Quantitative	CNOs/AuNPs/PEG	0.1 fg/mL	0.1 fg/mL–1 ng/mL	[31]
Laschi *et al*.	Electrochemical combo-strip	Point-of-care	Combo-strip	1.5 ng/mL × 10–2 ng/mL	N/A	[32]
Teixeira *et al*.	Graphene electrochemical sensor	Quantitative	Chitosan/AuNPs	0.016 ng/mL	0.1–25 ng/mL	[33]
The current study	Voltammetry-based electrochemical biosensor	Quantitative	Carbon	0.11 mIU	5–100 mIU/mL	-

The table clearly shows that cost-effectiveness, environmental friendliness, ease of design, and high detection ability are the advantages of the present method compared to previous techniques. N/A – Not available; LOD – Limit of detection; CNOs – Carbon nano-onions; AuNPs – Gold nanoparticles; PEG – Polyethylene glycol

In addition, the findings of the present study demonstrated that the designed electrochemical-based method is able to identify hCG levels favorably with a recovery percentage of 98.3%–101.5% compared to the common standard method. In addition, the linear range of detection was 5–100 mIU/ml. In recent years, researchers have made a great effort to design and propose a novel method to detect hCG levels with high performance. In this regard, researchers recently developed a gold–zinc–salen metal-organic framework for the detection of hCG in patients with polycystic ovarian syndrome that exhibited an efficacy range of 96.6%–99.44% for plasma samples, 98.84%–100.50% for serum samples, and 96.1%–97.48% for urine samples.[7] In addition, a designed method using the silver nanoparticles peptide-based aptamer represented a 0.4 mIU/ml LOD and appropriate efficacy compared to a standard ELISA method.[22] Nevertheless, it is documented that studies on the ecotoxicity of metal oxides used in the mentioned biosensors are rare, and there are major concerns regarding the bioavailability and biosafety of metal-based biosensors, particularly heavy metals,[39] the application of carbon paste can be one of the advantages of the present method when compared to similar designed techniques. Hence, in addition to the fact that the current approach is adaptable, effective, and sensitive for the quantification of hCG levels, its significant biosafety for the environment can be mentioned as one of its merits. In addition, the use of expensive metals may limit the availability of designed techniques, a concern that the present study addresses using carbon paste. Furthermore, the researchers have used the photonic crystal waveguide approach to detect the urinary levels of hCG for the diagnosis of pregnant women, which resulted in a favorable performance.[40] However, the lack of comparison with standard methods can be considered one of the main disadvantages of the mentioned study and an advantage of the present study.

A number of previous studies have investigated the selectivity of the designed approaches by evaluating the determination of hCG levels in comparison with other hormones with the same structure, such as follicle-stimulating hormone, thyroid hormone, thyroid-stimulating hormone, and thrombin.[22,41-43] An assessment could be considered one of the most important limitations of the current study. In addition, the lack of evaluation of the performance of the current diagnostic strategy on different physiological and pathological samples can be considered a limitation. Nevertheless, significant merits such as promptness, affordability, cost-effectiveness, high sensitivity and efficiency, and being environmentally friendly can be considered the most significant findings of the present study.

Conclusion

In this study, a facile and low-cost electrochemical biosensor with high sensitivity was fabricated that showed an LOD of 0.11 mIU/ml for hCG, enabling its detection in the early stages of pregnancy. Furthermore, this sensor shows promise for the diagnosis of various physiological and pathological conditions, including malignancies, such as testicular tumors and prostate cancer, as well as nontrophoblastic neoplasms and gestational trophoblastic disorders, by accurately measuring hCG levels. Current electrochemical biosensors offer several advantages, including promptness, affordability, cost-effectiveness, high sensitivity, efficiency, and environmental friendliness. These features represent a significant improvement over existing diagnostic methods, which often suffer from limitations, such as prolonged analysis times, low sensitivity, and high costs. While further investigation and validation on larger sample sets are necessary to establish the efficacy of the biosensor in clinical settings, its potential to overcome current diagnostic limitations is evident.

Financial support and sponsorship

This research received support from grants provided by Shiraz University of Medical Sciences, with grant number 11139.

Conflicts of interest

There are no conflicts of interest.

References

1. Hemberger M, Dean W. The placenta: Epigenetic insights into trophoblast developmental models of a generation-bridging organ with long-lasting impact on lifelong health. Physiol Rev 2023;103:2523-60.
2. McMahon LM, Stewart WW, Cuthill L. Human chorionic gonadotrophin assays to monitor GTD. Best Pract Res Clin Obstet Gynaecol 2021;74:109-21.
3. Lahoud RM, O'Shea A, El-Mouhayyar C, Atre ID, Eurboonyanun K, Harisinghani M. Tumour markers and their utility in imaging of abdominal and pelvic malignancies. Clin Radiol 2021;76:99-107.
4. Solano ÓI, Espitia ID, Mejía CA, Loaiza HJ, Giraldo AH, Salcedo MM, *et al*. Human Chorionic Gonadotropin As A Marker of Testicular Cancer*;* 2021;5:1-7.
5. Bhakta S, Mishra P. Molecularly imprinted polymer-based sensors for cancer biomarker detection. Sens Actuators Rep 2021;3:100061.
6. Desai S, Guddati AK. Carcinoembryonic antigen, carbohydrate antigen 19-9, cancer antigen 125, prostate-specific antigen and other cancer markers: A primer on commonly used cancer markers. World J Oncol 2023;14:4-14.
7. Mohamed RM, El-Sheikh SM, Kadi MW, Labib AA, Sheta SM. A novel test device and quantitative colorimetric method for the detection of human chorionic gonadotropin (hCG) based on Au@Zn-salen MOF for POCT applications. RSC Adv 2023;13:11751-61.
8. Ding X, Yang KL. Antibody-free detection of human chorionic gonadotropin by use of liquid crystals. Anal Chem 2013;85:10710-6.
9. Parrettini S, Caroli A, Torlone E. Nutrition and metabolic adaptations in physiological and complicated pregnancy: Focus on obesity and gestational diabetes. Front Endocrinol (Lausanne) 2020;11:611929.
10. Fan J, Wang M, Wang C, Cao Y. Advances in human chorionic gonadotropin detection technologies: A review. Bioanalysis

2017;9:1509-29.
11. Herkert D, Meljen V, Muasher L, Price TM, Kuller JA, Dotters-Katz S. Human chorionic gonadotropin-A review of the literature. Obstet Gynecol Surv 2022;77:539-46.
12. Poudineh M, Poudineh S, Jalalifar T, Vakili S, Rostami A, Samare-Najaf M, *et al*. Aptamer-functionalized localized surface plasmon resonance sensor for the rapid and affordable detection of *Staphylococcus aureus* alpha-toxin. Optik 2022;270:169902.
13. Banakar M, Hamidi M, Khurshid Z, Zafar MS, Sapkota J, Azizian R, *et al.* Electrochemical biosensors for pathogen detection: An updated review. Biosensors (Basel) 2022;12:927.
14. Singh A, Sharma A, Ahmed A, Sundramoorthy AK, Furukawa H, Arya S, *et al.* Recent advances in electrochemical biosensors: Applications, challenges, and future scope. Biosensors (Basel) 2021;11:336.
15. Sharma A, Singh A, Gupta V, Sundramoorthy AK, Arya S. Involvement of metal organic frameworks in wearable electrochemical sensor for efficient performance. Trends in Environ Anal Chem 2023;38:e00200.
16. Dinu A, Apetrei C. A review of sensors and biosensors modified with conducting polymers and molecularly imprinted polymers used in electrochemical detection of amino acids: Phenylalanine, tyrosine, and tryptophan. Int J Mol Sci 2022;23:1218.
17. Divya KP, Keerthana S, Viswanathan C, Ponpandian N. Bimetallic coreshell-hemoglobin complex immobilized MXene based voltammetric biosensor for the electrochemical detection of acrylamide. J Electrochem Soc 2022;169:127510.
18. Ronkainen NJ, Halsall HB, Heineman WR. Electrochemical biosensors. Chem Soc Rev 2010;39:1747-63.
19. Damiati S, Haslam C, Søpstad S, Peacock M, Whitley T, Davey P, *et al*. Sensitivity comparison of macro- and micro-electrochemical biosensors for human chorionic gonadotropin (hCG) biomarker detection. IEEE Access 2019;7:94048-58.
20. Kassanos P, Iles RK, Bayford RH, Demosthenous A. Towards the development of an electrochemical biosensor for hCGbeta detection. Physiol Meas 2008;29:S241-54.
21. Koterwa A, Bojko M, Ryl J, Łukaszuk K, Kozłowska K, Sieklicki W, *et al*. An electrochemical biosensor for the determination of hormone Human Chorionic Gonadotropin (hCG) in human serum. Electroanalysis 2023;35:e202300095.
22. Xia N, Chen Z, Liu Y, Ren H, Liu L. Peptide aptamer-based biosensor for the detection of human chorionic gonadotropin by converting silver nanoparticles-based colorimetric assay into sensitive electrochemical analysis. Sens Actuators B Chem 2017;243:784-91.
23. Yuksel M, Luo W, McCloy B, Mills J, Kayaharman M, Yeow JT. A precise and rapid early pregnancy test: Development of a novel and fully automated electrochemical point-of-care biosensor for human urine samples. Talanta 2023;254:124156.
24. Leeners B, Geary N, Tobler PN, Asarian L. Ovarian hormones and obesity. Hum Reprod Update 2017;23:300-21.
25. Bollig KJ, Senapati S, Sammel MD, Takacs P, Robins JC, Haisenleder DJ, *et al.* Validation of a multiple marker test for early pregnancy outcome prediction. J Assist Reprod Genet 2023;40:837-44.
26. AlZahrani YM, Alharthi S, AlGhamdi HA, Youssef AO, Ahmed SS, Mohamed EH, *et al.* Highly selective optical sensor N/S-doped carbon quantum dots (CQDs) for the assessment of human chorionic gonadotropin β-hCG in the serum of breast and prostate cancer patients. RSC Adv 2023;13:21318-26.
27. Winter MC, Tidy JA, Singh K, Sarwar N, Aguiar X, Seckl MJ. Efficacy analysis of single-agent carboplatin AUC4 2-weekly as second-line therapy for methotrexate-resistant (MTX-R) low risk gestational trophoblastic neoplasia (GTN). Gynecol Oncol 2023;175:66-71.
28. McMahon LM, Joyce CM, Cuthill L, Mitchell H, Jabbar I, Sweep F, *et al.* Measurement of hCG in women with gestational trophoblastic disease. Gynecol Obstet Invest 2023. [doi: 10.1159/000531499].
29. Rusman ZW, Thambiah SC, Samsudin IN, Anas SS, Staneshwar P. Limitations of the Human Chorionic Gonadotropin (hCG) assay in the diagnosis of gestational trophoblastic disease. Malays J Med Health Sci 2022;18 (Suppl 21):134-6. doi:10.47836/mjmhs18.s21.22.
30. Shen X, Ma Y, Zeng Q, Huang J, Tao J, Wang L. Ultrasensitive determination of human chorionic gonadotropin using a molecularly imprinted electrochemical sensor. ChemistrySelect 2017;2:6549-55.
31. Rizwan M, Hazmi M, Lim SA, Ahmed MU. A highly sensitive electrochemical detection of human chorionic gonadotropin on a carbon nano-onions/gold nanoparticles/polyethylene glycol nanocomposite modified glassy carbon electrode. J Electroanal Chem 2019;833:462-70.
32. Laschi S, Sfragano PS, Ranaldi F, Palchetti I. Progesterone and β-hCG determination using an electrochemical combo-strip for pregnancy monitoring. Int J Mol Sci 2023;24:15381.
33. Teixeira S, Ferreira NS, Conlan RS, Guy O, Sales MG. Chitosan/AuNPs modified graphene electrochemical sensor for label-free human chorionic gonadotropin detection. Electroanalysis 2014;26:2591-8.
34. Braunstein GD, Rasor J, Danzer H, Adler D, Wade ME. Serum human chorionic gonadotropin levels throughout normal pregnancy. Am J Obstet Gynecol 1976;126:678-81.
35. Korevaar TI, Steegers EA, de Rijke YB, Schalekamp-Timmermans S, Visser WE, Hofman A, *et al.* Reference ranges and determinants of total hCG levels during pregnancy: The generation R study. Eur J Epidemiol 2015;30:1057-66.
36. Gailani S, Chu TM, Nussbaum A, Ostrander M, Christoff N. Human chorionic gonadotrophins (hCG) in non-trophoblastic neoplasms. Assessment of abnormalities of hCG and CEA in bronchogenic and digestive neoplasms. Cancer 1976;38:1684-6.
37. Dieckmann KP, Simonsen-Richter H, Kulejewski M, Anheuser P, Zecha H, Isbarn H, *et al*. Serum Tumour Markers in Testicular Germ Cell Tumours: Frequencies of Elevated Levels and Extents of Marker Elevation Are Significantly Associated with Clinical Parameters and with Response to Treatment. BioMed research international 2019;2019:5030349, doi:10.1155/2019/5030349.
38. Cole LA. The hCG assay or pregnancy test. Clinical chemistry and laboratory medicine. 2012;50:617-30.
39. Kahru A, Dubourguier HC, Blinova I, Ivask A, Kasemets K. Biotests and biosensors for ecotoxicology of metal oxide nanoparticles: A minireview. Sensors (Basel) 2008;8:5153-70.
40. Krupa V, Triveni C. Design of Biosensor for the Detection of Pregnancy based on hCG Presence in urine Using Photonic Crystal Waveguide. Proceedings of the 2020 IEEE International Conference on Electronics, Computing and Communication Technologies (CONECCT). IEEE; 2020. p. 1-4.
41. Chiu NF, Kuo CT, Chen CY. High-affinity carboxyl-graphene oxide-based SPR aptasensor for the detection of hCG protein in clinical serum samples. Int J Nanomedicine 2019;14:4833-47.
42. Kumar R, Leng Y, Liu B, Zhou J, Shao L, Yuan J, *et al.* Ultrasensitive biosensor based on magnetic microspheres enhanced microfiber interferometer. Biosens Bioelectron 2019;145:111563.
43. Xia N, Wang X, Yu J, Wu Y, Cheng S, Xing Y, *et al*. Design of electrochemical biosensors with peptide probes as the receptors of targets and the inducers of gold nanoparticles assembly on electrode surface. Sens Actuators B Chem 2017;239:834-40.