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# Fabrication of ultra-sensitive and disposable electrochemical biosensor: Detection of kidney injury molecule-1 protein in urine for diagnosis of kidney injury

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ABSTRACT

This research study indicates the development of the indium tin oxide-polyethylene terephthalate (ITO-PET) coated electrode-based electrochemical biosensor system to sensitively and specifically detect kidney injury molecule-1 (KIM-1), an acute kidney injury (AKI) biomarker. The ITO-PET electrode surface was modified with 3-(Trimethoxysilyl)-1-propanethiol (3-TMESP) silane agent. Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and single frequency impedance (SFI) techniques were utilized to examine the interactions between anti-KIM-1 antibody and KIM-1 antigen. The ITO-PET electrode surface was observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM) to analyze the morphological characterization at each electrode development stage. Optimization studies have been conducted to determine the optimal concentrations for detecting the 3-TMESP silane agent, the NHS crosslinker, the anti-KIM-1 antibody, and the optimum incubation period for the anti-KIM-1 antibody and the KIM-1 antigen. Analytical characteristic properties of the developed biosensor, including linear determination range, and the studies of reproducibility, regeneration, repeatability, storage life, and selectivity were investigated. The KIM-1 biosensor system, based on 3-TMESP, has a broad linear range and is capable of providing sensitive measurements between 0.1 fg/mL and 1000 fg/mL. The values of low limits of detection (0.26 fg/mL) and of quantification (0.87 fg/mL) were calculated, highlighting its high sensitivity and precision in detecting KIM-1. In addition, five different commercially purchased human urine samples were tested to validate the practicability of the developed biosensor.

## 1. Introduction

This paper presents the design of an electrochemical biosensor for the sensitive detection of KIM-1. KIM-1 protein, a urinary biomarker that has been validated by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the detection of nephrotoxicity, is a type-1 transmembrane protein [1]. The KIM-1 protein is usually not significantly present in the body but is expressed in response to kidney injury [2]. In the initial phases of AKI, the expression of the KIM-1 protein is detected significantly earlier than the elevation of serum creatinine levels [3,4]. Hence, the KIM-1 protein is regarded as a promising biomarker for AKI and has the potential to be employed in the early detection and diagnosis of this condition. Thus, it is reasonable to postulate that the detection of the KIM-1 protein could be advantageous for patients with AKI by facilitating timely medical interventions, thereby potentially mitigating further renal damage and enhancing overall patient outcomes. AKI is defined as a sudden and rapid kidney deterioration within a short period of timeframe [5]. AKI leads to electrolyte imbalances and accumulation of water, sodium, metabolites, and metabolic waste products [2]. Approximately 15 % of hospitalized patients experience AKI [6], and it affects an estimated 13 million people annually [7,8].

Electrochemical biosensors are devices with an electrochemical transducer that converts biochemical information [9,10]. Nowadays, electrochemical biosensors are widely utilized in fields such as medical analysis and food safety detection [11,12]. Commonly used electrochemical techniques include cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV), and square wave voltammetry (SWV). These techniques are crucial due to their ability to provide precise and sensitive measurements, which are

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**Table 1**
The comparison of the ITO-PET-coated electrode-based electrochemical biosensor system with the other techniques for the KIM-1 detection.

| Immobilization | Material/Method | Linear range | Detection range | LOD | Ref. |
|---|---|---|---|---|---|
| Carboxylated Polystyrene Beads/EDC/Sulfo-NHS/Goat Polyclonal Antibody/$NaHCO_3$/PBS | Nuclear Magnetic Resonance Service (μNMR) | - | 0.2–2 ng/mL | 100 pg/mL | [17,18] |
| HAp/Graphite Powder | Hydroxyapatite | 0.01–0.20 μg/mL | - | 0.017 μg/mL | [19] |
| Urinary Total Protein/NAG/Reagent Buffer/Diluted Sample/Particles Coated with Polyclonal Anti-cystatin C Antibodies | Microbead-based Sandwich ELISA | - | 0.04–160 ng/mL | 4.4 pg/mL | [20] |
| GCE/$CuCl_2$ NWs/Au/Ab1/BSA | PdPtBP NPs/MXene | 0.5–100 ng/mL | - | 86 pg/mL | [2,21] |
| TAB + PTA/COFs/AuNPs/GCE/Anti KIM-1-Ab1/BSA | Electrochemical Immunosensor | 0.01–50.00 pg/mL | - | 2.00 fg/mL | [2] |
| SPA/Anti-KIM-1/BSA | Electrochemical Immunosensor | 0.1–1000 ng/mL | - | 64 pg/mL | [22] |
| Protein A/KIM-1 mAb + HMGB-1 mAb/PEG2000 + BSA | SPRi-based Nano-Immunosensor | - | 0.005–50 ng/mL | 5 pg/mL | [18,23] |
| **OH/3-TMSPE/NHS/Anti KIM-1/BSA** | **ITO-PET-coated Electrode-Based Electrochemical Biosensor System** | - | **0.1–1000 fg/mL** | 0.26 fg/mL | **This work** |

essential for accurate diagnostics and quality control. Electrochemical biosensors are highly valued for their numerous advantages, including simplicity in operation, rapid detection capabilities, portability, real-time monitoring, and cost-effectiveness, all of which contribute significantly to their effectiveness in various applications [12,13].

ITO-PET film electrodes indicate excellent conductivity and are particularly affordable in comparison with other biosensor electrodes, such as screen printed (SPE), and glassy carbon (GCE) electrodes [14–16]. In view of the millions of individuals affected by AKI annually, the implementation of an affordable ITO-PET-coated electrode-based biosensor system that can detect the KIM-1 protein could significantly lower diagnostic costs by providing a more accessible and cost-effective alternative for early detection, thereby enhancing global healthcare accessibility.

In this study, optimization experiments were conducted to determine the optimal concentrations of the 3-TMESP silane agent, NHS crosslinker agent, and anti-KIM-1 antibody for KIM-1 protein detection. Additionally, the optimal incubation times for the anti-KIM-1 antibody and KIM-1 protein were evaluated. Storage studies were performed to assess changes in biosensor stability. The developed electrochemical biosensor was tested for repeatability, regeneration, reproducibility, and selectivity. An ITO-PET-coated electrode-based electrochemical biosensor system was developed to detect KIM-1 protein concentrations ranging from 0.1 to 1000 fg/mL. The CV technique was used as a secondary validation method for EIS results, while the SFI technique was also employed. The newly developed biosensor is compared to several KIM-1 biosensor systems described in the literature, and the results are shown in Table 1.

## 2. Materials and methods

### 2.1. Devices

Throughout the entire research study, ultra-pure water was supplied from PURELAB flex 3 Ultrapure Water Purification System (ELGA LabWater, UK) device. All electrochemical studies were conducted using the Gamry potentiostat (Reference 600, Gamry Instruments, Warminster, PA, USA) device via EChem Analyst software. The reference and the counter electrodes were obtained from BASi (Bioanalytical Systems, Inc., IN, USA). The SEM and AFM measurements were implemented at the Science and Technology Application and Research Center of Çanakkale Onsekiz Mart University (ÇOBİLTUM).

### 2.2. Chemicals and equipment

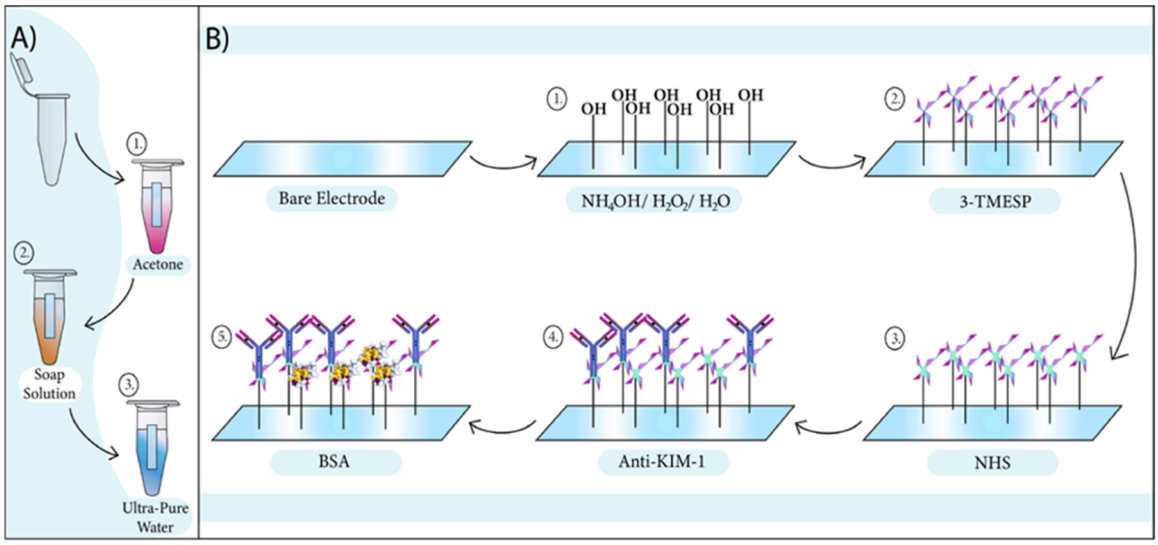
In a three-electrode system, the reference (Ag/AgCl, saturated with KCl) and the counter (platinum wire) electrodes were utilized and purchased from BASi (Bioanalytical Systems, Inc., IN, USA). The ITO-PET-coated electrode strips (2 mm × 20 mm) were employed as the working electrode. ITO-PET-coated film sheet, hydrogen peroxide solution, ammonium hydroxide solution, and the N-hydroxysuccinimide crosslinker agent were obtained from Sigma-Aldrich (Sigma-Aldrich Solutions, M.O., USA) company. Ethanol Absolute was supplied by Isolab (INTERLAB Laboratory Products, TR). The KIM-1 antigen and the anti-KIM-1 antibody were used by diluting in a phosphate buffer (50 mM, pH 7.4). BSA (0.5 %) was prepared with phosphate buffer (50 mM, pH 7.0). The anti-KIM-1 antibody and the KIM-1 antigen were obtained from MyBioSource company. A solution of 5 mM ferricyanide/ferrocyanide (1:1) redox probe was prepared for the electrochemical measurement stages of the study. Pooled human urine samples were purchased from Innovative Research (Novi, MI) and stored at − 20 °C.

### 2.3. Principle of measurement

In this study, the measurement process was determined by the EIS, CV, and SFI techniques. The frequencies of 50,000 Hz (initial frequency) and 0.05 Hz (final frequency) were used for the EIS measurements. The determined range for the CV measurements was between − 0.5 V to 1 V (Scan rate:100 mV/s, step size:10 mV). The measurements were conducted at 22 °C in the previously mentioned ferricyanide/ferrocyanide redox probe.

### 2.4. Immobilization method of the KIM-1 biosensor

The ITO-PET-coated electrodes were cleaned with acetone, soap solution, and ultra-pure water in turn (10 min for each) by the ultrasonication procedures in the ultrasonic cleaner device (Jeiotech, Republic of Korea). Following the end of the cleaning process, the hydroxylation stage began. In the process, the working electrodes were incubated for 90 min in a 1:1:5 ratio of ammonium hydroxide solution, hydrogen peroxide solution, and ultra-pure water, respectively. After the incubation process, the ITO-PET-coated electrodes were passed through ultra-pure water. Thereafter, they were kept in the silane solution (3-TMEPS, 0.05 %) overnight. After approximately 18 h, the working electrodes were passed through ethanol and ultra-pure water. Then, the electrodes were dried with argon gas and incubated for one hour with the NHS crosslinker agent at the rate of 2 mg/mL. After passing the electrodes through ultra-pure water, the electrodes were incubated in an antibody solution (anti-KIM-1, 5 ng/mL) prepared with PBS buffer (50 mM, pH:7.4) for 45 min. The immobilization process was completed after incubating the electrodes for 60 min. with the BSA (0.5 %) protein to block the remaining active sites to help prevent the occurrence of non-specific interactions. The cleaning process and the immobilization steps (OH/3-TMSPE/NHS/Anti KIM-1/BSA) of the




**Scheme 1.** A representation of the immobilization process of the KIM-1 biosensor.

developed biosensor are illustrated in Scheme 1.

### 2.5. Urine sample preparations

Five different commercially purchased human urine samples were used for this study. The standard addition method was used because it was not certain if the samples contained the KIM-1 protein. KIM-1 concentrations of 75 fg/mL and 100 fg/mL were selected for the standard addition method. The human urine samples were not diluted.

## 3. Results and discussion

### 3.1. SEM and AFM images of the KIM-1 biosensor

SEM was used to investigate the surface characterization of the developed KIM-1 biosensor, and changes in the surface morphology were examined by taking SEM images of each surface modification step of the biosensor. Fig. 1A illustrates the AFM (A1, A2) and SEM (A3) images of the hydroxylation step. During this surface modification process, it can be observed that the surface is smoother and emptier than in the upcoming steps. Upon examining the images of the second row in Fig. 1, which depict the modification step using 3-TMESP silane agent (B1–B3), it is evident that the surface morphology has undergone a significant change. At this stage, the electrode surface has become more coated, and the surface has become rougher. At the NHS cross-linking agent stage (Fig. 1. C1–C3), the voids on the electrode surface became even more filled, and the density of surface protrusions increased. When the SEM image (Fig. 1. D3) of the KIM-1 antibody application (Fig. 1. D1–D3) is examined, it is apparent that there are significantly more biochemical agents attached to the surface than in previous steps. As shown in the BSA protein images (Fig. 1. E1–E3), the corresponding AFM topography image (Fig. 1. E1) showed peaks from many locations. This is because BSA is a large molecule, and when bound to the surface, it can significantly increase the surface roughness and elevation. The last step shows AFM (Fig. 1. F1, F2) and SEM (Fig. 1. F3) images of the developed KIM-1 biosensor with KIM-1 antigen application. As can be seen in the microscopy images, the biosensor immobilization process was successful.

### 3.2. Immobilization process of the KIM-1 biosensor

The immobilization process is crucial for reliable biosensor development. Thus, the immobilization steps were carried out with caution. The EIS spectra and CV voltammograms that indicate the alteration in the electrode surface are shown in Fig. 2A and Fig. 2B, respectively. A hydroxylation process was accomplished on a pre-cleaned ITO-PET-coated electrode surface. Thereafter, a siloxane bond was created on the electrode surface by treating it with the 3-TMESP (0.05 %) silane agent. The methoxy groups of the 3-TMESP silane agent caused issues with electrochemical measurements due to the redox probe's negative charges compared to the hydroxylation measurements. Accordingly, the signal of the 3-TMESP silane agent in the EIS spectrum escalated, which was attributed to the presence of a more significant number of molecules on the electrode surface and increased ionic interactions. Subsequently, the electrode surface was incubated with the NHS crosslinker agent (2 mg/mL) for 60 min. In the next step, the electrode surface was incubated with the anti-KIM-1 antibody (5 ng/mL) for 45 min. The NHS crosslinker agent reacted with primary amines on the antibodies and created a covalent linkage. A slight signal increase in the EIS spectrum was observed for both steps. The electrode surface was lastly incubated with 0.5 % BSA solution to prevent non-specific interactions. A significant increase in the signal was observed in the EIS spectrum due to the greatly increased charge transfer resistance. The CV technique was also used to support and validate the electrochemical measurement results. The CV voltammograms of the immobilization stages overlapped with the EIS spectra measurement results of the same working electrodes. Formation of the hydroxyl groups on the electrode surface caused high peak currents. A significant alteration in the surface insulation was observed after the 3-TMESP silane agent immobilization step. The NHS crosslinker agent immobilization step did not give significantly different results for the surface insulation. The electrode surface became more insulated after the antibody and the BSA immobilization steps.

The Kramers-Kronig transformation, given in Fig. 2C, was performed to analyze the impedance spectrum of the KIM-1 biosensor system. This helped to determine how external factors affect the system [24,25].

### 3.3. Optimization process of the KIM-1 biosensor

Optimization studies have been conducted carefully to determine the optimal concentration for detecting the 3-TMESP silane agent, the NHS crosslinker agent, the anti-KIM-1 antibody, and the optimum time for the anti-KIM-1 antibody and the KIM-1 antigen. Three different concentrations of the 3-TMESP silane agent (0.05 %, 0.01 %, and 0.1 %, w/v) were tested for the first optimization study of the proposed biosensor (Fig. S1.A). The 0.1 % concentration was immoderately concentrated for the biosensor, resulting in a significantly decreased sensor response

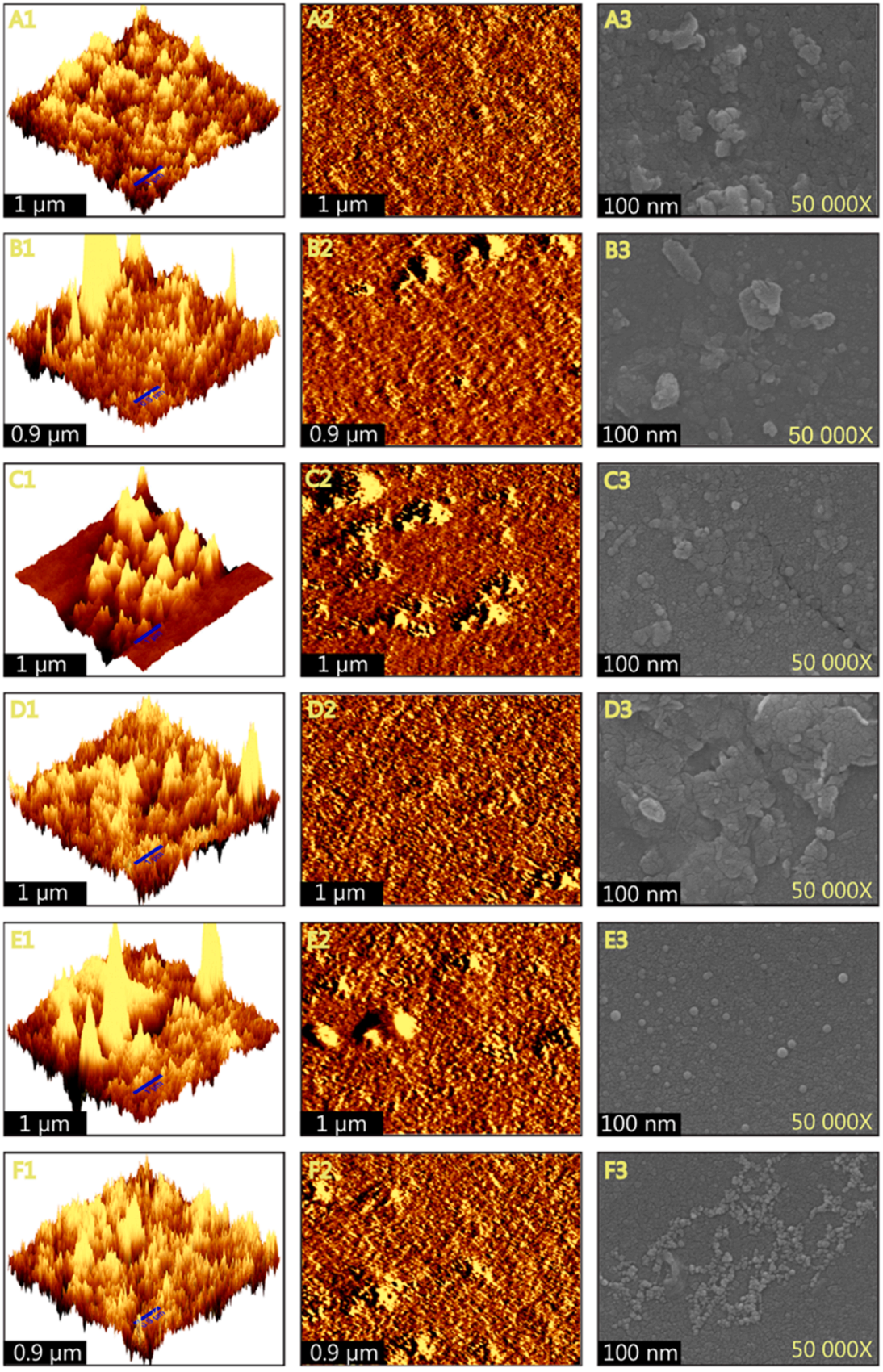



**Fig. 1.** AFM and SEM images of the KIM-1 biosensor of each surface modification step. **A1–A2)** AFM-OH **A3)** SEM -OH, **B1–B2)** AFM-3-TMESP **B3)** SEM -3-TMESP, **C1–C2)** AFM-NHS **C3)** SEM-NHS, **D1–D2)** AFM-anti-KIM1 **D3)** SEM -anti-KIM1, **E1–E2)** AFM-BSA **E3)** SEM-BSA, **F1–F2)** AFM-KIM1 **F3)** SEM-KIM.

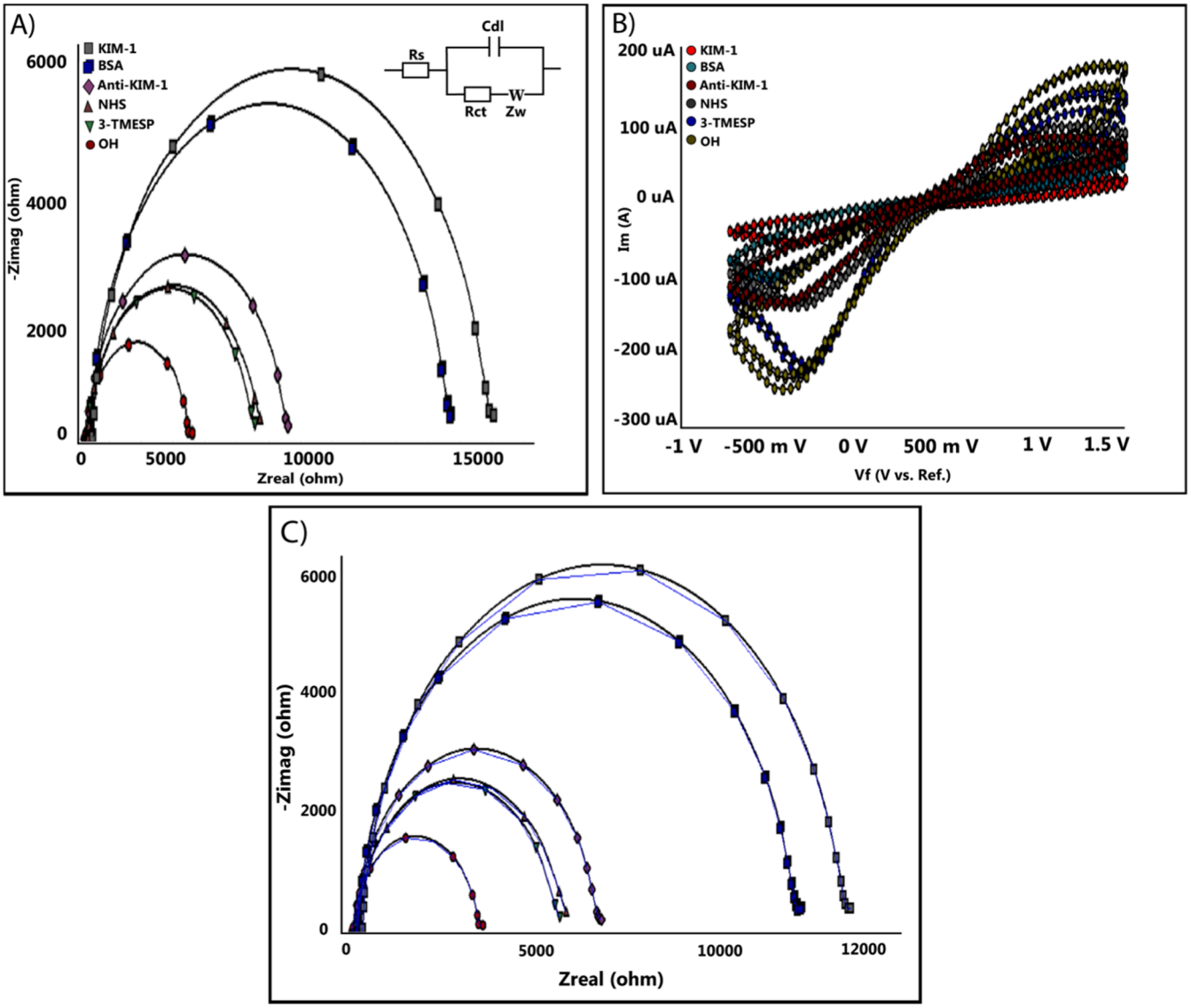



**Fig. 2.** A) EIS Spectra, B) CV voltammograms, C) Kramers-Kronig Transform of the proposed immunosensor.

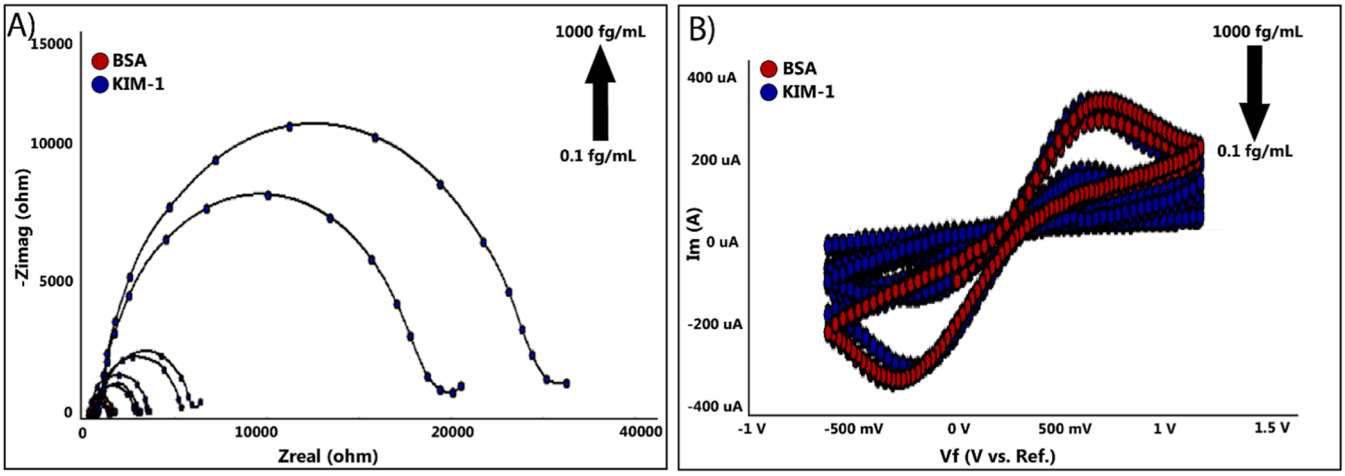



**Fig. 3.** A) EIS Spectra, B) CV voltammograms of the KIM-1 antigen detection at increased concentrations.

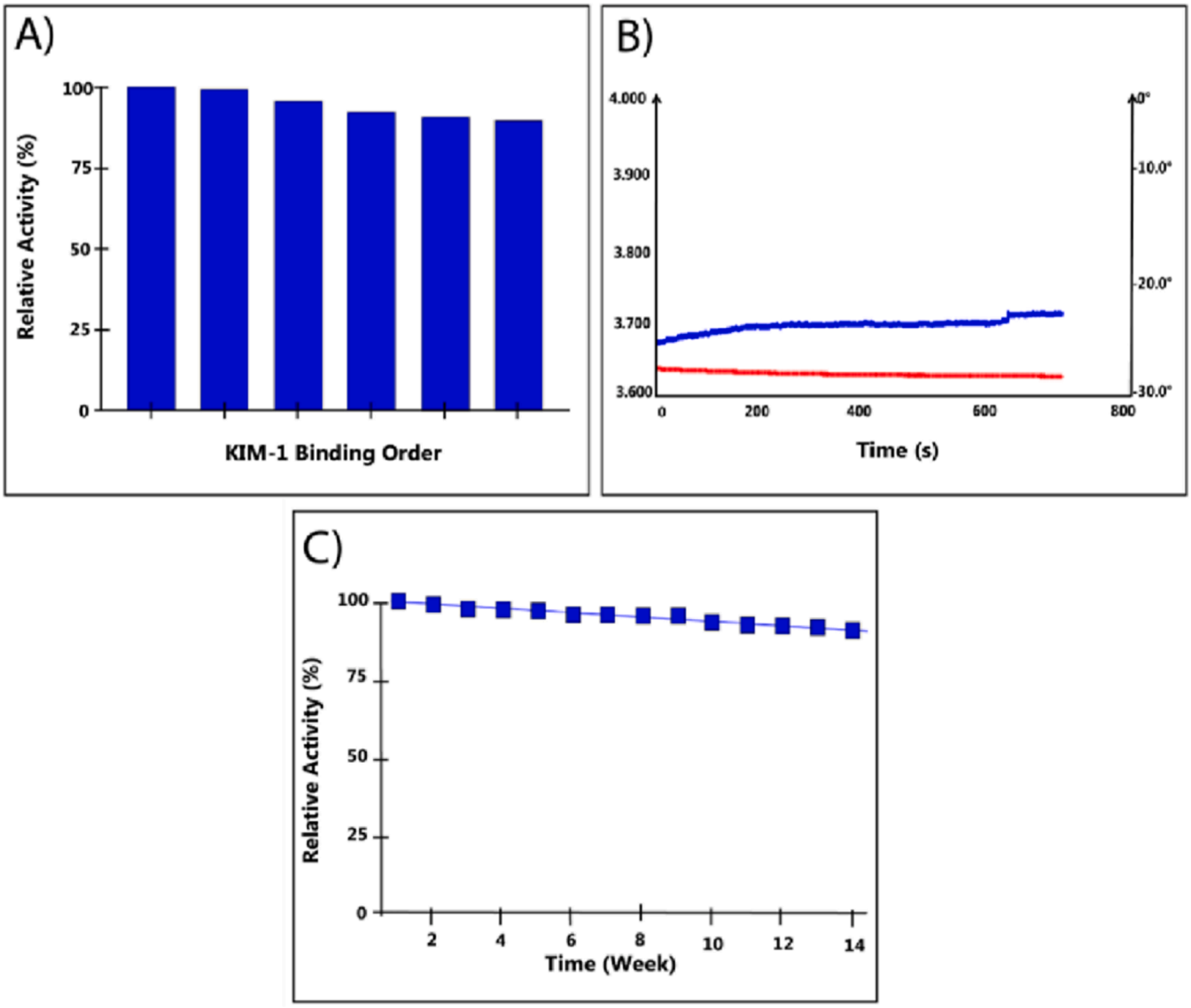



**Fig. 4.** A) Regeneration, B) SFI analysis, C) Storage life of the KIM-1 biosensor.

compared to the other ones. The sensor response for the 0.01 % concentration was slightly higher than that of the 0.05 %. However, the 0.05 % concentration provided a similar response and initiated a sensor response earlier in the measurement. Therefore, the 0.05 % concentration was determined as the optimal concentration.

Three different concentration ratios (1 mg/mL, 2 mg/mL, 3 mg/mL) of the NHS crosslinker agent were tested for the second optimization study step of the proposed biosensor. The sensor response to the 1 mg/mL NHS concentration was significantly lower than the other tested concentrations. The 2 mg/mL concentration gave a close sensor response to the 3 mg/mL concentration and also started responding at the measurement's early stages. Therefore, the 2 mg/mL concentration was determined as the optimal concentration, helping to prevent chemical product waste whilst also obtaining highly similar Results (Fig. S1.B).

Afterward, three different concentrations (3 ng/mL, 5 ng/mL, and 7.5 ng/mL) of the anti-KIM-1 antibody were tested. The 5 ng/mL concentration gave a remarkably higher signal in the sensor response than the other selected concentration values. Hence, the 5 ng/mL concentration was determined as the optimal concentration because both going over and under that concentration ratio gave notably weaker signals (Fig. S1.C).

One of the other important optimization parameters for the developed biosensor was the incubation periods of the anti-KIM-1 antibody and the KIM-1 antigen. 45 min, 30 min and 60 min incubation periods were tested for the anti-KIM-1 antibody. The 30 min and 60 min of incubation periods performed similarly in the sensor response. The 45 min incubation period started giving higher signals at the earlier stages of the measurement and was remarkably higher in sensor response. The 45 min incubation period was determined to be the optimal incubation period for the anti-KIM-1 antibody (Fig. S1.D).

45 min, 30 min, and 60 min incubation periods were tested for the KIM-1 antigen. All incubation periods performed similarly at the earlier stages of the measurement. However, the 30 min incubation time was determined to be the optimal incubation period because of the increased sensor response (Fig. S1.E).

The optimization process graphs (Fig. S1.) of the KIM-1 biosensor are given in the supplementary file.

### *3.4. Analytical studies of the KIM-1 biosensor*

Fig. 3A demonstrates the EIS measurements of KIM-1 antigen at different concentrations. The measurements were taken in the range of 0.1 fg/mL to 1000 fg/mL. The figure also shows the EIS measurement of

**Table 2**
The analysis of the KIM-1 biosensor system for different urine samples.

| Serum number | KIM-1 concentration value (fg/mL) | Standard addition value (fg/mL) | Measurement concentration value (fg/mL) | RSD (%) (n = 3) | Recovery (%) |
|---|---|---|---|---|---|
| 1 | 17.98 | 75 | 93.88/94.65/94.63 | 1.51 | 101.51 |
| | | 100 | 119.38/116/118.17 | 0.09 | 99.91 |
| | | 75 | 94.03/96.66/101.86 | 0.84 | 99.17 |
| 2 | 23.34 | 100 | 121.89/125/127.60 | 1.21 | 101.21 |
| | | 75 | 98.20/103.55/102.21 | 0.04 | 99.96 |
| 3 | 26.36 | 100 | 125.90/125.71/128.98 | 0.40 | 100.40 |
| | | 75 | 86.74/91.80/83.17 | 0.12 | 100.12 |
| 4 | 12.14 | 100 | 115.40/114.66/112.72 | 1.89 | 101.89 |
| | | 75 | 86.00/85.21/93.68 | 1.14 | 98.86 |
| 5 | 14.32 | 100 | 108.85/110.19/120.26 | 1.06 | 98.94 |

BSA overlaid with the antigen concentration measurements. The EIS measurement increased with each increase in concentration level. The highest signal was observed when the working electrode was incubated with 1000 fg/mL of the KIM-1 antigen.

The overlay of the CV measurement results with the BSA measurement result (Fig. 3B) revealed a consistent narrowing as antigen concentration increased.

The calibration curve (Fig. S2. A) was plotted in the linear range of 0.1–1000 fg/mL. The LOD (limit of detection) and LOQ (limit of quantification) values were calculated as 0.2628 fg/mL and 0.8759 fg/mL, respectively. The formula "k.S (standard deviation)/m (slope of curve)" was used for calculations. The "k" value was accepted as 3 for LOD and 10 for LOQ [24,26].

Repeatability (Fig. S2. B) is one of the critical properties of developing a biosensor to maintain healthy results. Eight ITO-PET-coated working electrodes were prepared as part of a repeatability study. Once they were immobilized, the electrodes were incubated in different concentrations of the KIM-1 antigen, ranging from 0.1 to 1000 fg/mL. The same conditions, incubation periods, and concentrations of the calibration process were repeated for this study, and the results ($y = 19.9x + 194$, $R^2 = 1$) showed a significant overlap when compared to the calibration graph. The repeatability study equation was These findings indicate that the developed KIM-1 biosensor has a remarkably high level of repeatability.

### *3.5. Reproducibility, regeneration, selectivity and storage life of the KIM-1 biosensor*

The regeneration study (Fig. 4A) demonstrated that the ITO-PET-coated electrode can be reused multiple times despite being a low-cost disposable electrode. The chosen working electrode was incubated with an antigen concentration of 75 fg/mL for 30 min, followed by an EIS measurement. After washing through ultra-pure water, the electrode was incubated with a 10 mM HCl solution for 5 min. With the application of HCl solution, the electrode surface was cleaned of bound proteins. After the acid treatment, the electrode was rewashed and incubated in the same amount of antigen concentration for 30 min. The same electrode successfully gave appropriate EIS measurements after five acid treatments but could not be continued further because the electrode surface was clearly degraded after the sixth acid treatment. Based on the first antigen measurement prior to acid application, a graph was generated according to relative activity, as shown in Fig. 4A. When the graph is analyzed, a decrease in relative activity can be observed after each acid application.

In the SFI study (Fig. 4B) of the KIM-1 biosensor, frequencies were determined using a Bode plot. The real-time interaction of the antigen-antibody interaction was studied. The SFI technique allowed for the real-time measurement of changes occurring on the electrode surface. A frequency of 125.56 Hz was selected for the measurement. KIM-1 antigen (10 ng/mL) and PBS buffer (50 mM, pH 7.4) were used for the measurement. The measurement was completed in approximately 30 minutes. The SFI plot, illustrated in Fig. 4B, shows increased antigen-antibody interaction over time.

The storage life (Fig. 4C) of the KIM-1 biosensor system was studied for 15 weeks. The ITO-PET-coated electrodes were prepared, dried with argon gas, and finally stored at 4 °C. The EIS and CV measurements were taken from a pre-prepared study electrode each week. The first week was a baseline of 100 %, and the relative activity decrease was calculated for the following weeks, as shown in Fig. 3C. The working electrodes maintained over 90 % activity for 14 weeks, indicating the KIM-1 biosensor can be stored for long periods under appropriate conditions.

For the reproducibility (Fig. S3. A) study, 10 different KIM-1 biosensor systems were used. After carefully preparing the electrodes under the same conditions and in the same concentration range (0.1–1000 fg/mL), the EIS measurements were taken, and the graph shown in Fig. S3. A was drawn. All obtained $R^2$ coefficients were above 0.99, and the trendlines overlapped greatly.

The selectivity (Fig. S2) study was conducted and different biochemical agents were tested, including KIM-1, BSA, glucose, and creatine kinase. Additionally, two mixed solutions were tested. One of the mixed solutions contained a mix of BSA, glucose, creatine kinase, and urea, while the other one contained KIM-1 along with the same agents. The KIM-1 protein activity served as a reference point at 100 %, and the other biochemical agents were measured comparatively. The solution containing creatine kinase (11.44 %) produced the weakest signal, whereas the mixed solution containing KIM-1 (125.29 %) showed the highest signal. These results suggest that the developed biosensor is capable of providing clear and specific detections. The selectivity study graph of the KIM-1 biosensor is given in the Fig. S4.

### *3.6. The practicality test of the KIM-1 biosensor system for different human urine samples*

After optimization and characterization studies, a serum study was conducted as the final step. The response obtained in the serum study was analyzed, and comparisons were made. Impedance data obtained in the study were calculated using the equation found in the calibration curve. The aim of this study is to determine the clinical applicability of the developed biosensor with commercially purchased pooled urine. The standard addition method was used for this study. EIS measurements were utilized by adding 75 fg/mL and 100 fg/mL KIM-1 antigen concentrations to the urine samples. The calculations and the results are given in Table 2.

## 4. Conclusion

An electrochemical biosensor system, which has the ability to detect KIM-1 antigen concentrations ranging from 0.1 f to 1000 fg/mL, has been developed successfully. This newly developed biosensor is unique for being an ITO-PET-coated electrode-based system whilst having the aforementioned combination of chemical agents for its immobilization. Various optimization and characterization studies were conducted to

analyze the optimal concentrations and incubation periods as well as the biosensor system characteristics. For this novel biosensor, LOD and LOQ values were calculated to be 0.26 fg/mL and 0.87 fg/mL, respectively. Five different human urinal serums were tested to examine the biosensor practicability. The storage life study provided data to indicate that the biosensor system could be stored for up to 14 weeks when kept at 4°C. It is plausible to claim that this biosensor might have the potential to be used for the detection of acute kidney injury.

## CRediT authorship contribution statement

**Mustafa Kemal Sezgintürk:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Burçak Demirbakan:** Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Sude Aras:** Validation, Methodology, Investigation, Formal analysis. **Elif Ceren Ankara:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis.

## 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.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpbao.2024.100042.

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