Regular Article

ANALYTICAL
AND BIOANALYTICAL
CHEMISTRY
RESEARCH

Published by the
Iranian Chemical Society

***Anal. Bioanal. Chem. Res.**, Vol. 10, No. 4, 375-385, September 2023.*

Electropolymerized β-cyclodextrin as an Efficient Recognition Element Toward a Highly Sensitive Electrochemical Sensor for Sumatriptan

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(Received 29 December 2022, Accepted 15 April 2023)

The present study aims to introduce a highly sensitive electrochemical sensor for the quantification of trace amounts of Sumatriptan (SUM) in biological fluids. To immobilize a stable nanofilm of β-cyclodextrin (β-CD) on a glassy carbon electrode (GCE), electropolymerization of monomer was carried out within 0.1 M phosphate buffer with pH 6.0 utilizing cyclic voltammetry to yield polymerized β-CD (pβ-CD). The morphological characterization of pβ-CD/GCE was examined by Field emission scanning electron microscopy (FESEM). The electrochemical redox action of SUM on pβ-CD/GCE was scrutiny studied by cyclic voltammetry and chronocoulometry. The electrochemical parameters including the electron transfer coefficient (α), the standard heterogeneous rate constant (k_s), the surface area of the electrode (A), the electron transfer number (n), and the surface coverage (Γ) were estimated to be 0.38, 1.23×10^{-3} cm s^{-1}, 0.06 cm^2, 1, 1.07×10^{-8} mol cm^{-2}, respectively. At optimized criteria, a substantial enhancement was attained toward the electrooxidation of SUM on the developed electrode compared to the bare GCE, resulting in wide linear ranges of 0.062-2.47 µM and 2.47-52.1 µM with a low detection limit of 27 nM. The developed sensor was successfully employed for the quantification of SUM in human blood serum and urine samples with good selectivity and acceptable recoveries, proving its utility for further applications as a sensitive and reliable sensor.

Keywords: Sumatriptan, Cyclodextrin, Cyclic voltammetry, Electropolymerization, Glassy carbon electrode

INTRODUCTION

As an intricate condition, Migraine shows broad symptoms, and among them is a painful, disabling headache. It influences about 1 person in 8 worldwide, mostly 30 to 50 years old women [1]. Sumatriptan (SUM) belongs to the triptan family of drugs utilized in the treatment of this painful disease, by prescription via four main ways: oral, subcutaneous, intranasal, and rectal [2,3]. However, its overuse may result in an overdose with several side effects including tremors, skin irritation, breathing problems, blue-colored lips, vision problems, watery eyes, weakness, and lack of coordination [4]. Hence, it's worthwhile to determine SUM in real biological fluids and pharmaceutical formulations. Up to now, quantitative analysis of SUM has been carried out by several methods; like UV spectrophotometry [5], fluorescence [6], liquid chromatography [7], high-performance liquid chromatography (HPLC) [8], and capillary electrophoresis [9], all of which are sophisticated to handle, expensive and time-consuming techniques.

Electrochemical techniques have been widely used in pharmaceutical analysis, which in contrast possess many advantageous features such as high sensitivity, selectivity, rapidness, and simple procedures [10]. Thus, some electroanalytical sensors have been developed for the determination of SUM using various types of chemically modified electrodes including

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ZnO/NiO/Fe_3O_4/MWCNTs/GCE [11], Ni-Co layered double hydroxide/SPE [12], MXene/MWCNT/chitosan/GCE [13], pencil graphite electrode modified with molecular imprinted polymer/sol-gel/polyoxometalate/rGO [14], screen printed graphite electrode modified by Fe_3O_4@ZIF-8 nanoparticles [15], and carbon paste electrode with Pt/ZrO_2 nanoparticles modifier [16]. Although all these sensors provided nanomolar range detection of SUM, they suffer from tedious electrode preparation steps, which reduces their utility. On the other hand, the specific interactions of the electrode surface and target was just considered in molecular imprinted polymer-based electrode [14]. More recently, electropolymerization of p-aminophenol on the GCE surface was utilized for the voltammetric determination of sumatriptan succinate in the μM range [17]. Unlike previous works, this method benefits from a simple fabrication step accompanied by high repeatability, reproducibility, and stability. More studies are still required to develop sensitive, simple, and selective sensors for the determination of SUM.

The β-Cyclodextrins (CDs) are oligosaccharides composed of seven glucose units with a toroidal form consisting of a hydrophilic outer side and a hydrophobic inner cavity [18]. Benefiting from special cavities with variable ring sizes and plentiful approaches to chemical modification, they are currently the matter of concentrated electrochemical research [19,20]. As a macrocyclic oligosaccharide, the β–CD and its electropolymerized form have been used as an electrode modifier in voltammetry for the quantification of several analytes [21-25]. It acts as a promising molecular recognition agent in the creation of electrochemical sensors, chiral sensors, and biosensors by selective pre-concentrating of target species on the electrode surface and consequently improving its sensitivity for the detection of analytes of interest [26]. In this regard, electropolymerization provides a facile and controllable approach towards sensor development [27].

In the present study, an electropolymerized β–CD-modified glassy carbon electrode was prepared and its electrochemical response against SUM was studied. The electrooxidation current of SUM at the pβ–CD/GCE increased 5.2-fold compared with that of bare GCE using cyclic voltammetry, indicating that pβ–CD/GCE has much better electrocatalytic properties compared to the unmodified electrode. The effect of several experimental variables, such as the pH of the supporting electrolyte, the number of electropolymerization cycles, the concentration of β–CD, and the accumulation time were all studied and optimized. The cyclic voltammetry technique was used to study various kinetic parameters of the redox reaction including standard heterogeneous rate constant (k_s), electron transfer coefficient (α), and the number of exchanged electrons (n). In addition, the application of the introduced electrode for the quantification of SUM in the urine sample and blood serum was examined.

EXPERIMENTAL

Materials and Reagents

The analytical-grade reagents and double-distilled water were used for the preparation of solutions. A pure sample of Sumatriptan was obtained from Tehran Chemie pharmaceutical company (Tehran, Iran). A stock solution of 1 mM SUM was set in Millipore water. The β–CD and all the other reagents were gained from Sigma or Merck (Darmstadt, Germany). Phosphate buffer with different pHs from 2.0 to 9.0 was prepared by mixing the appropriate volume of 0.1 M NaH_2PO_4/Na_2HPO_4 and then changing the pH with 0.2 M HCl/0.2 M NaOH.

Equipment

Voltammetric measurements were conducted with an Autolab PGSTAT-12 (Eco Chemie B.V., Ultrecht, the Netherlands) electroanalytical instrument run by GPES software. The electrochemical cell is composed of unmodified/modified GCE (diameter = 2 mm, physical surface area of 0.0314 cm^2) as a working electrode, Ag/AgCl, and platinum wire as reference and counter electrodes, respectively. The pH values of buffer solutions were set with a Metrohm pH meter (Model: 691Herisau, Switzerland). Scanning electron microscopy (SEM) images of interfaces of electrodes were attained with field emission scanning electron microscopy (FESEM Mira 3-XMU).

Modification of GCE with pβ–CD

The bare GCE was polished with Al_2O_3 slurry with mesh values of 0.3 and 0.05 μm on paper and chamois leather, then rinsed with double-distilled water and ultrasonicated in distilled water for 10 min. Then, the polished electrode was

deep in a 0.1 M PBS (pH 6.0) comprising 1 mM β–CD solution under agitation, and cyclic sweeping of electrode voltage was carried out between -2 to +2 V at a scan rate of 100 mV s^{-1} for 20 scans [28].

Analysis of Real Ssamples

Human plasma and urine samples were analyzed by the modified electrode to elucidate its utility for real applications. The former specimens were obtained from a local Laboratory (Pastor, Khoy- Iran) and aliquots were then transported into micro tubes and frozen at -4 °C up to analysis. For daily analysis, the samples were thawed at room temperature and vortexed to guarantee homogeneity. Thereafter, to precipitate plasma proteins, methanol with a volume ratio of 2:1 was added to the sample. After centrifugation for 5 min at 3000 rpm, the residues of plasma proteins were separated. 2 ml of it was then spiked with SUM and added to supporting electrolyte in the cell to reach a total volume of 10 ml for subsequent analysis.

A healthy volunteer gave the urine specimen before the experiments. It has been reported that Acetonitrile eradicates urine proteins more efficaciously, and adding 1-1.5 volume of acetonitrile in urine is adequate to eliminate proteins. To separate urine protein residues the mixture was vortexed for 30 s, centrifuged for 10 min at 3000 rpm and the supernatant was engaged for spiking different amounts of SUM.

RESULTS AND DISCUSSION

Surface Characterization of pβ–CD/GCE

The FESEM analysis was carried out to study the interface morphologies of the GCE and pβ–CD/GCE and reach evidence for the formation of pβ–CD polymer. Figure 1A and Fig. 1B show the changes in surface morphology obtained from FESEM images for bare and modified GCE, respectively. As realized in Fig. 1A, the surface of bare GCE is almost plane and uniform while after the modification, some branch-like structures are observed, confirming that β–CD was successfully electropolymerized on the GCE surface. The other evidence is that unlike some other studies, which used drop-casting of polymerized β–CD on electrode surface [29], wherein it is observed that well-attached structures of polymer have formed on the GCE surface that can enhance further its performance. Previous studies showed that the mechanism of electro-oxidation of β–CD follows three stages (Fig. S1): in stage 1, oxidation generates a radical cation on the carbon bearing the hydroxyl group, and the active center interacts with the GCE through a covalent bond, producing a ketone. The oxygen involved in the mechanism is probably obtained from the electropolymerization solution. Therefore, for the mechanism to occur effectively, electropolymerization must be performed in the presence of oxygen [30]. To fabricate pβ–CD/GCE, we followed the optimal criteria reported in previous research and we did not carry out further research to

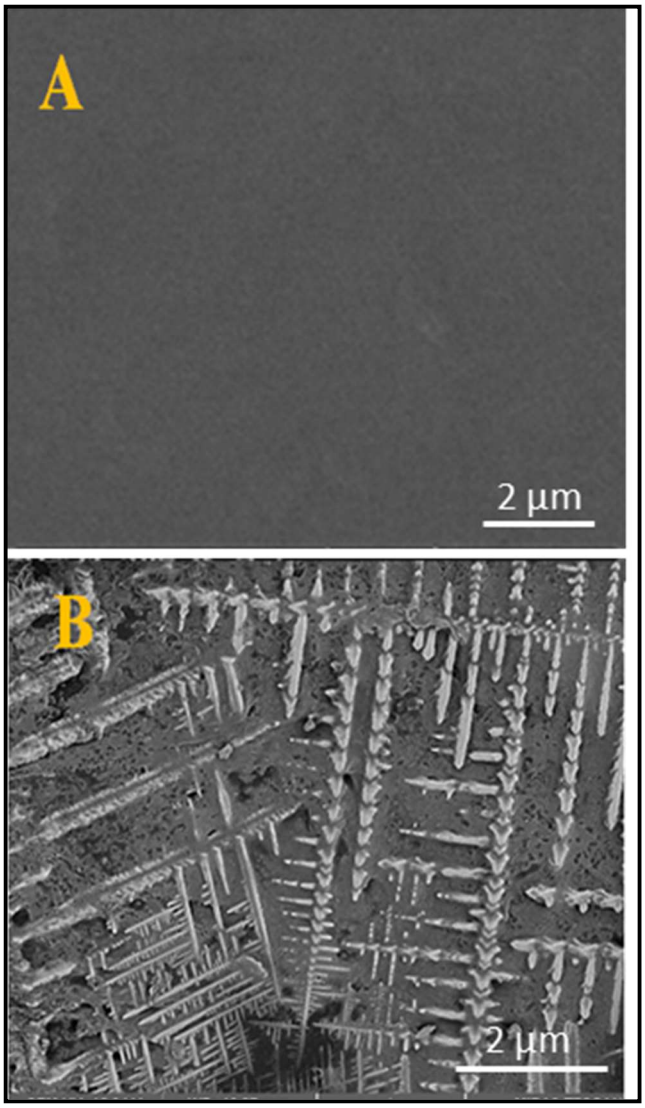


Fig. 1. FE-SEM micrographs of (A) GCE and (B) pβ–CD/GCE.

examine the hydrogen evolution reaction and oxygen evolution reaction on electro polymerization of β-cyclodextrin but such phenomena can be studied in future works.

Performance Evaluation of pβ–CD/GCE

The redox action of SUM in a phosphate buffer solution pH 7.0 was studied on bare GCE and pβ–CD/GCE using CV in a range of 0.2-1.0 V *vs.* Ag/AgCl. The CVs for 4.9 µM SUM were recorded after a 20 s accumulation time (Fig. 2A). During the accumulation step, stirring of the solution was applied without imposing a potential step on the electrode. The results confirm that the oxidation of SUM at the surface of the bare GCE (Fig. 2A, curves a) has a weak peak current, whereas on the surface of pβ–CD/GCE (Fig. 2A, curves b) resulted in a dramatic rise in the oxidation peak current (5.2-fold, 3.23 *vs.* 0.62 µA) around 0.71 V, which exhibited the electrocatalytic effect of pβ–CD for oxidation of SUM. The decline of the surplus of the anode potential and the increase in the intensity of the anodic stream suggests this effect. These indicate that β–CD facilitates electron transfer at the electrode surface. No peaks regarding reduction were observed, representing irreversible oxidation of SUM on both electrodes. To elaborate a better quantitative comparison of bare GCE and pβ–CD/GCE, differential pulse voltammetry was used as a more sensitive technique. A very sharp and well-defined peak was obtained on pβ–CD/GCE around 675 mV (Fig. 2B). Compared with unmodified GCE, the modified electrode with pβ–CD/GCE clearly showed higher sensitivity towards SUM due to the formation of the polymer layer. In addition, this is attributed to the preferable adsorption interaction of pβ–CD with SUM through the formation of hydrogen bonding between the −(HO)s on the relatively less polar cavity β-CD and the amino group (-NH_2) of SUM, resulting in the formation of SUM: β–CD inclusion complexes that act as selective adsorbent and enrichment agent. The inclusion of SUM into β-cyclodextrin has been previously proved via FT-IR spectroscopy, solid-state NMR with magic angle spinning condition, ^{1}H and ^{13}C MAS NMR, and differential scanning calorimetry methods [31]. Such interactions have been used in other studies for the enantio-recognition of tryptophan enantiomers [29,32] as well as the recognition of dopamine from ascorbic acid and uric acid [33].

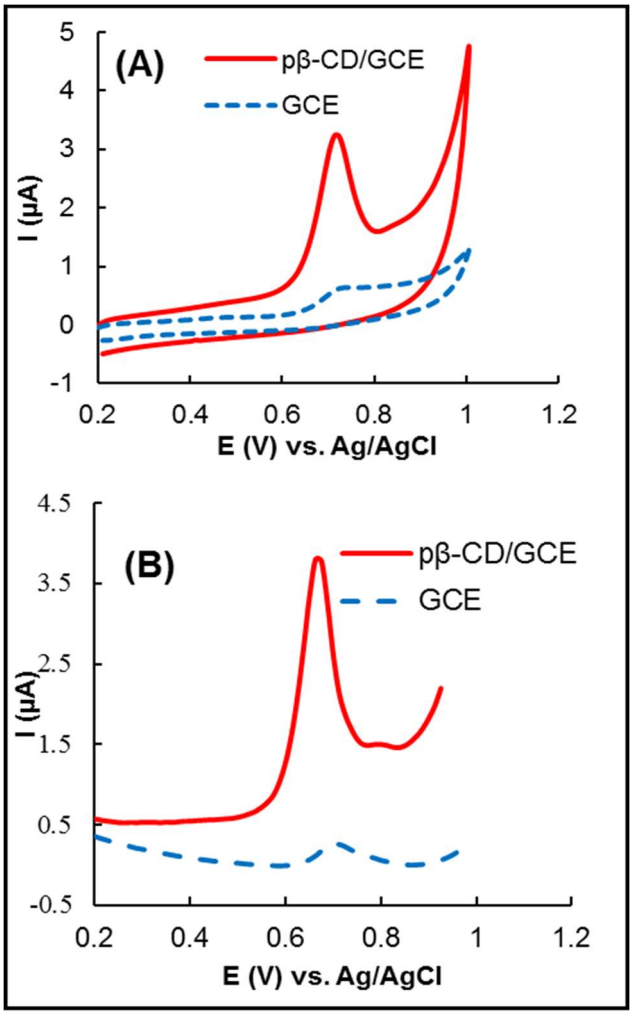


Fig. 2. (A) Cyclic voltammograms and (B) Differential pulse voltammograms of 4.9 µM SUM in 0.1 M PBS for dash lines: GCE and solid lines: pβ-CD/GCE.

Optimization of Experimental Settings

In the absence of control on the synthesis of electropolymerized β-CD on the GCE surface, the severe steric hindrance could reduce the efficiency of the modified electrode towards SUM electro-oxidation and that's why we optimized the synthesis conditions of pβ-CD on GCE before any quantitative studies. The effects of several determining factors on the performance of the developed sensor were examined including the pH of the electrolyte, the number of cycles in CV, the concentration of β–CD in the electropolymerization step, and finally accumulation time of the analyte.

Effect of the number of cycles of electropolymerization on sensor performance. The relation between peak current intensity and the number of cycles of electropolymerization of pβ-CD was studied from 5 to 25 cycles and the outcomes are exposed in Fig. 3A. The peak intensity amplified drastically with an augment in the number of cycles from 5 to 20, due to the formation of a polymer layer; however, at higher cycles, it results in the decrease of current may be due to the hindering of the pβ-CD cavities for entrapping SUM molecules as electroactive species and henceforth triggering a decrease of response [34]. The maximum current was observed at 20 cycles, hence polymerization by 20 cycles was chosen for further experiments.

Optimization of the initial concentration of β-CD. The initial monomer concentration affects the electropolymerization rate and film thickness and hence determines the analytical performance of the sensor. To clarify the effect of it on the response of 2.97 µM SUM, the films were grown in solutions with varying β-CD concentrations in the range of 0.5-3.0 mM by cycling potential between -2.0 V-2.0 V. Figure 3B represents the alteration of the current values for SUM as a function of the monomer concentration. As seen, increasing the monomer concentration causes rapid polymerization and increases sensor sensitivity due to the formation of a polymer layer; however, at higher concentrations, it results in the decrease of current may be due to the blocking of the active sites and hence causing a decrease of response [34]. As seen in Fig. 3B, the highest response of the electrode to SUM was attained at 1.0 mM of monomer, as an optimal value.

Influence of accumulation time. The accumulation time (t_{acc}), as a determinant step, was also optimized for measuring target SUM, while the stirring of the solution was applied without imposing a potential step on the electrode. As indicated in Fig. 3C, for the quantitative detection of SUM, the maximum current signal was obtained when the accumulation time was 20 s. By the extra rise in the accumulation time, the peak currents start to level off, showing the occupation of interaction sites within p β-CD cavities. Accordingly, 20 s was set as the optimum value for t_{acc} in the following experiments.

Effect of pH on voltammetry responses. The impact of pH within the range of 2 to 9 on pβ–CD/GCE for 3.9 µM

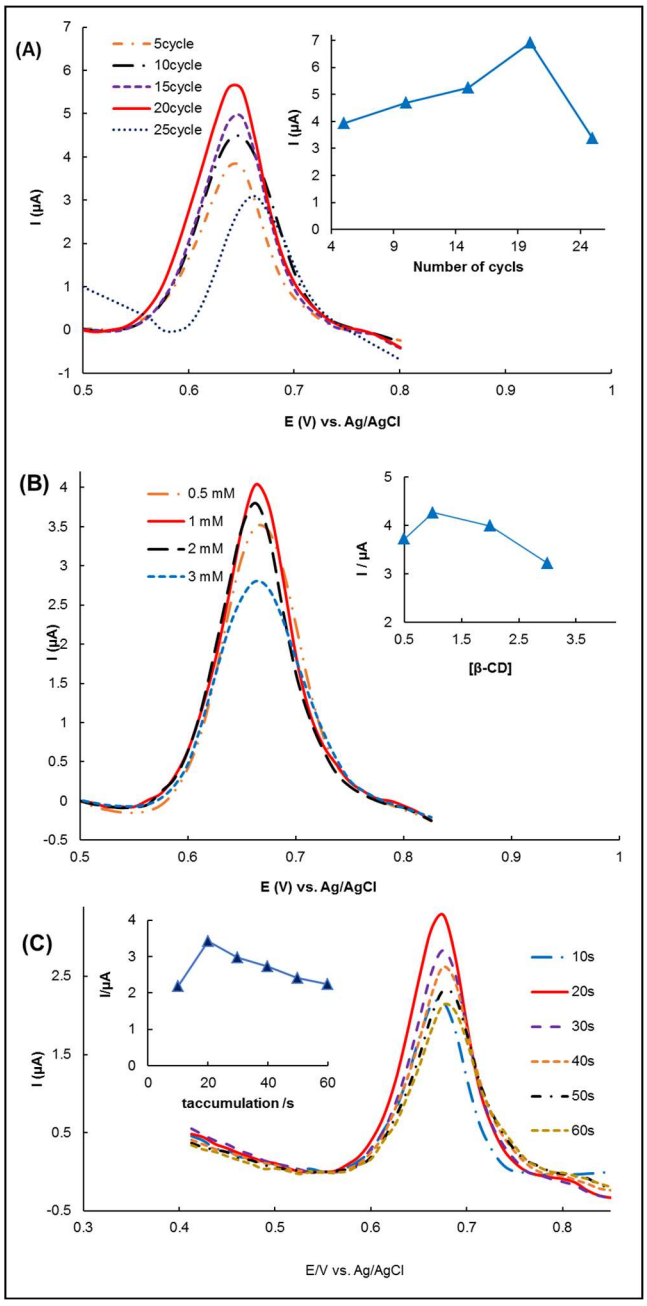


Fig. 3. (A) Effect of electropolymerization cycles, (B) Effects of the amount of β–CD, and **C)** Effect of accumulation time on the oxidation peak current of 2.97 µM SUM.

SUM was studied by linear sweep voltammetry (LSV) at a scan rate of 100 mV s^{-1} (Fig. 4A). From the I_p *vs.* pH plot in Fig. 4B, a non-linear rise in the anodic peak current was

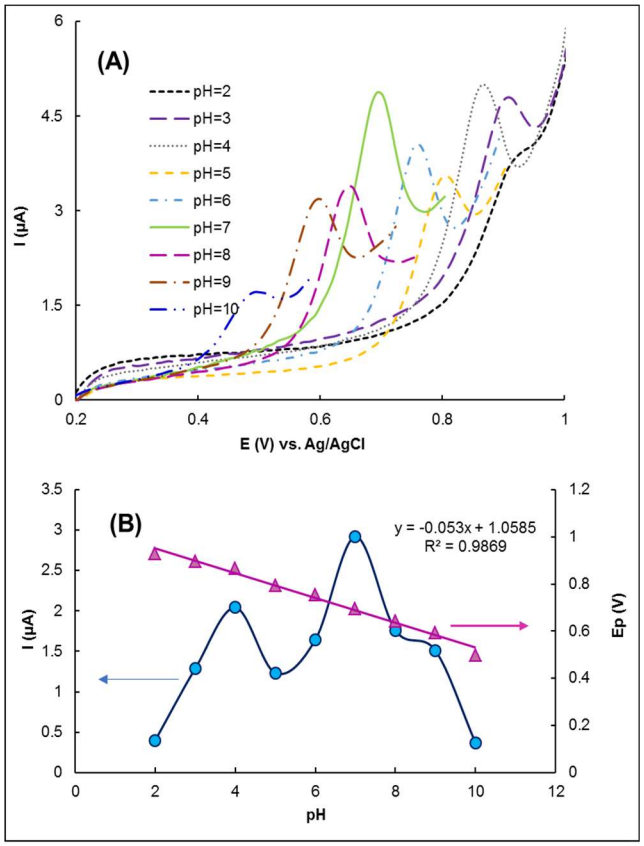
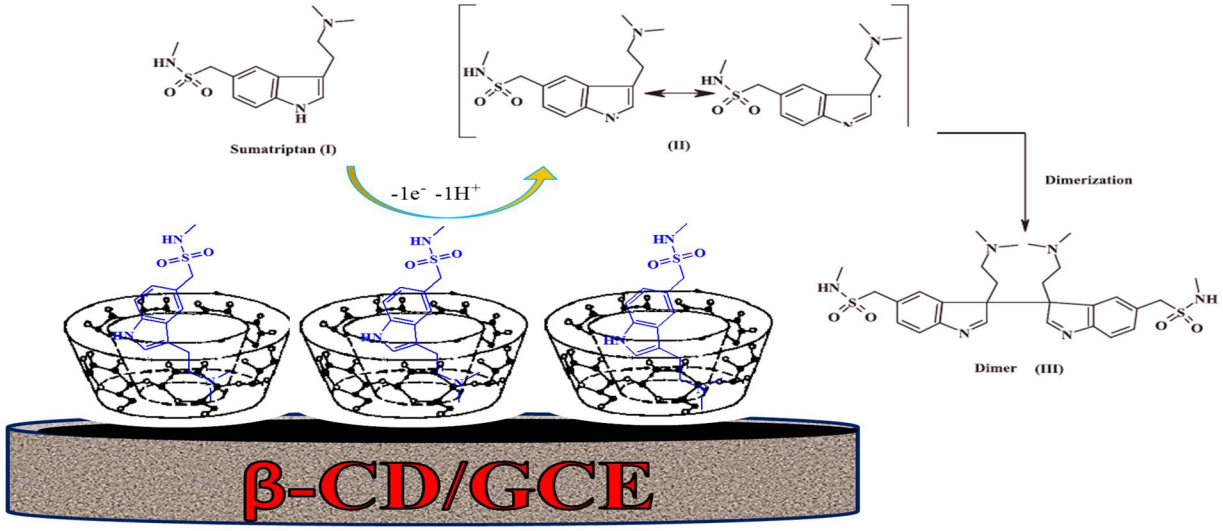


Fig. 4. (A) LSVs of 4.9 µM SUM on the surface of β–CD/GCE at various pHs (from 2.0 to 9.0) of the buffer solution; (B) dependence of anodic peak current (●) and anodic peak potential (▲) on the solution pH (Potential scan rate: 100 mV s^{-1}; accumulation time: 20 s).

detected as a function of pH, in which the maximum peak intensity was attained at pH 7.0. Therefore, for further studies, it was chosen as the working condition, which yielded higher responses. Furthermore, as seen in Fig. 4A, the oxidation peak of SUM on the surface of the improved electrode moves toward fewer positive potentials while increasing the pH. Such a linear shift in Fig. 4B indicates that protons are participating in the oxidation reaction and that it follows the equation: E_{pa} (mV) = 105.85 -53 pH, R^2 = 0.98. With a slope of 53 mV/pH, it satisfies the Nernst equation accompanying the equal number of electrons and protons [35]. Hence, the anticipated mechanism for the electrooxidation of SUM on pβ–CD/GCE is summarized in Scheme 1, which involves one electron-one proton mechanism as reported elsewhere [12]. It has been reported that the oxidation takes place at the indole moiety of SUM, which then gives a free radical (II) followed by combining with another SUM molecule and yields a dimer (III) in which two units are joined at β position [36].

Influence of Scan Rate on Electro-oxidation of SUM

The effect of scan rate on the electrochemical behavior of SUM (4.9 µM) on pβ–CD/GCE was studied by cyclic voltammetry. Figure 5A displays that at scan rates from 10 to 130 mV s^{-1}, the oxidation peak currents (I_{pa}) of SUM rise linearly with the scan rate through an equation of I_{pa} (µA) = 0.0156 ν (mV s^{-1}) + 0.1973 (R^2 = 0.9951) (Fig. 5B).



Scheme 1. The proposed mechanism for inclusion complex formation between pβ–CD and SUM as guest molecules and electrooxidation of SUM on pβ–CD/GCE

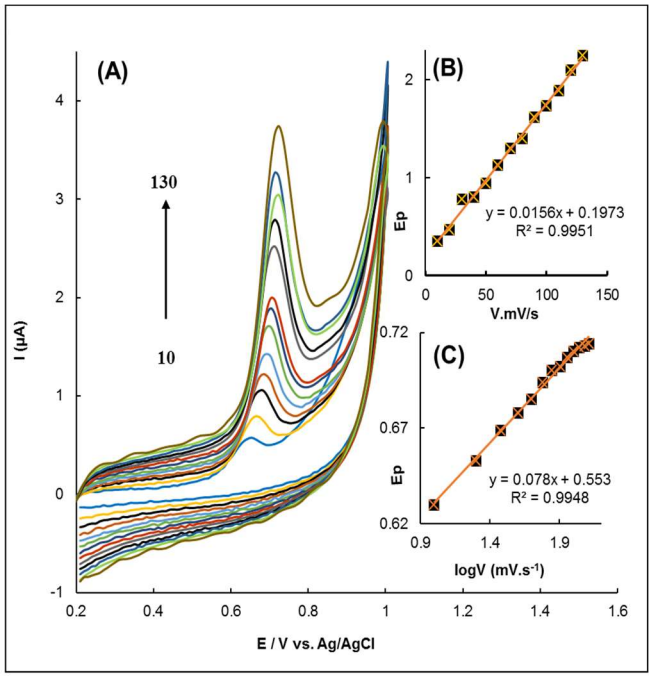


Fig. 5. (A) Cyclic voltammograms at various scan rates of 10 to 130 mV s^{-1} in PBS buffer (pH 7.0), (B) Plot of I *vs.* ν for 4.9 μM SUM at the modified glassy carbon electrode, and (C) Dependence of the peak potential, Ep on log (v) for the oxidation of SUM on pβ–CD/GCE

The outcomes designate that the reaction was adsorption-controlled. Additionally, by augmenting the scan rate, the oxidation peak potential lifted to high positive potentials, approving the kinetic restriction involved in the electrochemical process. Furthermore, as stated by Eq. (1), a linear relationship between peak potential (E_p) and the logarithm of scan rate (log ν) is seen, like for an irreversible process [37].

$$E_p = (b/2)\log\nu + \text{constant} \quad (1)$$

Where E_p is the anodic peak potential and b/2 is the Tafel slope. Figure 5C reveals the linear association between Ep and log v as stated by Eq. (1) and the slope was obtained to be 0.156 for the developed electrode. By considering a transfer coefficient of α = 0.38, this slope shows that the rate-limiting step is a one-electron transfer process. From the calculations, the number of electrons associated with the oxidation of SUM was estimated to be 0.97 (≈ 1). This result was experimentally evident that the electrooxidation of SUM was accompanied by a one-electron process [35].

Calculation of Electrochemical Parameters

The values of electrochemical effective surface area (A) for bare GCE and pβ–CD/GCE were estimated by chronocoulometry using the Anson equation (Eq. (2)). A potential step of 0.3 V was applied on the electrode within 1 mM $K_3[Fe(CN)_6]$ as an electroactive probe considering a diffusion coefficient of 7.6 × 10^{-6} cm^2 s^{-1} [38]:

$$Q = 2nFACD^{½}t^{½}\pi^{-½} + Q_{dl} + Q_{ads} \quad (2)$$

wherein n is the number of electrons transferred, A is the effective surface area of the electrode, C is the reactant concentration, D is the standard diffusion coefficient, and Q_{dl} and Q_{ads} are the double-layer charges and the Faradaic charge, respectively. By plotting Q *vs.* $t^{1/2}$ as shown in Fig. 6A, the electroactive area of the electrode was calculated to be 0.02, and 0.06 cm^2 for bare GCE and pβ–CD/GCE, respectively, confirming that the pβ–CD considerably increased the surface area of the electrode, as evidenced by FESEM images. Since the number of electrons involved in the oxidation of SUM is 1.0 and A = 0.06 cm^2, C = 3.96 μM and the slope is 12.717 μC $s^{-1/2}$ (according to Fig. 6B), the D value was estimated to be 2.42 × 10^{-6} cm^2 s^{-1}. The surface coverage Γ can be assessed from the equation:

$$Q_{ads} = nFA\Gamma \quad (3)$$

By assuming that Q_{dl} is not changed, Q_{ads} can be calculated by the difference of the intercepts of the plot of Q *vs.* $t^{1/2}$ in the presence and absence of SUM. Hence, Q_{ads} and Γ were calculated to be 61.926 μC and 1.07 × 10^{-8} mol s^{-1}, respectively. In addition, the standard heterogeneous rate constant (k_s) for irreversible oxidation of SUM on pβ–CD/GCE was estimated based on Eq. (4) [39]:

$$k_s = 2.415\exp\left(\frac{-0.02F}{RT}\right)D^{½}\left(E_p - E_{p/2}\right)^{-½}\nu^{½} \quad (4)$$

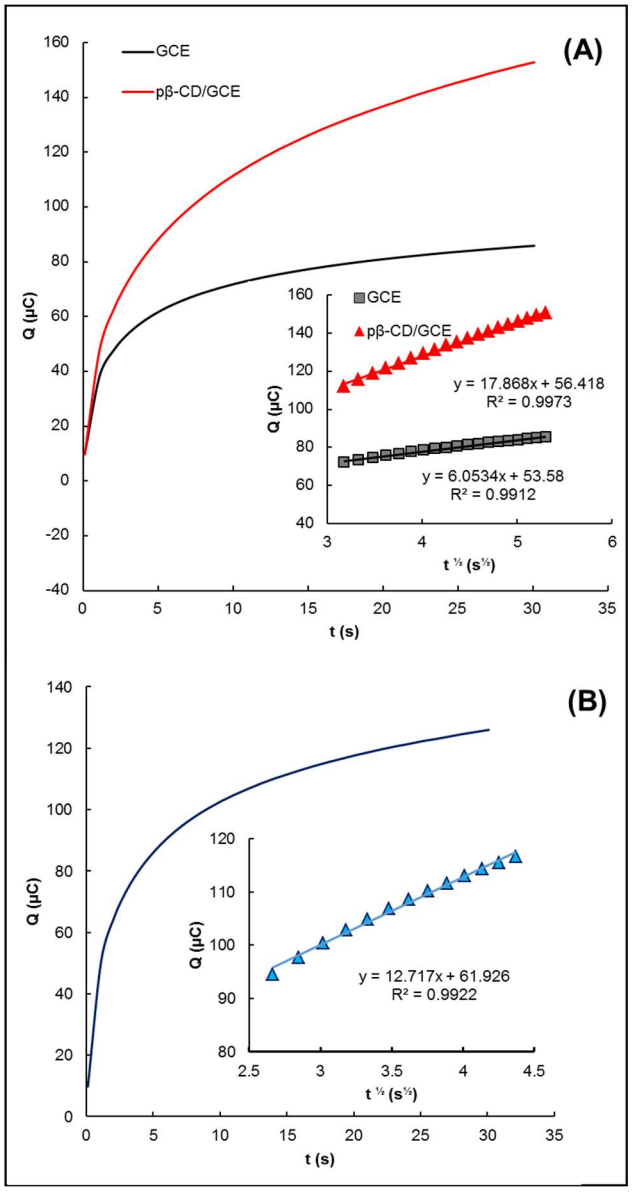


Fig. 6. (A) Plot of Q–t curves for the bare GCE (a), pβ– CD/GCE (b) in 1 mM $K_3[Fe(CN)_6]$; Inserts: plot of Q–$t^{1/2}$ curves for the bare GCE (a), pβ–CD/GCE (b), (B) Plot of Q–t curve for pβ–CD/GCE in 0.1 M pH 7 PBS containing 3.96 µM SUM after background subtraction. Insert: plot of Q–$^{1/2}$ curve for pβ–CD/GCE.

In which E_p and $E_{p/2}$ indicate the peak potential and the potential at which $I = I_{p/2}$ in LSV, respectively. Herein, for $E_p - E_{p/2} = 36$ mV, $v = 100$ mV s^{-1}, $D = 2.42 \times 10^{-6}$ cm^2 s^{-1} and $T = 298$ K, the k_s value was estimated to be 1.23×10^{-3} cm s^{-1}, demonstrating a relatively fast electrooxidation of SUM on the modified electrode.

Calibration Curve and Detection Limit

The electrochemical sensing performance of the pβ–CD/GCE towards SUM was examined by differential pulse voltammetry (DPV) at the optimized criteria. Figure 7 shows the DPVs for increasing concentrations of SUM. The plot of peak current *vs.* SUM concentration comprised two linear parts with slopes of 1.4256 and 0.0519 µA $µM^{-1}$ in the concentration ranges 0.062-2.47 µM and 2.47-52.1 µM, respectively. The saturation of the electrode surface with SUM molecules decreases the sensitivity of the second linear segment in the calibration graph. The limit of detection (LOD) was computed using the relation 3S/m, where S represents the standard deviation of blank (n = 3), and m represents the slope of the calibration curve for SUM, which was found to be 27 nM. Table 1 presents comparisons of the results obtained from the proposed method and previously reported works. The attained analytical features including the detection limit and linear dynamic range are comparable to and even better than previously developed electrochemical sensors. In addition, the cost-effectiveness and simple modification process of GCE with pβ–CD make it an interesting feature of our developed sensor.

Interference Studies, Reproducibility, Stability, and Regeneration-reuse of the Electrode

Considering the attained optimized conditions, the influence of some possible interference species in the determination of SUM *via* pβ–CD/GCE was evaluated in 500-fold including glucose, sucrose, NaCl, KCl, $CaCl_2$, and redox-active compounds including 100-fold epinephrine and ascorbic acid, 20-fold L-tryptophan, L-tyrosine, and acetaminophen. These substances represented almost negligible impact on the current responses of SUM (the variation in current signal was below 5.0%). The structures of the electroactive compounds along with the Ep values for GCE or its modified forms (see Tables S1 in supplementary data) clarify the selectivity of the sensor. Compounds including Epinephrine, Ascorbic acid, and L-tyrosine have oxidation potentials at lower potential values on GCE, so the lack of their interference comes from this interesting fact. For oxidation of L-Tryptophan and Acetaminophen on bare

Table 1. Comparison of Analytical Features of Reported Electrochemical Sensors for Determination of SUM

Electrode	Linear range	Detection limit (μM)	Ref.
ZnO/NiO/Fe_3O_4/MWCNTs/GCE	6.00 nM-380.00 μM	0.002	[11]
Ni-Co layered double hydroxide/SPE	0.01-435.0 μM	0.002	[12]
MXene/MWCNT/chitosan/GCE	0.0033-61 μM	0.00042	[13]
Molecular imprinted polymer/sol-gel/polyoxometalate/rGO/PGE	0.02-3 μM	0.004	[14]
Fe_3O_4@ZIF-8 nanoparticles/SPE	0.1-700.0 μM	0.03	[15]
Pt/ZrO_2 nanoparticles/CPE	55 μM-10 nM	0.003	[16]
Poly (p-aminophenol)/GCE	1.0-100.0 μM	0.294	[17]
pβ–CD/GCE	0.062-2.47 μM 2.47-52.1 μM	0.027	This work

GCE, potential values near SUM oxidation at pβ–CD/GCE are observed but our studies showed neglectable interference from these compounds on pβ–CD/GCE. This observation may be a result of changes in oxidation potentials of L-Tryptophan and Acetaminophen at pβ–CD/GCE. Therefore, we assume that the selectivity of this sensor is related to both the host-guest chemistry of the recognition element as well as the redox potential of compounds on pβ–CD/GCE.

Although the fabrication process of sensors was straightforward, it is still necessary to evaluate the reproducibility of the preparation of electrodes and measurements. It was examined by measuring the current response to six successive mixed samples containing 2.97 μM SUM. Relative standard deviations (RSDs) of 2.39% were obtained, showing good reproducibility of the method. Due to the attachment of the polymeric layer on the GCE surface, it was necessary to check its long-term stability. The stability of the pβ–CD/GCE was tested, in which the modified electrode was stored at room temperature, after three weeks, this modified electrode was used to detect SUM. It was found that the peak current intensities only decreased by about 3.7%, confirming the good stability of the sensor. The RSD values (n = 3) for all these species was less than 6.0%. The inter-day and intraday measurements were investigated too. The inter-day experiments were carried out in the 1, 3, and 5 days, and the intraday experiments are for three separate measurements in a day. The t-test results with a confidence level of 95% (alpha = 0.05) showed that there is no significant difference between the results obtained (P values were 0.79 and 0.93 for 5 and 20 μM of SUM), ensuring the robustness of the developed method (Fig. S2). All the outcomes proved that the sensor benefits from high selectivity and reproducibility, and satisfies the stability criteria. In addition, we investigated the regeneration-reuse of the modified electrode and found that by cycling the electrode voltage in a 0.1 M PBS (pH 6.0) between -2 to +2 V at a scan rate of 100 mV s^{-1} for 5 scans, the pβ–CD/GCE electrode could easily be regenerated and reused for analysis of SUM. For instance, the relative standard deviation for 2.97 μM SUM was below 5%. This property is a good achievement that boosts further the real utility of the sensor.

Analysis of Real Samples

To examine the utility of the proposed sensor for analysis of SUM in real samples, it was measured in urine and blood serum samples using the standard addition method. The recovery values presented in Table 2 are in the range of %97.4-%105, confirming the ability of the sensor to the determination of SUM in real samples. Moreover, via utilizing the two-tailed paired t-test, for $P < 0.05$ the results showed no significant difference between spiked and detectable values. P values of 0.177 and 0.9061 were obtained for urine and blood sample analysis in Table 2. So, this method has good precision, high accuracy, and robustness of the results obtained.

Table 2. Recovery Values for Quantification of SUM in Real Samples *via* pβ–CD/GCE

Urine sample	Added (μM)	Founded (μM)	Recovery (%)
1	0	Not detected	
2	1.99	1.98	99.4
3	3.98	3.93	98.7
4	7.93	7.83	98.8
Human blood serum	Added (μM)	Founded (μM)	Recovery (%)
1	0	Not detected	
2	0.99	1.04	105
3	1.99	1.94	97.4
4	2.99	2.96	99
5	3.98	4.023	101

CONCLUSIONS

To measure trace amounts of SUM in biological fluids, a highly sensitive electrochemical sensor was developed utilizing electropolymerization of β–CD on GCE. Firstly, the electrochemical behavior of SUM was studied on a pβ–CD/GCE and it was characterized by CV, CC, and FESEM techniques. The electrochemical reaction was adsorption controlled and irreversible involving one electron accompanied by a transfer of one proton. The pβ–CD/GCE showed good operational features of sensitivity, stability, low detection limit, and a broad working range. In addition, utilizing electrochemical impedance spectroscopy could further underscore more details of the electrooxidation of SUM on pβ–CD/GCE.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Research Council of Payame Noor University for their financial and technical support.

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