Electrochemical Determination of Chloramphenicol in Milk and Eye-drop Using Easily Activated Screen Printed Carbon Electrodes

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Abstract

Background

An analytical method that has fast response with cheap price and simple sample preparation is an ideal technique that the science need. An electrochemical sensor is an analytical method that suit for the above criteria to determine various samples using different electrode material. Providing detection method for chloramphenicol in farm product and drug sample using cheap electrode material is required due to its wide application in farm.

Result

In this study, direct and cost-effective activated SPCE was made electrochemically to determine chloramphenicol in eye-drop and pasteurized milk. The activation of the SPCE was straightforward and done by cycling 0.5 M KOH using LSV techniques. The employed surface imaging, spectroscopy, and electrochemical analysis show that there is an effective formation of new functional groups during the activation. The activated SPCE gave a superb current response for Chloramphenicol during the CV and SWV study using PBS of pH 6.5 compared to that of the bare one. Utilizing the optimal SWV conditions, a linear calibration curve was obtained using the range of 0.05–100 μM with quite a small detection limit of 20 nM.

Conclusion

In general, the higher sensitivity, stability and remarkable recovery results on the real sample analysis indicate that the activated screen printed carbon electrode can be applied to analyses sample in the field cause it have high stability, excellent reproducibility and long-term stability.

Introduction

Chloramphenicol (CAP) is a powerful antibiotic used specifically to avoid infections in small wounds, in addition to treating many sicknesses such as cystic fibrosis, conjunctivitis, cholera, typhoid fever, plague, and ear and skin infection ¹. However, because of its availability and reasonable price, CAP is administered in animal husbandry and meal merchandise though it is banned inside the European Union and the United States ². Effluent from sewage dealt with or untreated has diverse types of contaminates from pharmaceutical merchandise, cosmetics, and different waste contaminants ³. Antibiotic tablets from now on should be utilized and applied only through considering their health-beneficial outcomes because the chance of producing an antibiotic resistant genes inside the bacterium that lessen the long-term effect of it ⁴. Public cognizance of the utility of CAP in farming and human beings directed the studies for its detection in milk ⁵⁻⁷, honey, ^{8,9} urine, ^{10,11} feed water ¹², and pharmaceutical merchandise ¹³.

A range of analytical strategies had been studied for the determination of CAP such as Solid-Phase Extraction Based on Molecularly Imprinted Polymer ¹⁶, Capillary Electrophoresis ¹⁵, Fluorescence ¹⁶, Ultra-High Pressure Liquid Chromatography-Tandem Mass Spectrometry ¹⁷⁻¹⁸ Liquid Chromatography with tandem mass spectrometry ¹⁹ High-performance liquid chromatography ²⁰ and through Gas Chromatographic ²¹ in pure or in aggregate with different drugs. Analyses of low concentration of drugs like CAP in biological samples including urine and milk products frequently required analytical techniques that have low detection limits and are applicable to small samples. Electrochemical techniques are effective and flexible analytical strategies that provide high sensitivity, accuracy, and precision in addition to a huge linear dynamic range, with distinctly low-cost instrumentation ²². The improvement of screen-printing technology and the serial manufacturing of one time use cheap SPEs for sensing of a huge variety of materials are broadly growing ²³⁻²⁴. The technology used for making screen printed electrodes offers the production of massive numbers of electrodes in a reproducible, low-cost, and disposable format. The opportunity to incorporate chemically functionalized carbon material in the printing process gave a considerable benefit for SPCEs ²¹. In this work, the oxidation–reduction behavior of CAP was investigated on electrochemically activated SPCEs. The incorporation of carbonyl group on the SPCEs during activation was found to make the SPCE behave more or less like an edge plane graphite electrode or similar to that of CNT modified electrodes ²⁵.

Experimental

Chemicals and Materials

Chloramphenicol, ascorbic acid, uric acid, lactose, glucose, glycine, calcium chloride, magnesium chloride, zinc nitrate hexahydrate, sodium nitrate, potassium nitrate, urea, glacial acetic acid, sodium chloride, and potassium hydroxide were purchased from Sigma-Aldrich. Potassium hydrogen phosphate, potassium dihydrogen phosphate, phosphoric acid, and p-nitrophenol were purchased from BDH. For real sample analyses, commercial pharmaceutical eye drop of chloramphenicol formulation (MBL Pharma, Pakistan) was purchased from a local drug store from Addis Ababa, Ethiopia, and pasteurized milk (Shola milk, Lame Dairy (Shola) PLC, Ethiopia) containing 20 cigarettes per pack was bought from a shop in Addis Ababa.

Instrumentation

Cyclic Voltammetry (CV), Linear Sweep Voltammetry (LSV) Square Wave Voltammetry (SWV), and Electrochemical Impedance Spectroscopy (EIS) were performed with a PC-integrated CHI760D electrochemical workstation (CH Instruments, USA) instrument. IRTracer-100 Fourier Transform Infrared Spectrophotometer (SHIMADZU) was used for FTIR spectra recoding and JEOL JSM-7401F Field Emission Scanning Electron Microscope was used for imaging. Zensor screen-printed carbon electrodes (ET077-40 Zensor TE100 SPEs - Pack of 40) were purchased from Taiwan with working area 3 mm

diameter and made of graphitic carbon powder (working and auxiliary electrodes), Ag/AgCl pellet (reference), and counter electrodes.

Activation of SPCE

Fabricated SPCE makes use of an insulating polymer withinside the printing inks for enhancing the adhesion of the graphite with the substrate. These polymers ink block the electrochemically active carbon particles and decrease the electron transferability of the electrode therefore, activation of the SPCE reverses the insulating property of the bare SPCE [26] and in our case, this performed electrochemically through cycling 0.5 M KOH in the potential range of -1.5 to 1.0 V for 10 cycles at 100 mV s⁻¹ scan rates.

Preparation of Milk Sample

Preceding the determination, the milk that is pasteurized was treated as mentioned in [27]. In brief, 5 ml milk sample that is pasteurized had been spiked with varying concentrations of CAP standard solution in 5 mL ethyl acetate. After mixing the sample very well and 10 mins of sonication the aggregate became centrifuged at 4000 rpm for 5 mins and the supernatant was evaporated. Then the dissolution of the residue was done using 10 mL 0.1 M PBS of pH=6.5.

Chloramphenicol Formulation

50 µL of CAP eye drop become pipetted out and poured right into a 50 mL volumetric flask, then filled with the supporting electrolyte (0.1 M PBS pH 6.5) to acquire the very last concentration of 15.5 µM which is in the range of the calibration curve. The solute on was then taken and transferred to 25 mL volumetric flasks and spiked with varying concentrations of CAP standard to get the desired concentration.

Results And Discussion

Surface Characterization

From the IR assessment withinside the activated SPCE, the absorption band has become observed at 3500 cm⁻¹, at 1600 cm⁻¹, and at 1200 cm⁻¹ this is as a result of the formation of hydroxyl and carbonyl containing groups in the route of activation of SPCE Fig. S1 [28, 29]. The SEM imaging for the activated SPCE exhibits a greater cracked surface together with massive defects as compared to the bare SPCE. It is seen that the small ball-like structures have been allocated over the activated SPCE surface with an average length of 56.4 to 86 nm Fig. S2. This confirms that the electrochemical activation significantly affects the surface morphology of SPCE. The Nyquist plot diameter of the semicircle is quite large on the bare SPCE in contrast to the activated one suggesting that the surface of the bare SPCE acts has an insulating property. And the smaller diameter length of the activated SPCE is a piece of evidence for superior electrical conductivity of the activated SPCE Fig. S3.

Electrochemical Property of the Electrodes

Upon scanning from positive to negative potentials, a well-defined irreversible cathodic peak (F1) was observed at -0.59 V. Also, one additional anodic peak (F2) is obtained at -0.069 V during the second scanning a new cathodic peak (S1) at -0.036 V was observed only after the second run. The irreversible cathodic peak at -0.59 V (F1) as a result of the nitro functional group reduction in the CAP to phenylhydroxylamine, which is due to four-electrons and four-protons transfer mechanisms. Whereas the anodic peak (F2) is due to the oxidation of hydroxylamine to the nitroso group derivative and the cathodic peak (S1) is the reverse of the nitroso group derivative to hydroxylamine. This redox process follows a two-electron and two-proton transfer mechanism. The displayed result in the Figure 1 show, no obvious electrochemical response for CAP was observed at the bare SPCE. On the contrary, a well-defined peak was observed for the activated SPCE indicating its high electrocatalytic activity Fig. S4.

The Effect of pH

The result of varying pH for CAP oxidation was investigated in the pH range 4.0 to 8.0, using cyclic voltammetry. It was noted that the current response and the peak potential of CAP changed with increasing pH from 4.0 to 6.5 which implies the oxidation is pH-dependent. As displayed in Figure 2, the oxidation peak current after increasing up to pH 6.5, then gradual decrease was observed till it reaches 8.0 therefore pH 6.5 was selected for further analysis. A decrease in the current peak of CAP with increase in pH after 6.5 revealed that the detection of CAP at the activated SPCE is feasible only in a neutral medium.

A potential peak shift of the CAP to the negative way was observed when the electrolyte pH solution increases suggesting that proton is involved in the redox reaction process. The linear regression equation obtained from the cathodic peak potential vs. pH plot is $E_{pc}(V) = -0.05153pH - 0.25276$; $R^2 = 0.99534$. The slope value from the linear graph 51.53 mV.pH indicates that complete reversible redox reaction of CAP the surface of activated SPCE involving the transfer of equal number of protons and electrons [30, 31].

The Effect of Scan Rate

The impact of changing the scan speed of on the electrochemical property of CAP was investigated using cyclic voltammetry (Figure 3) in PBS pH 6.5 with 50 μ M CAP the reduction due to nitro functional group on CAP was small and not investigated. The plot of the peak current vs. scan rate study in the range 50–275 $mV s^{-1}$ for the oxidation and reduction of CAP and is linear with equations of: $I_{pa} (\mu A) = 0.00285v (mV s^{-1}) + 1.175$; with $R^2 = 0.99342$ and $I_{pc} (\mu A) = -0.004887v (mV s^{-1}) + 1.175$; with $R^2 = 0.99343$. The peak potential shows little shift positively while the scan rate was increased. Moreover, the plot of $\log I_{pc}$ vs $\log v$ is linear with equation: $\log I_{pc} (\mu A) = 0.83171 \log v - 0.84933$, $R^2 = 0.9919$ and gave slope value

of 0.83171 which is near to 1. The obtained results suggest that the oxidation-reduction of CAP at the surface of Activated-SPCE is principally an adsorption controlled process [31].

Optimization of SWV Parameters

The three parameters of square wave were evaluated with the objective of obtaining highest signal for CAP determination. The peak current rely on the values of the parameters step potential, amplitude and frequency and this was studied in by varying 1–15 mV, 10–120 mV and 10–100 Hz range respectively. The study was done varying one parameter by maintaining the other two at constant value. By considering the optimum signal and good square wave voltammetric peak shape for CAP, the optimized parameters were 8 mV step potential; 90 mV amplitude and 30 Hz frequency.

The Effect of Accumulation Potential and Time

The effect of varying the accumulation potential on the determination current of CAP was studied in the range 0.0 to -1.0 V with a difference of 0.1 V at an accumulation time of 15 s. The current response increased when the potential is reduced up to -0.8 V, then after the peak current starts to decrease (Figure 4 (A)). Therefore, the accumulation potential of -0.8 V was chosen as an optimum potential for further measurements. The effect of accumulation time on the reduction peak current of CAP was also investigated by varying from 15–90 s with a difference of 15 s. As illustrated in Figure 4 (B), the reduction peak current increased with increasing accumulation time till it reached 60 s, and then a plateau was observed as a result of surface saturation. Therefore, 60 s was selected as the optimal time for the accumulation of CAP in the activated SPCE.

Interference Study

The influence of various potentially interfering substances in the determination of CAP was studied under the optimum conditions. Oxidation peak currents of CAP were compared with and without the potential interferents: ascorbic acid, lactose, urea, D-glucose, glycine, *p*-nitrophenol, Zn^{2+} , Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Cl^- and NO_3^- and the result shows that the percent changes in the peak currents were less than 5% for the studied substances except for, *p*-nitrophenol. This point out that the activated SPCE exhibited no response to potentially interfering excipients and the *p*-nitrophenol must be separated before analysis.

Determination of NA by Square Wave Voltammetric Techniques

The determination of CAP was performed using the optimized square-wave parameter. Figure 5 shows the SW voltammograms of varying concentration of CAP in 0.1 M PBS of pH 6.5 at the Activated-SPCE. The peak current versus concentration plot for CAP is linear within 0.05 to 100 μM range, Figure 5 (B) with linear equation: $I_p(\mu\text{A}) = -1.68[\text{CAP}](\mu\text{M}) - 3.6$ and $R^2 = 0.99517$. The calculated value for quantification

and detection limit for the blank measurements ($n = 6$) were discovered as $0.067 \mu\text{A}$ and $0.02 \mu\text{A}$, respectively.

As shown in the Table 1 the developed electrode has wider linear range and better sensitivity to all except to graphene oxide hierarchical zinc oxide nanocomposite modified glassy carbon electrode.

Analytical Application

The practical applicability of the developed sensor was demonstrated by analysing CAP-eye drop from commercial pharmaceutical products (Table 2) and pasteurized milk sample (Table 3). The experimentally detected values were compared with the labelled one. And it was found that the results obtained using the proposed sensor is in good agreement with the labelled values. Moreover, the accuracy and reliability of the proposed method was checked by adding CAP standard to the pharmaceutical product and pasteurized milk sample and calculating the percent recovery values. As can be seen from Table 2 and 3, the recovery results lie between 95% and 108%, indicating that the effect of the sample matrix is not significant and hence the activated SPCE has excellent potential to be used in real sample analysis.

Conclusions

In conclusion, the sensitive determination CAP was performed using activated SPCE that was achieved in a simple and fast way electrochemically. The activated SPCE gave outstanding results in an electrochemical determination of CAP. The cost-effectiveness of the electrode material with its simple preparation is another advantage of the electrode in addition to the use of a smaller sample size. The developed electrode also shows excellent analytical characteristics such as high sensitivity, repeatability, reproducibility, and stability. The selectivity of the electrode towards potential interferents is good. The real sample analyses revealed that the electrode can be suitable for the determination of CAP in pharmaceutical products and milk samples.

Declarations

Data Availability

The *Microsoft Word* data used to support the findings of this study are included within the article and supplementary material.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.”

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Tables

Table 1 Comparison of the performance of the proposed method with other electrochemical sensors used for the determination of CAP

Electrodes	Method	Linear Range (μM)	LOD (μM)	Reference
^a Gr/CuPc/GCE	DPV	0.01- 20	0.027	[31]
^b EPC/GCE	SWV	0.01-1 and 1-4	0.0029	[30]
^c GO/ZnO/GCE	DPV	0.2–7.2	0.01	[33]
^d Z-800/rGO/GCE	DPV	1-180	0.25	[34]
^e MoS ₂ -IL/GO/GCE	DPV	0.1–400	0.047	[35]
^f rGO/PdNPs/GCE	DPV	0.05-100	50	[36]
^g Mn ₂ O ₃ TNS/SPCE	DPV	0.015–1.28 and 1.35–566.3	4.26	[37]
Activated SPCE	SWV	0.05-100	0.02	This Work

^aGraphene/Copper Phthalocyanine Nanocompositse, ^bexfoliated porous carbon, ^cgraphene oxide hierarchical zinc oxide nanocomposite, ^dzeolitic imidazolate framework reduced graphene oxide, ^ede-layered molybdenum disulfide/graphene oxide nanocomposites, ^fpalladium nanoparticles decorated reduced graphene oxide, ^gmanganese(III) oxide tiny nanostructures screen printed carbon electrodes.

Table 2 Recovery of CAP spiked in CAP-eye drop sample (n=3)

Added (μM)	Found (μM)	Recovery ($\pm\text{sd}$) (%)
0	15.12	–
10	26.18	104.2

Table 3 Detection of CAP in milk products (n=3)

Added (μM)	Found (μM)	Recovery ($\pm\text{sd}$) (%)
10	9.34	93.6
20	20.46	102.3
50	51.55	103.1

Figures

Figure 1

(A) Cyclic voltammograms of 50 μM CAP at Bare SPCE (black), first cycle (blue), and second cycle at Activated-SPCE in 0.1 M PBS at scan rate of 100 mV s⁻¹ (B) SWVs of 50 μM CAP in the same solution at bare SPCE (a) and Activated-SPCE (b)

Figure 2

(A) CV of 50 μM CAP as a function of pH in activated SPCE in PBS of pH 4.0-8.0 with 100 mV s⁻¹ scan rate. Inset: plot of peak current vs, pH (B) Plot of peak potentials of 50 μM CAP as a function of pH at activated SPCE

activated SPCE in PBS of pH 6.50 with 100 mV s⁻¹ scan rate

Figure 3

(A) CVs of 50 μM of CAP at activated SPCE at scan rates of 50–275 mV s^{-1} in PBS pH 6.50 (B) Peak current of CAP vs scan rate

Figure 4

(A) Current response of 50 μM CAP vs. deposition potential (B) Current response of 50 μM CAP vs. deposition time

Figure 5

(A) SWVs for varying concentrations of CAP: 0.0, 0.05, 0.5, 5.0, 7.5, 15, 30, 45, 65, 80 and 100 μM in 0.1 M PBS pH 6.50 at activated SPCE (B) the plot of peak current vs. CAP concentration

Supplementary Files

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