Scientific paper

Voltammetric Determination of Sulfaclozine Sodium at Sephadex-modified Carbon Paste Electrode

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Abstract

The electrochemical behavior of Sulfaclozine Sodium (SLC) was studied at a bare and sephadex-modified carbon paste electrodes by cyclic voltammetry and square wave voltammetry. The cyclic voltammetry (CV) showed a well-defined irreversible oxidation peak at 0.94 V in Britton- Robinson buffer pH 7.0. The strong affinity of SLC to sephadex allowed accumulation of SLC at the surface of electrode and thus higher electrochemical sensitivity to SLC. The influence of sephadex loading, the pH of the solution and the scan rate on the peak current was studied. A linear calibration curve covering the concentration range from 0.005 to 1 mM was obtained using SWV. The method was successfully applied for the determination of SLC in the veterinary pharmaceutical formulations with satisfactory accuracy and precision.

Keywords: Sulfaclozine Sodium; square wave voltammetry; sephadex; carbon paste electrode.

1. Introduction

Coccidiosis is a parasitic disease that attacks the intestinal tract of poultry caused by protozoan parasites of the genus Eimeria. This disease is of worldwide occurrence and costs the poultry industry many millions of dollars every year to control.¹ Although live vaccines were introduced, prophylactic chemotherapy is still preferred for coccidiosis control in most countries. The last half of the twentieth century marked improvements in the performance of commercially reared poultry. These improvements would not be possible without the introduction of a succession of ever more effective anticoccidial agents to control coccidiosis.²

Sulfaclozine, N^1 – (6-chloropyrazinyl) sulfanilamide (Figure 1) is a sulfonamide antibacterial that has been used

H₂N O N N

Figure 1. Chemical structure of Sulfaclozine

in veterinary medicine.³ it is effective in the treatment of clinical coccidiosis as well as prevention of the disease.⁴

The analytical methods which have been reported for the determination of sulfaclozine include chromatographic methods with different detectors.⁵⁻⁹ and capillary electrophoresis.¹⁰ These methods are either time-consuming or use expensive instrumentation.

In contrast to the reported techniques, the voltammetric techniques are simple and rapid with high sensitivity and selectivity for drug analysis. Moreover, the carbon paste electrodes which are used for voltammetric measurements have several advantages for the electrochemical investigation of organic compounds. They are cheap, easy to prepare and use; and offer surface regeneration and modification, low background current, a large potential domain, no memory effects and adsorption-extraction capabilities. 11,12 Furthermore, including surfactants in the experimental protocol and modification of the carbon paste (chemically or by nanomaterials) have been reported to influence the electrochemical process occurring at the surface of the electrode. 13,14 Sephadex is a cross-linked dextran which is used as a stationary phase in gel filtration chromatography. 15 Carbon paste electrodes modified with sephadex have been used for the sensitive determination of nifuroxazide and glibenclaide. Indeed, Numerous pharmaceutical compounds were analyzed using carbon paste electrodes.

The present work reports for the first time a SWV method for the determination of SLC in veterinary formulation. The proposed method utilizes the electrochemical oxidation of SLC at a carbon paste electrode modified with sephadex in a micellar medium. The effect of sephadex on the oxidation peak current was investigated. The method was validated According to the ICH guidelines.¹⁸

2. Experimental

2. 1. Reagents and Materials

Sulfaclozine sodium was obtained from Yangzhou Tianhe Pharmaceutical Co., LTD, China with potency 99.75%. Clozicocc® W.S.P (Each 100 g contains 32 g sulfaclozine sodium) was obtained from Pharco Pharmaceuticals, Alex., Egypt. Graphite powder, paraffin oil, Sephadex G-50, C18 silica gel and chitosan were supplied from Sigma-Aldrich. Methanol was purchased from Loba Chemie Co., India. Sodium dodecyl sulfate (SDS), Phosphoric acid and boric acid were supplied from Adwic Co., Egypt. Acetic acid was obtained from Piochem Co., Egypt. Briton Robinson buffer (BR) buffer was prepared by adding equal volumes of phosphoric acid 0.04 M, acetic acid 0.04 M and boric acid 0.04 M, the pH of the buffer was adjusted by NaOH 0.2 M to cover the pH range from 2.0 to 10.0. SDS 10.0 mM was prepared by dissolving an appropriate amount of SDS in water. Double- distilled water was used throughout the study and referred to by "water".

2. 1. 4. Standard Solution

Sulfaclozine stock solution (10.0 mM) was prepared by dissolving 30.66 mg of SLC in 1.0 mL methanol, then diluting with water to 10 mL.

2. 2. Apparatus

Bio-logic SP 150 electrochemical work station with a three-electrode configured stand (model C-3) was used for the voltammetric measurements. The working electrode was a bare carbon paste electrode or a sephadex-modified carbon paste electrode (SMCPE); the reference electrode was Ag/AgCl/3 M KCl (BAS, USA) and the counter electrode was a platinum wire (BAS, USA).

2. 3. Procedures

2. 3. 1. Preparation of Modified Carbon- Paste Electrode

Sephadex-modified carbon paste (SMCPE) electrode was made by hand mixing of 0.4 g Sephadex and 0.8 g of

graphite powder with 0.4 mL paraffin oil. Plain (unmodified) carbon paste was made by mixing 1.0 g graphite powder with 0.6 mL paraffin oil. The paste was packed into the electrode body and smoothed on a filter paper till a shiny appearance of the electrode surface was obtained.

2. 3. 2. Analytical Procedure

The CV at the carbon paste was repeated between 0 and 1.4 V several times in the buffer solution (pH 7.0) till the CV becomes stable. Then the electrode was transferred into another cell containing BR buffer solution (pH 7.0), 0.005 mM to 1.0 mM SLC and 0.03 mM SDS. The solution was stirred for 30 s at an open circuit potential, afterwards, the CV was recorded between +0.4 and +1.4, at 100 mVs $^{-1}$ scan rate.

2. 3. 3. Calibration Curve of SLC

The SWV was performed to determine SLC in bulk powder and pharmaceutical formulations. Different aliquots were accurately transferred from the stock standard solution to an electrochemical cell containing 10 mL buffer (pH 7.0) and 0.03 mM SDS. The SWV was recorded at SMCPE. The peak current was plotted against drug concentration of SLC in (μ M).

2. 3. 4. Application to Veterinary Pharmaceutical Formulation

An accurately weighed 0.96 g Clozicocc® W.S.P. containing 306.7 mg of sulfaclozine sodium was transferred into a 100-mL volumetric flask and dissolved in 10 mL methanol. The solution was sonicated for 15 min, then, the flask was completed to the mark with water to obtain 10.0 mM SLC (solution I). Further dilution was carried out from solution 1 into 10-mL volumetric flask to obtain 1.0 mM SLC (solution II).

The accuracy and precision of the method was studied using 0.005, 0.47 and 0.65 mM of the sample solution, each solution was prepared in triplicate. The accuracy and precision solutions were prepared by transferring 50 μL from solution 1; 500 μL and 700 μL from solution II, each into an electrochemical cell containing BR pH 7 and 0.03 mM SDS. The concentration of the sample was determined by the standard addition method using the SWV.

3. Results and Discussion

3. 1. Sulfaclozine Electrochemical Oxidation Behavior

The electrochemical behavior of SLC was studied at the carbon paste by recording the CV from 0 to 1.4 V in BR pH 7. The CV (Figure 2) shows one anodic peak current

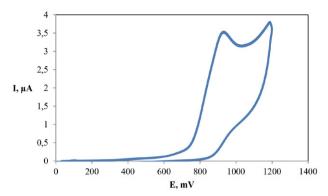


Figure 2. The results are the average of five separate determinations Cyclic voltammogram of 0.1 mM SLC in BR buffer of pH 7.0 at a bare carbon paste lectrode.

 $((Ip) = 3.5 \,\mu\text{A})$ at 0.94 V) with no cathodic peak in the reverse scan, it means that the oxidation process of SLC is irreversible. The anodic peak could be due to the oxidation of the amine group in SLC.¹⁹

3. 2. Optimization of the Experimental Conditions

3. 2. 1. Effect of pH

The electrochemical oxidation of organic compounds depends, in most cases, on the pH of the solution. Herein, the effect of changing the pH of the solution on the oxidation of SLC was studied in BR buffer over the pH range from 2.0–10.0. It was observed that the peak potential of SLC is shifted towards less positive values when the pH was increased. The relationship between the $E_{\rm p}$ and pH at the sephadex-modified carbon paste electrode was found to be linear and controlled by the equation $E_{\rm p} = -51 {\rm pH} + 1284~({\rm R}^2 = 0.995)$ (Figure 3a). The slope (~ 51 mV per pH) is close to the expected 59 mV per pH indicating that equal number of protons and electrons involved in the oxidation process of SLC. The highest oxidation current was obtained at pH 7 (Figure 3b), therefore, all measurements were carried out at pH 7.0.

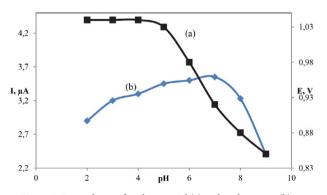


Figure 3. Dependence of peak potential (a) and peak current (b) on The pH of 0.1 mM SLC. The scan rate is 100 mV s⁻¹

3. 2. 2. Effect of Sephadex

Different materials including C_{18} modified silica, chitosan and sephadex were tested for possible enhancement of the oxidation current and, hence, increasing sensitivity of the electrode. (Figure 4) shows no difference in the electrochemical behavior of sulfaclozine when 30% C_{18} modified silica was added to the carbon paste electrode, while the addition of 30% chitosan to the paste make a little improvement in the current response. In contrast, carbon paste modified with 30% (w/w) sephadex exhibited a considerable oxidation current that indicating the high affinity of the drug to sephadex. This affinity has been utilized for preconcentration of the drug onto the electrode surface to increase the sensitivity to SLC. The effect of sephadex loading on the peak current is shown in Figure 5.

3. 2. 3. Effect of Sodium Dodecyl Sulfate

Sulfaclozine oxidation behavior in a micellar medium was also studied using SDS.

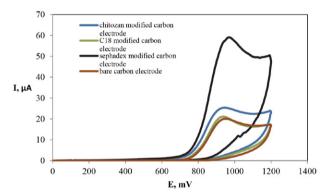


Figure 4. Cyclic voltammograms of $1.0~\mathrm{mM}$ SLC in PH $7.0~\mathrm{using}$ different modified and unmodified electrodes.

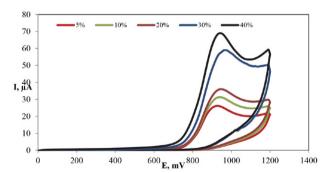


Figure 5. Cyclic voltammograms of SLC (1.0 mM) in PH 7.0 at carbon paste electrode containing different amounts of sephadexL; the scan rate is 100 mV s^{-1}

SDS is a hydrophobic ionic surfactant, which can be adsorbed onto the electrode surface. As a result, the electrochemical process such as the mass and electron transfer energy at the electrode/solution interface are affected.²⁰ It

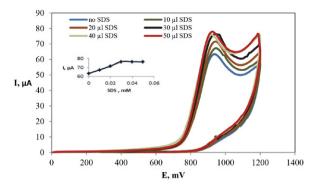


Figure 6. Cyclic voltammograms recorded in 1.0 mM SLC containing different concentrations of SDS, the measurements were carried out using 40% sephadex carbon paste electrode in BR buffer pH 7.0.

has been reported that SDS can remove the oily binder (insulator) and hence lower the uncompensated resistance at the electrode/solution interface. ^21, 22, 23 Herein, the effect of SDS was studied by the addition of different volume of 0.01 M SDS (10–50 μL) to the SLC solution of pH 7 and recording the CV. Figure 6 shows the relationship between the anodic current and the SDS concentrations. It was observed that the peak current increases with increasing SDS in the measuring solution, and the highest oxidation current was observed when the SLC solution contains 30 μL of 0.01 M SDS; no further improvement in the peak current was observed above this concentration.

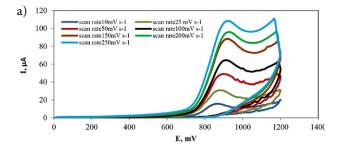
3. 2. 4. Effect of Scan Rate

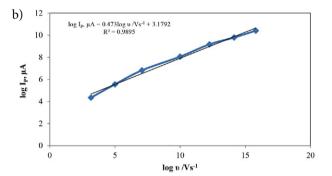
The effect of the scan rate (v) on the peak potential $(E_{\rm p})$ and the peak current $(i_{\rm p})$ was studied between 10 mVs⁻¹and 250 mVs⁻¹ in 1.0 mM sulfaclozine solution in BR buffer (pH 7.0) containing 0.03 mM SDS (Figure 7a), The relationship between the oxidation peak current of SLC and the square root of scan rate (v^{1/2}) was found to be linear, indicating that electrochemical oxidation of SLC is a diffusion controlled process.²⁴

Plotting the logarithm of the peak current against the logarithm of the scan rate resulted in a straight line with a slope of 0.47 (Figure 7b), this value is close to the theoretical value of 0.5 for a purely diffusion-controlled process. 24 It was also found that the E_p (oxidation peak potential) was dependent on scan rate, the peak potential was shifted to more positive values when the scan rate increased, which confirms that the oxidation process is irreversible. Furthermore, the relationship between the peak potential and the logarithm of the scan rate was found to be linear (Figure 7C) in accordance with Laviron's equation (1). 25

$$E_p = E^{0'} + (\frac{2.303RT}{\alpha nF}) log (\frac{RTk0}{\alpha nF}) + (\frac{2.303RT}{\alpha nF}) log v$$
 (1)

Here α is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, ν is the scan





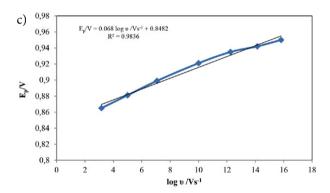


Figure 7. (a) The CV of 1.0 mM SLC containing 0.03 mM SDS in BR buffer of pH 7.0 at 40% SMCPE at different scan rates, (b) Dependence of the logarithm of peak current Ip/ μ A on logarithm of scan rate (ν /Vs⁻¹). (c) Relationship between peak potential E_p /V and logarithm of scan rate log (ν /Vs⁻¹).

rate, and E_0 is the formal redox potential, n is the number of electrons transferred. So, the value of αn can be obtained from the slope of Ep vs log ν . The slope was found to be 0.068, when T=298K and R=8.314 JK $^{-1}$ mol $^{-1}$ and F=96485 C/mol, αn was found to be 0.85. According to Bard and Faulkner. 26 α can be calculated from the following equation (2).

$$\alpha = \frac{47.7}{\text{Ep-Ep/2}} \,\text{mV} \tag{2}$$

 k^0 value can be calculated from the intercept of the above plot if the value of E^{0} is known.

 E^0 ' in Eq. (1) can be obtained from the intercept of Ep versus υ curve by extrapolating to the vertical axis at $\upsilon = 0^{27}$. All values of αn , α , n, E^0 ' and k^0 are summarized in table 1.

Table 1. The calculated values of αn , α , n, E^{0} and k^0 for the electro-oxidation of SLC by cyclic voltammetry (CV) at SMCPE.

Parameters	SMCPE	
αn	0.8476	
α	0.611	
n	1.38	
E ⁰ '	0.85	
k^0	3.1439	

3. 2. 5. Square Wave Voltammetry (SWV)

Under optimal experimental conditions, the calibration curve was constructed using the SWV over the concentration range from 0.005 to 1 mM. The parameters of SWV are 50 mV pulse height, 200 ms pulse width, 10 ms step height and 100 ms step time. The solution was stirred for 30 s at 400 rpm at an open circuit potential followed by 30 s quiescent time before any measurements.

3. 3. Calibration, Detection Limit and Reproducibility

A linear relationship between SLC anodic peak current of and its concentration was found in the concentra-

Table 2. Performance data of the proposed SWV method for determination of SLC

Parameters	SLC		
Linearity range (mM)	0.005mM to 1.0 mM		
Slope (μA. mM ⁻¹)	50.67		
Intercept (μA)	5.86		
Correlation coefficient (r)	0.9995		
LOD (µM)	1.04		
LOQ(µM)	2.99		
Accuracy (mean ± S.D.)	100.18 ± 0.01		
Precision (RSD %)			
Interday	2.08		
Intraday	2.12		

tion range from 0.005 mM to 1.0 mM (R = 0.999) with a slop of 50.67 μ AmM $^{-1}$ and a limit of detection 0.001 mM. The reproducibility (%RSD, n = 3) of the peak current for 0.005 mM sulfaclozine was 2.08% as shown in Table 2.

3. 4. Determination of Sulfaclozine in Veterinary Formulation

SLC was determined in Clozicocc® W.S.P. using standard addition method; the obtained results were sta-

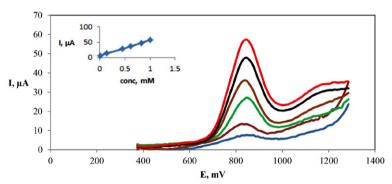


Figure 8. SWV of SLC over the concentration range from 0.005 to 1.0 mM in BR pH 7.0 containing 0.03 mM SDS using 40% SMCPE.

 $\textbf{Table 3.} \ \ determination \ \ of SLC \ in \ pharmaceutical \ dosage \ form \ and \ statistical \ comparison \ of \ the \ proposed \ voltammetric \ and \ the \ published \ HPLC \ method \ ^5$

Pharmaceutical formulation	Standard addition technique				Reference method ⁵
	Taken(mg)	Added (mg)	Found(mg)	%Recovery ^a	metnod
al .	0.015	0.015	0.0149	99.96	
Clozicocc w.s.p	0.120	1.45	1.43	98.87	
batch No.564	0.120	2.00	2.01	100.31	
Mean				99.59	99.33
S.D				1.01	1.51
n			5		
variance				1.02	2.28
Student's <i>t</i> -test (2.132) ^b				0.64	-
F-test (6.39)b				2.24	-

^a The results are the average of five separate determinations ^b the tabulated t and F values, respectively, at P = 0.05

tistically compared with those obtained by a reference method.⁵ The calculated t- and F-values are found to be less than the theoretical ones, confirming that accuracy and precision of the two methods are comparable at 95% confidence level (Table 3).

4. Conclusion

Herein, we report for the first time a novel simple and rapid SWV method for SLC determination in veterinary formulations. The method is based on a carbon paste electrode modified with sephadex. The sephadex modified carbon paste electrode showed a dramatic increase in the oxidation peak current over the plain carbon paste. The SWV method was linear over a wide concentration range of SLC from 0.005 mM to 1.0 mM with a detection limit of 1 μM . The method was applied successfully for the determination of SLC in the veterinary formulation with satisfactory accuracy and precision. The student's t-test and F-ratio test showed no significant difference regarding the accuracy and precision between the present method and the reported method.

List of abbreviations

SLC: Sulfaclozine Sodium SWV: Square wave voltammetry SDS: Sodium dodecyl sulfate BR: Briton Robinson buffer

SMCPE: Sephadex-modified carbon paste electrode

CV: Cyclic Voltammetry W.S.P: Water soluble powder

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Povzetek

Elektrokemijsko obnašanje natrijevega sulfaklozina (SLC) je bilo proučevano na golih in sephadex-modificiranih elektrodah iz ogljikove paste s ciklično voltametrijo in kvadratno valovno voltametrijo. Ciklična voltametrija (CV) je pokazala dobro definiran nepovratni vrh oksidacije pri 0.94 V v Britton-Robinson pufru pri pH 7.0. Močna afiniteta SLC do sefadeksa je omogočila kopičenje SLC na površini elektrode in s tem večjo elektrokemično občutljivost za SLC. Proučen je bil vpliv nalaganja sefadeksa, pH raztopine in hitrost skeniranja na največji tok. Z uporabo SWV smo dobili linearno kalibracijsko krivuljo, ki pokriva območje koncentracije od 0.005 do 1 mM. Metodo smo uspešno uporabili za določanje SLC v veterinarskih farmacevtskih formulacijah z zadovoljivo točnostjo in natančnostjo.



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