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VOLTAMMETRIC DETERMINATION OF STREPTOZOTOCIN IN PHARMACEUTICAL AND HUMAN URINE SAMPLES USING GLASSY CARBON ELECTRODE MODIFIED WITH POLYANILINE BASED NANOSENSORS

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ABSTRACT

The electrochemical behavior of streptozotocin (STPZ) drug was studied using glassy carbon electrode modified with polyaniline based nanosensors in phosphate buffer solutions using cyclic and differential pulse voltammetric techniques. The reduction process was shown to be irreversible over the pH range (2.0–10.0) and the well defined reduction peaks are obtained at -0.53 V based on diffusion controlled. Effects of cathodic peak potential, current (I_{pa}), scan rate, pH, etc have been discussed. A possible electro reduction mechanism was proposed. An analytical method was developed for the determination of streptozotocin in phosphate buffer solution at pH 4.0 as a supporting electrolyte. The cathodic peak current varied linearly with streptozotocin concentration in the range 3.0×10^{-6} M to 5.0×10^{-3} M with a limit of detection (LOD) of 1.25×10^{-10} M and limit of quantification (LOQ) of 2.35×10^{-8} M. The proposed method was successfully applied to the determination of streptozotocin in pharmaceutical and human urine samples.

Key Words:- streptozotocin, polyaniline, glassy carbon electrode, differential pulse voltammetry and cyclic voltammetry.

INTRODUCTION

Diabetic cardiomyopathy is a distinct entity independent of coronary artery disease (Spector KS, 1998; Poornima IG *et al*., 2006) despite earlier beliefs common in the diabetic population (Bell DS, 2003). Experimental models of diabetes mellitus, such as streptozotocin (STPZ)-induced type 1 diabetes mellitus, imitate the structural and cellular abnormalities of diabetic cardiomyopathy (Tschope C *et al*., 2004). These abnormalities include, among others, cardiac fibrosis (Asbun J *et al.*, 2006) and cardiac inflammation, which lead to left ventricular (LV) dysfunction, mediated mainly by the diabetic milieu (e.g. high glucose levels, oxidative stress, angiotensin II) (Tschope C *et al*., 2004). Diabetes mellitus has adverse effects on male sexual and reproductive functions in diabetic patients and animals (Baker HW, 1998; Meyer K, 2000; Sexton WF *et al*., 1997). There are many hypotheses to explain diabetic nephrotoxicity, hyperglycemia itself also leads to excess free-radical generation and induces oxidative stress (Allen DA *et al*., 2005). Excessive oxidative stress may result in oxidative damage to proteins, lipids and DNA, and subsequently leads to apoptosis (Brownlee M, 2007). Oxidative stressinduced apoptosis is described in the pathophysiology of streptozotocin (STZ)-induced diabetic nephropathy models (Tesch GH *et al.*, 2006). Some reports have provided important concepts regarding the role of p53 in cisplatin-induced nephrotoxicity (Jiang M, 2008).

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On the other hand Polyaniline (PAN) is a key material in the family of conductive polymers and numerous studies have addressed its electrochemical conductivity and photoelectrochemical functions (Huang MJ et al., 1986; Negi YS, 2007; Kinyanjui JM *et al*.,2007) Numerous applications of PAN were reported, including the fabrication of electronic devices the use of the polymer for electrochemical triggering of mechanical movements of micro-objects and the use of the polymer as an active matrix for sensing (Zaidi NA *et al*., 2004; Bhattacharya A *et al*,. 1996) PAN itself reveals redox functionality only in acidic media, pH<3 (Ohsaka T *et al*, 1984; Diaz AF *et al*, 1980) a feature that limits its use.

In the present work focused on an electro chemical analysis of STPZ in human urine samples with polyaniline modified glassy carbon electrode. It was chosen to get the reduction mechanism of carbonyl group by employing electrochemical techniques such as cyclic voltammetry, differential pulse voltammetry. Therefore, a rapid and sensitive voltammetric method has been applied for the determination of STPZ in pharmaceutical and human urine samples.

EXPERIMENTAL

Apparatus

Electrochemical studies were carried out by Autolab PG STAT101 supplied by Metrohm Autolab B.V., The Netherlands. A three electrode system comprising of a glassy carbon electrode as a working electrode. Glassy carbon electrode (GCE-3mm) obtained from Metrohm India Ltd Chennai. Saturated Ag/AgCl/KCl as a reference electrode and Pt wire as a counter electrode. An Elico LI-120 pH meter supplied by Elico Ltd, Hyderabad, India was used to determine the pH of the buffer solution.

Chemical and reagents

A stock solution of STPZ (1.0×10^{-3} M) was prepared by dissolving of STPZ in methanol and it was further with same solvent diluted up to 100 ml to give appropriate concentrations. The solution was stable for 2 weeks in a refrigerator at about 4°C. The working solutions of the drug were prepared daily by diluting the stock solution with a selected supporting electrolyte. The supporting electrolyte was usually phosphate buffer containing Na_2HPO_4 and NaH_2PO_4. Double distilled water was used throughout the work. All other solvents and reagents used throughout this study were of analytical grade.

Analytical procedure

After 10 ml of phosphate buffer (PBS) of pH 4.0 was placed in the electrochemical cell, to this certain volume of standard solution (3.0×10^{-6} M) of STPZ was added and de-aerated with pure nitrogen for 10min. Then the voltammograms were recorded using cyclic voltammetry and differential pulse voltammetry over the potential range -0.1 V to 1.0 V vs Ag/AgCl/KCl. All measurements were carried out at room temperature.

Assay of tablets

The tablets of STPZ were obtained from local commercial sources. Ten tablets were weighed accurately and ground to a fine powder. A portion of the powder equivalent to 1 mM STPZ was transferred to a 100 ml volumetric flask and completed to volume with distilled water and sonicated for 15 min to affect complete dissolution. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte. The content of the drug in tablet was determined referring to the calibration graph using differential pulse voltammetry.

Glassy carbon electrode modification with polyaniline (PAN/GCE)

Electrode preparation thoroughly polished GCE surfaces using alumina slurry on a soft cloth were sonicated in first ethanol and then doubly distilled water for 3 min each to remove possible contaminants. The PAN coatings were formed on the GCE surfaces by dipping the polished GCEs and electrochemically deposited at a constant potential of 0.80 V for 120 s in an aqueous solution of 0.1 M $LiClO_4$ and 0.1 M carbonate containing 0.15 M polyaniline as well as in a 0.25M H_2SO_4 electrolyte containing 7.3 mM aniline monomers via a CV process from −0.2 to 0.9 V at a scan rate of 50 mV/s for cycles under a nitrogen environment. After the polymerization of PAN, the fabricated PAN/GCEs were dipped into doubly distilled water for 3 min to remove unpolymerized aniline monomers remaining in the PAN coatings if any. After each polishing, the electrode was sonicated in ethanol and doubly distilled water for 5 min, successively, in order to remove any adsorbed substances on the electrode surface. Finally, it was dried under nitrogen atmosphere ready for use. The electrode was then transferred into 0.1 M $HClO_4$ solution for 12 h aging. The polyaniline modified electrode was denoted as PAN/GCE.

RESULTS AND DISCUSSIONS

Cyclic voltammetric study

The cyclic voltammograms of STPZ in 0.04 M phosphate buffer of pH 4.0 in concentration 3.0×10^{-6} M and scan rate $50mVs^{-1}$ (Fig 2). Reduction peak appears at -

0.65 V, which may be due to reduction of carbonyl group of the drug and no oxidation peak is observed in the anodic branch which suggests that the process is irreversible. The cycle 'a' shows the peak at blank, cycle 'b' shows the peak at GCE and cycle 'c' shows the peak at PAN/GCE of STPZ at PAN/GCE electrode. It shows that the peak current increases in the second cycles and this behaviour gives an indication of an adsorption character. A plot of logarithm of peak current versus logarithm of the scan rate gave a straight line relation with a slope of 0.55 which is close to the theoretically 0.72 for an ideal relation of surface species. The peak potential shifted to more negative values with increasing scan rate.

Effect of scan rate

The influence of the scan rate was investigated. The results suggested that differential pulse voltammetric peak current reached maximum value when the scan rate was $50mVs^{-1}$. As for the scan rate; the current response with increasing the scan rate from $0mVs^{-1}$ to 60 mVs^{-1} it gives the maximum response at $50mVs^{-1}$(Fig 3). Accordingly, the optimum conditions for recording a maximum developed and sharper peak for STPZ are t_{acc}: 60 sec., E_{acc}: -0.65, scan rate: 50 mVs^{-1} and pulse amplitude: 50 mV, optimum temperature: 25^{o}C.

Effect of pH

The effect of pH, on 3.0×10^{-6} M STPZ and adjusting the pH between 2.0-10.0 with phosphate buffer was studied. The results showed in Fig. 4, it show that the peak potential varies linearly with pH between 2.0 to 10.0, with for a reduction process in which the same number of protons as that of electrons is involved. That process, responsible for this behaviour, must be the reduction of the carbonyl group in the STPZ molecule and the maximum peak was observed at pH 4.0 on PAN/GCE.

Differential pulse voltammetric studies

An accurate volume of the clear supernatant solution was transferred into the electrochemical cell containing 10 mL of phosphate buffer of pH 4.0 to yield a final concentration of approximately 3.0×10^{-6} M of STPZ. The DPV was recorded under the optimum experimental conditions at PAN/GCE electrode. A typical differential pulse voltammogram recorded in various concentration ranges from 3.0×10^{-6} M to 5.0×10^{-3} M at PAN/GCE. Fig.5 depicts the differential pulse voltammograms of STPZ in different concentrations.

Calibration graph, limit of detection and limit of quantitation

Calibration curves for standard drug solution under the optimized parameters were obtained. A linear relationship was observed between 3.0×10^{-6} M to 5.0×10^{-3} M of STPZ on PAN/GCE. Fig. 6 represents the differential pulse voltammograms recorded using the standard addition method. The linear regression equation was I (μA) = $9.61+1.43\times10^{-7}$C mol/L with a correlation coefficient of 0.9943, the limit of detection (LOD = 3(sd)/b) and limit of quantitation (LOQ = 10(sd)/b), were calculated, where sd is the standard deviation of the intercept and b is the slope of the calibration graph. LOD and LOQ were found to be 1.25×10^{-10} M and $2.35\text{x}10^{-8}$ of STPZ on PAN/GCE, respectively. The analytical parameters for the calibration graph are summarized in Table 1.

Fig. 1 Structure of Streptozotocin (STPZ)

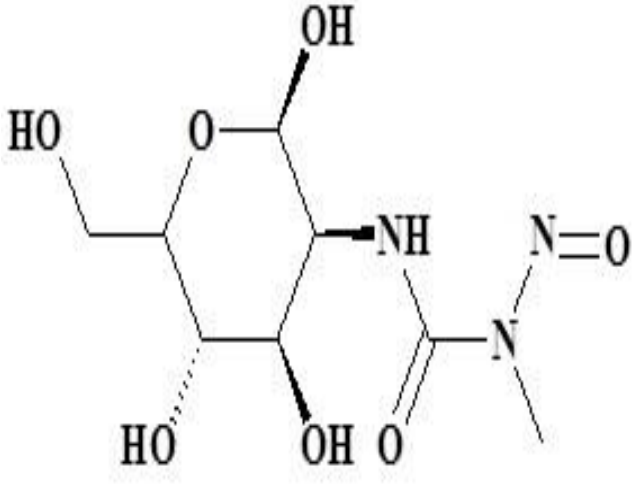

Fig 2. Typical cyclic voltammograms of 3.0×10^{-6} M STPZ at (a) without STPZ solution (Blank), (b) GCE and (c) PAN/GCE in 0.04 M phosphate buffer of pH 4.0 and scan rate of 50 mVs^{-1}.

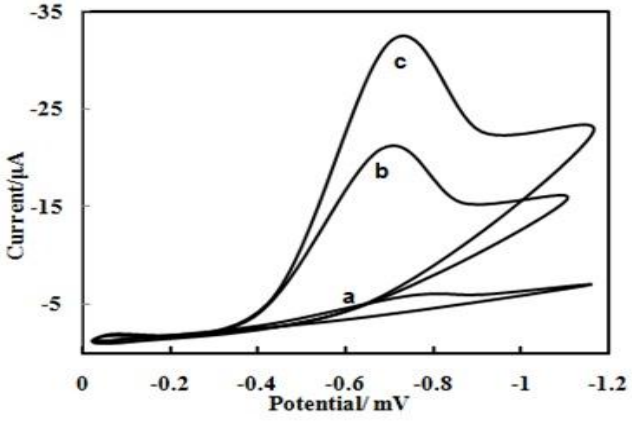

Fig 3. Effect of scan rate on STPZ at pH 4.0 in PB solution at concentration 3.0×10^{-6} M at different scan rates: 0 mV/s to 50 mV/s on PAN/GCE

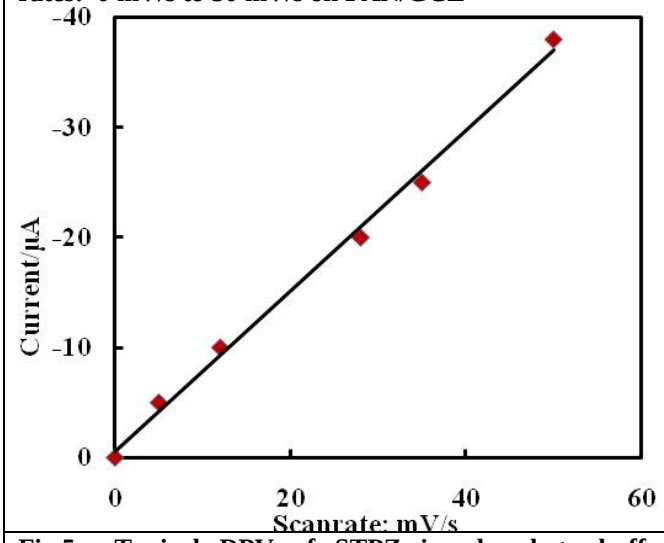

Fig 4. The plot the of current vs pH of STPZ in phosphate buffer solution, concentration: 3.0×10^{-6} M at PAN/GCE in different pH values

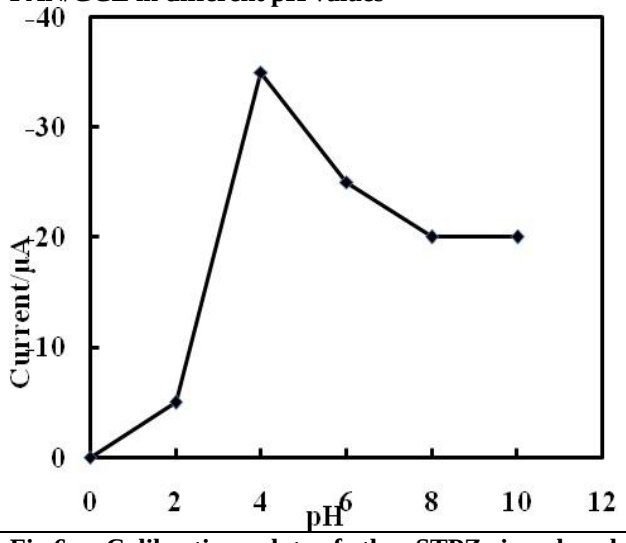

Fig 5. Typical DPV of STPZ in phosphate buffer solution of pH 4.0, concentration: 3.0×10^{-6} M to 5.0×10^{-3} M at PAN/GCE

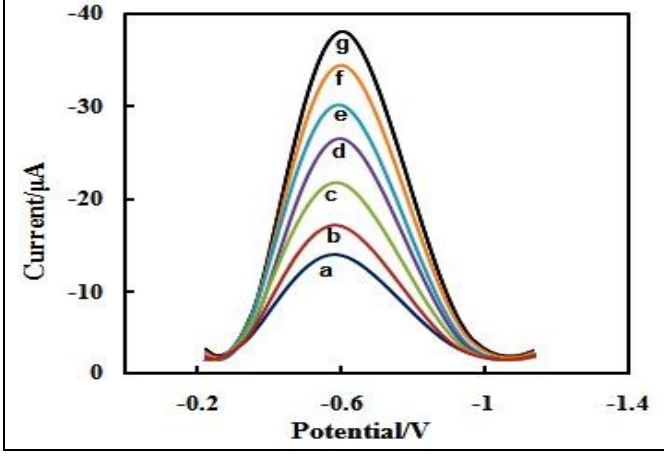

Fig 6. Calibration plot of the STPZ in phosphate buffer solution of pH 4.0, at different concentrations (10 µM-100 µM) at at PAN/GCE

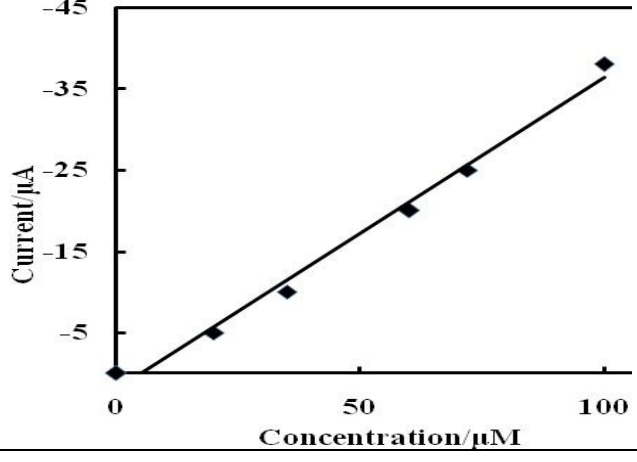

Table 1. Recovery study of the STPZ

Pharmaceutical samples			
Added(M)	**Found(M)**	***Recovery (%)**	**RSD**
$3.0x10^{-6}$	$2.98x10^{-6}$	99.33	1.69
$1.5x10^{-5}$	$1.48x10^{-5}$	98.66	1.48
$2.5x10^{-4}$	$2.48x10^{-4}$	99.20	1.62
$5.0x10^{-3}$	$4.9x10^{-3}$	98.00	1.35
Urine samples			
Sample	**Spiked(M)**	***Recovery (%)**	**RSD**
$3.0x10^{-6}$	$2.93x10^{-6}$	97.66	1.28
$1.5x10^{-5}$	$1.44x10^{-5}$	96.00	0.92
$2.5x10^{-4}$	$2.40x10^{-4}$	96.00	0.94
$5.0x10^{-3}$	$4.85x10^{-3}$	97.00	1.21

CONCLUSION

In this paper, the electrochemical behaviour of STPZ on PAN/GCE electrodes has been investigated by cyclic and differential pulse voltammetric techniques. The proposed procedure showed clear advantages, such as no pre-treatment or time consuming extractions steps were required prior to the analysis, ease of preparation and easy renewable of the electrode surface. The proposed method is less expensive than alternative techniques like HPLC and hence can be applied to the routine determination of the drug in quality control laboratories.

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