ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 22, No. 1 (2021) 172 – 179

http://doi.org/10.21743/pjaec/2021.06.18

Electrochemical Determination of Furosemide Drug at Tranexamic Acid Gold Nanoparticles Modified Glassy Carbon Electrode

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Received 07 October 2020, Revised 30 April 2021, Accepted 02 May 2021

Abstract

The reported work discussed the simpler and sensitive strategy for the electrochemical determination of furosemide by employed tranexamic acid derived gold nanoparticles modified glassy carbon electrode (GCE). The synthesis of tranexamic acid derived gold nanoparticles (Tr-AuNps) was carried out using single step approach. The synthesized Tr-AuNps were characterized by using atomic force microscopy (AFM), illustrated that the particles are spherical in shape with an average size of 35 nm. The synthesized AuNps have modified the sensing surface of GCE. The modified GCE demonstrated highly catalytic behavior for the oxidation of loop diuretic drug furosemide. The influence of pH and supporting electrolyte was examined and the working conditions were optimized. The amperometric determination of furosemide was also carried out at the Tr-AuNps modified GCE under stirred conditions using Britton Robinson buffer (BR buffer) as supporting electrolyte at pH 5. The linear calibration plot showed the dependence of the peak current on increasing concentrations of furosemide in the range of 50 µM to 500 µM furosemide with the detection limit of 5 µM. The proposed sensing plan has been successfully employed for the quantification of furosemide in human urine samples with satisfactory recoveries.

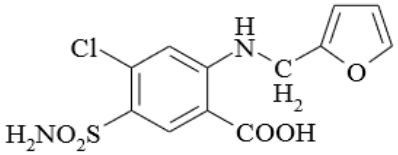
Keywords: Furosemide, Tranexamic acid gold nanoparticles, Amperometric, Glassy Carbon Electrode.

Introduction

Furosemide is commonly known as frusemide, which is a derivative of anthranilic acid inclusion to the class of compounds designated as high-ceiling diuretics [1]. Furosemide (4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid, is primarily labelled antibacterial agent as sulfonamide [2]. Owing to its fast and powerful diuretic results, this drug has prolonged usages as a prevailing acidic diuretic in veterinary medicine and humans [3]. Its prime action is also classified as a loop diuretic, which prevents the vigorous reabsorption of chloride in the diluting segment of the loop of Henle [4-6]. This drug was primarily used for the control of hypertension and later, it has found uses in the cure of edema related to nephrotic syndrome, heart failure, cirrhosis of renal and liver disease [7- 10]. The International Olympic Committee of Medical Commission in 1986 has banned all diuretics as well as furosemide in sports. In this regard, health experts in most

countries have already ruled out the constituent for the manufacture of drugs for bodyweight reduction. This compound caused weight loss by increasing the flow of urine. It is also used in the production of illegal drugs to lose weight in women and caused health issues in some cases in China [7].

Furosemide is a white or slightly yellow powder. It is frequently solvable in organic solvents i.e. methanol and acetone as well as in alkaline aqueous solutions. It is sparingly solvable in aqueous acidic solutions [11]. Its half-life in the blood plasma is about 1-2 h and bioavailability ranges from 60 to 70 % [12]. The structural formula of the drug is given below:



Furosemide

Large numbers of analytical procedures have been published for the endurance of furosemide in biological fluids and pharmaceutical products. These including spectrofluorimetry [13-18], titrimetry [19], spectrophotometry, [20-25], Liquid chromatography (LC) [20, 26, 27], simultaneous determination of diuretics by HPLC-EC [28], micellar electrokinetic chromatographic methods [29], HPLC methods [12,15, 30,31], capillary electrophoresis [32], variable-angle scanning fluorescence spectrometry, [33], potentiometry, GC-MS [34], voltammetry [35], glassy carbon electrode [36], carbon fiber microelectrodes [37], capillary electrophoresis [38], flow-injection, gold electrode, hanging mercury drop electrode [39], graphite electrode [1] and multi-walled carbon nanotubes-paraffin oil paste electrode [10]. Most of the above described methods involve the extraction, preparation of the sample and the use of toxic solvents. They are also time consuming or require expensive equipment. Thus, the development of an efficient and effective method for the quantification of furosemide in biological fluids and pharmaceutical preparation is a substantial imposition. Nowadays, electrochemical techniques have led to advances in the analysis due to their relatively short analysis time, sensitivity and low cost. The proposed electrochemical method has advantage over the available developed methods, owing to its low detection limit and experimental simplicity, relatively inexpensive, fast response, ultra-high sensitivity, selectivity and relatively and remarkable detectability.

In the presented work, the determination of furosemide has been done by using a glassy carbon electrode (GCE) modified with gold nanoparticles and nafion, and the suitability has been investigated.

Correct sequence of references from 34-40

Materials and Methods

Chemicals and Reagents

Analytical grade potassium chloride, acetic acid, hydrochloric acid, sodium hydrogen phosphate and disodium hydrogen phosphate reagents of E. Merck, Germany were used in the study. The standard for furosemide drug was obtained from Natural Pharma Brazil. 1 mM stock solution of furosemide was prepared in HPLC grade methanol and stored under refrigeration (4 ^{o}C) in the dark. Further diluted solutions of (10^{-4} to 10^{-6} mM) were prepared from a stock solution. BR buffer (mixture of boric acid, phosphoric acid and acetic acid), acetate buffer and chloride buffer solutions were used as supporting electrolyte. In order to obtain the appropriate pH value, buffer solutions

were adjusted by adding the necessary amounts of HCl or KOH.

Instrumentation

Electrochemical measurements were performed on Trace Analyzer (VA 797 of Metrohm version 1.1 Switzerland) with a personal computer together with a conservative three-electrode cell. Three-electrode scheme consisted of an Ag/AgCl (3M KCl) as reference electrode, a platinum wire as an auxiliary electrode and self-made gold nanoparticles and nafion modified GCE was used as working electrode. Analytical grade balance (Switzerland) was used for weighing the solid materials. The pH studies were carried out using a 781 pH/ ion meter of Metrohm with an internal reference electrode and glass electrode. For the transfer of analyte solutions, micro-pipette (Eppendorf Multipette plus) was used. The deionized water, purified with a Milli-Q Plus system (Millipore) was used throughout the study. An ultrason unique (ultrasonic model) was used for dissolution of all other reagents and furosemide. AFM studies were conducted by using an Agilent 5500, atomic force microscope, USA. This instrument is beneficial for imaging of dried deposits of AuNps or other species and is also capable of providing a 3-D image of the analytical species.

Synthesis of Tranexamic Acid Derived AuNps

The synthesis of tranexamic acid derived AuNps was carried out according to the reported work [43]. 5 mL of deionized water was added to 200 µL of 0.2 M NaOH with the subsequent addition of 150 µL of 0.5% $HAuCl_4$ and 120 µL of 0.5% tranexamic acid solutions. The resultant solution was heated at 150^{o}C under constant shaking at 200 rpm till the color of the solution turned red wine, indicating the formation AuNps.

Sample Preparation for AFM

To investigate the morphological features of the synthesized Tr-AuNps, 100 µL of the solution of Tr-AuNps was placed over mica slide and evaporated to a thin film. The film was subjected to AFM imaging.

Results and Discussion

AFM Characterization of Tr-AuNps

Various techniques are used for the characterization / morphological studies of nanoparticles. These techniques are helpful to examine the accumulation and dispersion of nanoparticles. For this purpose, three dissimilar scanning modes are available, including intermittent sample contact mode, non-contact mode, and contact mode for their size, structure, sorption and shape. The morphological features of Tr-AuNps were studied using AFM. It can be clearly seen (Fig. 1) that the synthesized AuNps are spherical in shape and possess an average diameter of around 35± nm.

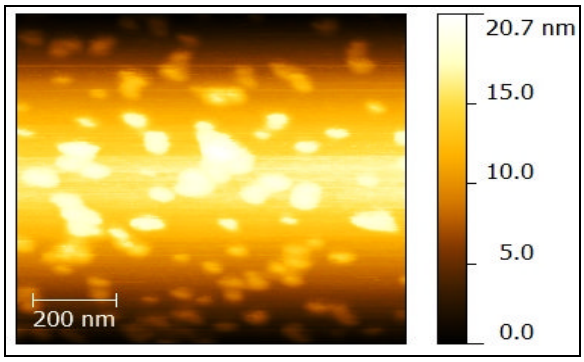


Figure 1. **AFM image of the Tr-AuNps**

Electrochemical Oxidation of Furosemide at Bare and Modified GCE

Furosemide electrochemical behavior was examined at bare and Tr- AuNps modified GCE at pH 5 in BR buffer (0.04 M). The oxidation behavior of furosemide showed no cathodic peak in the reverse scan, while

one anodic peak in the positive scan was observed.

The comparative voltammograms are shown in Fig. 2. It is evident that as the concentration of furosemide increases, there is an increase in peak current value in the case of Tr-AuNps modified GCE in comparison to bare GCE. This clearly indicates the electrocatalytic nature of Tr-AuNps for furosemide.

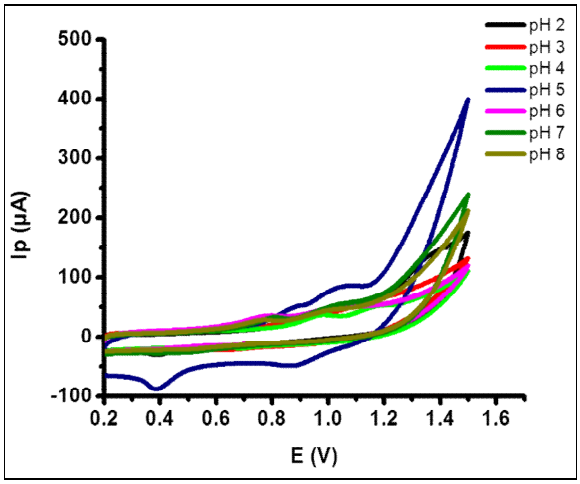


Figure 2. **Cyclic voltammograms recorded in 0.04 M BR buffer pH 4 with the absence (a) bare GCE, (b) Tr-AuNps modified GCE , and in the presence of 0.125 mM furosemide (c) bare GCE, (d) Tr-AuNps modified GCE with scan rate 0.05 Vs^{-1}**

Influence of pH

The pH effect was also studied for the voltammetric determination of furosemide using BR buffer (0.04 M) as a supporting electrolyte. The influence of pH was monitored in the range of pH 2 to 8. The influence of pH on peak current and peak shape is shown in Fig. 3. It could be observed that an increase in pH leads to the abrupt shift of peak potential and peak current, as well as. This may be due to the solubility of analytes. The highest peak current was observed at pH 5 due to better current response, pH 5 was selected for subsequent studies and considered as optimized pH.

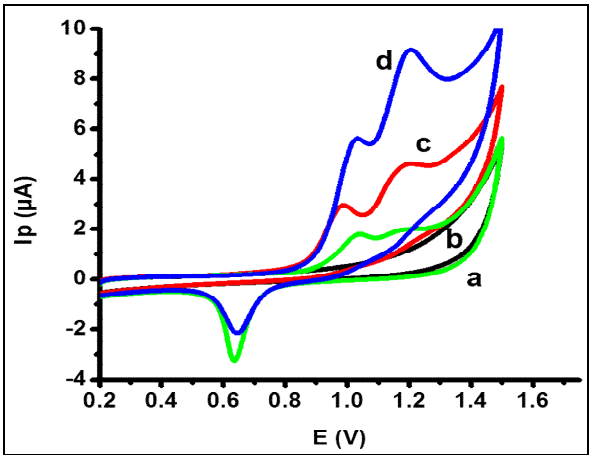


Figure 3. **Cyclic voltammograms of furosemide (0.125 mM) with Tr-AuNps modified GCE electrode in BR buffer (0.04 M) with 2 to pH 8 used as supporting electrolyte.**

Influence of Supporting Electrolyte

To study the influence of supporting on the current sensitivity marked medium of surrounding, three different kinds of buffer, such as acetate buffer (0.1 M), chloride buffer (0.1 M) and BR buffer (0.04 M) were used as supporting electrolytes. In acetate buffer system (pH-5) showed no anodic peak. Despite acetate buffer, chloride and BR buffer gave good responses, as shown in Fig. 4. It was noticed that the highest sensitivity response was observed at BR- buffer and was selected for further studies.

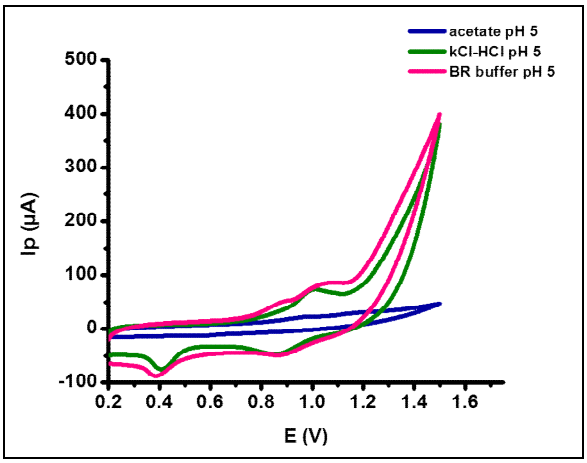


Figure 4. **Cyclic voltammograms recorded with 0.125 mM furosemide in different buffers i.e., BR buffer, acetate and KCl-HCl buffer each at pH 5.**

Amperometric Determination of Furosemide at Tr-AuNps Modified GCE

The amperometric response of the Tr-AuNps modified GCE was observed for the oxidation of furosemide in 0.04 M pH 5 BR buffer under stirring at 0.8 V. Fig. 5 shows that i-t curve for the stepwise addition of furosemide in BR buffer, each addition of 0.1 mL leading to an increment of 50 µM of furosemide concentration. A plot of peak current versus concentration is illustrated in the inset of Fig. 4 that follows a linear relationship in the range of 50 furosemide to 450 µM. The detection limit was found to 5 µM.

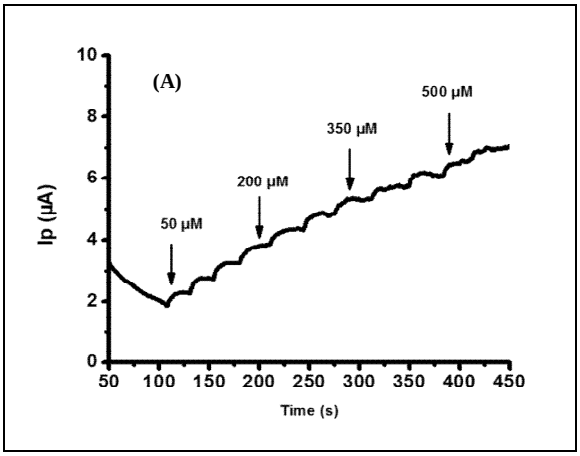


Figure 5. **(A) Amperometric current- time response curves for the successive addition of furosemide solution in stirred BR buffer 0.04 M pH 5 under applied potential of 0.8 V (B) the corresponding calibration plot for the dependence of peak current on furosemide concentration.**

Comparative study

The present study was compared with the reported methods in the literature was shown in the Table 1.

Table 1. **Electroanalytical procedures for determination of furosemide in the literature.**

Detection	Media	LOD (mol dm^{-3})	Ref
Amperometric detection at a GCE (+1.3 V vs. Ag/AgCl) coupled to HPLC	Water-acetonitrile (30:70)	4.5×10^{-8}	[28]
Amperometric detection at carbon fiber microelectrodes (+1.25 V vs. Ag/AgCl) coupled to HPLC and FIA	Acetonitrile-water (25:75), 5 mmol L-1 NaH2PO4 (HPLC), 5 mmol L-1 NaH2PO4 (FIA)	5.5×10^{-7}	[40]
Voltammetric detection at GCE (+1.2 V vs. Ag/AgCl)	Methanol-water (10:90)	1.5×10^{-7}	[28]
Graphite polyurethane composite electrode (+ 1.0 V vs. SCE)	1.0 mmol L^{-1} NaOH	2.8×10^{-6}	[41]
Multi-walled carbon nanotubes-paraffin Oil paste electrode	Methanol - water (10:90)	2.9×10^{-7}	[42]
Electrochemical oxidation at gold electrode	Methanol-Water (10:90)	4.12×10^{-8}	[43]
Voltammetric determination at GCE modified with gold nano particles	Buffer System (RB- Buffer & Chloride Buffer	5×10^{-6}	Present Work

Interference Study

The effect of multiple ions on the peak current of furosemide oxidation was also examined. Several interfering species such as citric acid, ascorbic acid, glucose, Na^{+}, Pb^{+}, Cl^{-}, and drugs such as paracetamol, diflunisal, piroxicam were added in equimolar ratios with

that of emide and change in signal for oxidation of furosemide was observed. Peak current value was considered as 100 % for the oxidation of furosemide in the absence of interfering ions and then change in peak current was observed after the addition of interfering species into the solution. % interference was calculated to observe the effect of various interfering species on the peak current of furosemide using the amperometric technique (Table 2). The results show that there was no appreciable interference observed and thus verified the validity of the proposed method for the analysis of furosemide in real samples. % interference was found below 5% for each interfering species.

Table 2. **Influence of interfering species for the amperometric determination of furosemide.**

Interferents	Interference (%)
Glucose	+1.5
Citric acid	+0.5
Uric acid	+2
Piroxicam	+2.2
Cephalothin	+0.98
Paracetamol	+0.15

Analysis of Furosemide in Real Samples

The applicability of the proposed method was checked in human urine samples. Before analysis, the urine samples were diluted 10 times with BR buffer. The standard furosemide solution for spiking of diluted samples was used to calculate % recovery values. Each sample was analyzed three times and the average was calculated and presented in Table 3. The values of % recovery ranges from 99.4% to 100.7% that indicates the applicability of the method to human urine samples.

Table 3. **Determination of Furosemide in human urine samples using recovery test (n=3).**

Samples	Detected (µM)	Spiked (µM)	Found (µM)	(%) Recovery	(%) RSD
Urine 1	150	100	248.5 ± 0.08	99.4	1.5
Urine 2	250	100	350.8 ± 0.02	100.2	1.25
Urine 3	350	100	453.2 ± 0.04	100.7	2.5

Conclusion

The current study proposed a fast and simple analytical process for the quantification of furosemide using Tr-AuNps. In this research work, GCE was modified with Tr-AuNps. AFM technique was used to find the shape and size of nanoparticles. The adopted strategy favors the catalytic oxidation of furosemide at the Tr-AuNps and minimizing the need for time consuming methods for the analysis of furosemide. The detection limit of 5 µM for furosemide estimation was observed in modified GEC. This method can also be used for the quantification of furosemide in human urine samples as an alternate means to check the toxicity of furosemide in the patients taking this drug.

Conflict of Interest

The authors declare that there is no conflict of interest.

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