

*Full Paper*

## **Application of Graphite Screen Printed Electrode Modified with Indium(III) Mixed-ligand Nanocomplex for Voltammetric Determination of Amlodipine**

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**Abstract-** The current work focuses on the development of a sensitive and selective electrochemical device based on a graphite screen printed electrode modified with indium(III) mixed-ligand nanocomplex (In(III) nanocomplex/SPE) for the analysis of amlodipine. The studies proved the sensor to have excellent electron-mediating behavior in the oxidation of amlodipine in a 0.1 M phosphate buffer solution (PBS) (pH 7.0), and the response of the sensor was found significantly depend on the charge and structure of the analyte. The detection limit of the method for amlodipine was 0.2  $\mu\text{M}$  (S/N=3) and the response was found to be linear in the concentration range of 1.0 to 500.0  $\mu\text{M}$ . The modified electrode was use for the detection of amlodipine in real samples and found to produce satisfactory results.

**Keywords-** Amlodipine, Indium(III) mixed-ligand nanocomplex, Graphite screen printed electrode, Voltammetry

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### **1. INTRODUCTION**

Hypertension is the most prevalent disease in adult population. Most of this hypertensive population requires treatment with antihypertensive agent [1]. Amlodipine, (3-ethyl 5-methyl-2-[2-aminoethoxy-methoxy]-4-(o-chlorophenyl)-1,4-dihydro-6-methyl-3,5-

pyridinedicarboxylate monobenzene sulfonate, AMP), a dihydropyridine derivative with calcium antagonist activity. It is used effectively for the treatment of hypertension [2]. It may be used alone or in combination with other active compounds as example, valsartan and hydrochlorothiazide [3]. Therefore, the development of a simple and selective analytical method for quantification of amlodipine in pharmaceutical preparations is very important to reach a high-quality drug regime for human health [4,5]. Several different analytical methods were reported in the literature for the determination of amlodipine alone or in combination with other drugs in pharmaceutical formulations and biological fluids, including spectrophotometric and high performance liquid chromatography [6], reversed phase high performance liquid chromatography [7], high performance thin layer chromatography [8], gas chromatography [9], spectrophotometry [10], liquid chromatography with tandem mass spectrometry [11], capillary electrophoresis [12] and electrochemical methods [13]. These methods require advanced technical expertise and time consuming and are expensive and often need the pretreatment step [14]. But electrochemical techniques as alternative methods have also received much interest due to their higher selectivity, faster and simple operation, lower cost, quick response, and therefore, have become of considerable importance for determination of amlodipine [15-17].

Screen-printing technology, which has been adopted for microelectronics, is significantly used to fabricate electrodes for disposable electrochemical sensors [18]. Screen printed electrodes (SPEs) are highly-versatile, easy to use, cost-effective analytical tools, also suitable to miniaturization and applied widely in the electroanalytical chemistry field [19,20].

Nowadays, nanotechnology has occupied a unique position in both science and technology sharing knowledge: tools, techniques, and information with electrochemistry and electroanalysis. In order to improve their electrochemical performance, electrodes have modified with nanosized materials [21-39]. Nanostructures modified electrodes have good electro catalytic activity, sensitivity, and selectivity; there have also a low detection limit compared to unmodified electrodes [40-54]. Especially, inorganic materials with organic frames have potential applications in catalysis, molecular adsorption, electro conduction, magnetics, magnetic, energy transfer and optical properties. Among many inorganic materials indium(III) complexes containing N and O donor ligands are important due to their application in catalysts, electronics and optics and their possession of antibacterial properties [55-58].

According to the previous points, it is important to create suitable conditions for the analysis of amlodipine in biological fluids. In this study, we describe application of novel In(III) nanocomplex as a nanostructure sensor for voltammetric determination of amlodipine. The proposed sensor showed good electrocatalytic effect on amlodipine. Eventually, we evaluate the analytical performance of the suggestion sensor for amlodipine determination in real samples.

## 2. EXPERIMENTAL

### 2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode.

All solutions were freshly prepared with double distilled water. Amlodipine and all other reagents were of analytical grade and were obtained from Merck chemical company (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

### 2.2. Synthesis of nano-sized [In(Me-phen)Cl<sub>3</sub>(DMSO)]

To prepare the nano-structure of [In(Me-phen)Cl<sub>3</sub>(DMSO)], using sonochemical method, an ultrasonic bath with 0.1 M salt to ligand solutions and the power of 100 W/45 min was used. The obtained precipitate was filtered and washed with double distilled water, then dried.

Yield, 75.9%. IR (cm<sup>-1</sup>): 3072 w, 2922 w, 1625 m, 1522 m, 1431 s, 1225 w, 1114 m, 1027 m, 989 s, 953 s, 430 m. Anal. Calcd. C, 36.50; H, 3.24; N, 5.67. Found: C, 36.21; H, 3.21; N, 5.60%.

### 2.3. Preparation of modified electrode

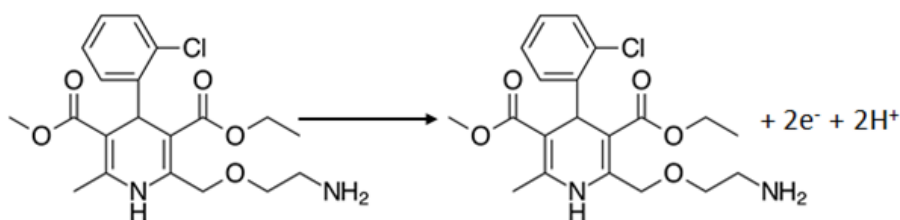
The bare screen printed electrode was coated with In(III) nanocomplex as follows. A stock solution of In(III) nanocomplex in 1 mL aqueous solution was prepared by dispersing 1 mg In(III) nanocomplex with ultrasonication for 1 h, and a 2  $\mu$ l aliquot of the In(III) nanocomplex/H<sub>2</sub>O suspension solution was casted on the carbon working electrodes, and waiting until the solvent evaporation in room temperature.

## 3. RESULTS AND DISCUSSION

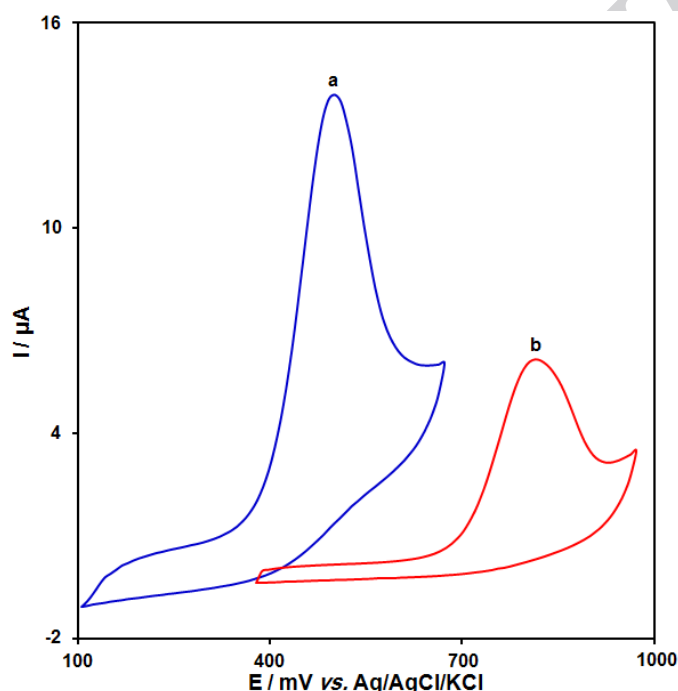
### 3.1. Electrocatalytic oxidation of amlodipine at a In(III) nanocomplex/SPE

The electrochemical behavior of amlodipine is dependent on the pH value of the aqueous solution (Scheme 1). Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of amlodipine. Thus the electrochemical behavior of amlodipine was studied in 0.1 M PBS in different pH values (2.0 < pH < 9.0) at the surface of In(III) nanocomplex/SPE by CV. It was found that the electrocatalytic oxidation of

amlodipine at the surface of In(III) nanocomplex/SPE was more favored under neutral conditions than in acidic or basic medium. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of amlodipine oxidation at the surface of In(III) nanocomplex/SPE.



**Scheme 1.** A tentative reaction scheme suggested for the oxidation of amlodipine



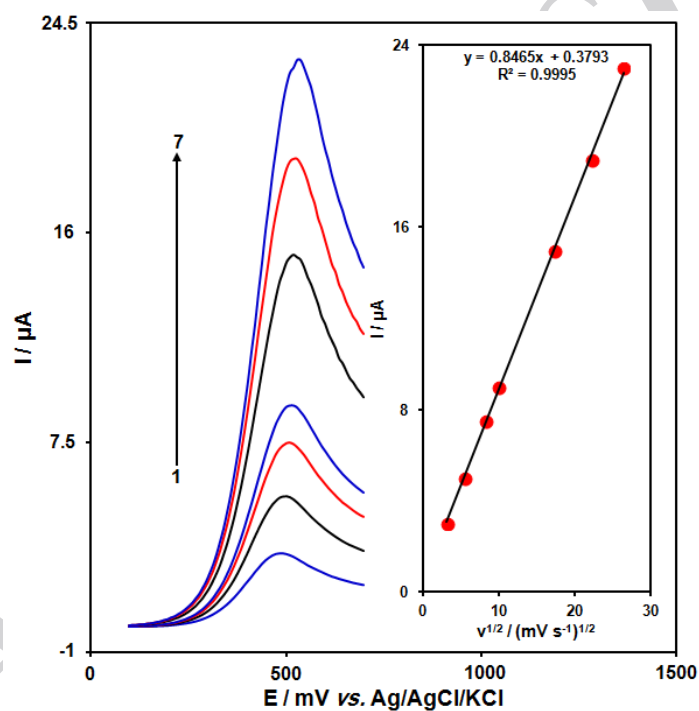
**Fig. 1.** Cyclic voltammograms of (a) In(III) nanocomplex/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 100.0  $\mu\text{M}$  amlodipine at the scan rate  $50 \text{ mVs}^{-1}$

Fig. 1 depict the cyclic voltammetric responses for the electrochemical oxidation of 100.0  $\mu\text{M}$  amlodipine at In(III) nanocomplex/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of amlodipine at In(III) nanocomplex/SPE (curve a) is about 500 mV compared with 810 mV for that on the bare SPE (curve b). Similarly, when the oxidation of amlodipine at the In(III) nanocomplex/SPE (curve a) and bare SPE (curve b) are compared, an extensive enhancement of the anodic peak current at In(III) nanocomplex/SPE relative to the value obtained at the bare SPE (curve b) is observed. In other words, the results

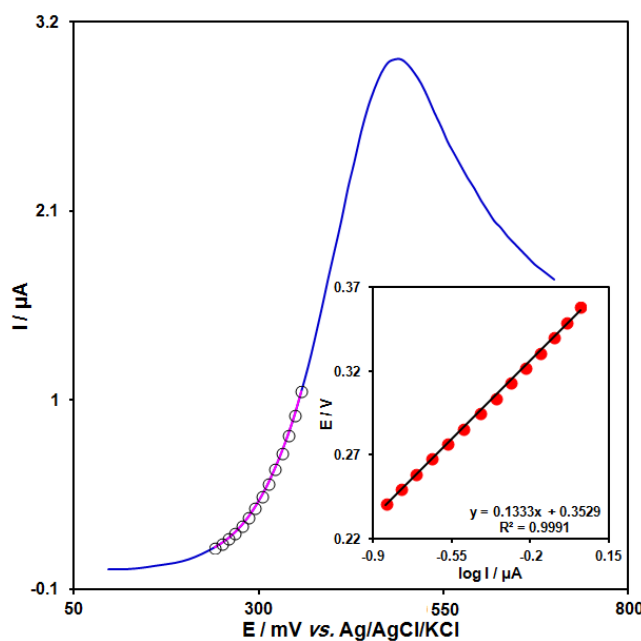
clearly indicate that the addition of In(III) nanocomplex to bare SPE improve the amlodipine oxidation signal.

The effect of potential scan rates on the oxidation current of amlodipine has been studied (Fig. 2). The results showed that increasing in the potential scan rate induced an increase in the peak current. In addition, the oxidation process is diffusion controlled as deduced from the linear dependence of the anodic peak current ( $I_p$ ) on the square root of the potential scan rate ( $v^{1/2}$ ) over a wide range from 10 to 700  $\text{mV s}^{-1}$ .

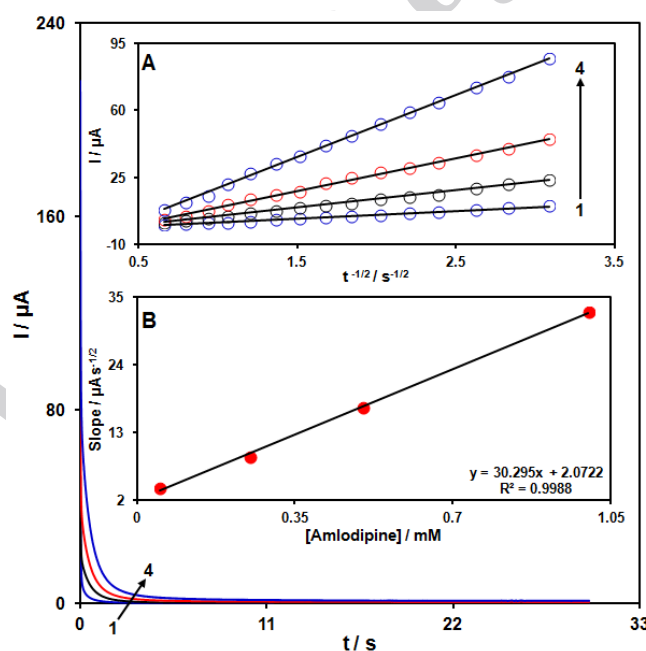
Fig. 3 show a Tafel plot that was drawn from points of the Tafel region of the LSV. The Tafel slope of 0.1333 V obtained in this case agrees well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of  $\alpha=0.56$  [59].



**Fig. 2.** LSV of In(III) nanocomplex/SPE in 0.1 M PBS (pH 7.0) containing 25.0  $\mu\text{M}$  amlodipine at various scan rates; numbers 1-7 correspond to 10, 30, 70, 100, 300, 500 and 700  $\text{mV s}^{-1}$ , respectively. Inset: Variation of anodic peak current vs. square root of scan rate



**Fig. 3.** LSV (at  $10 \text{ mV s}^{-1}$ ) of electrode in 0.1 M PBS (pH 7.0) containing  $25.0 \mu\text{M}$  amlodipine. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.



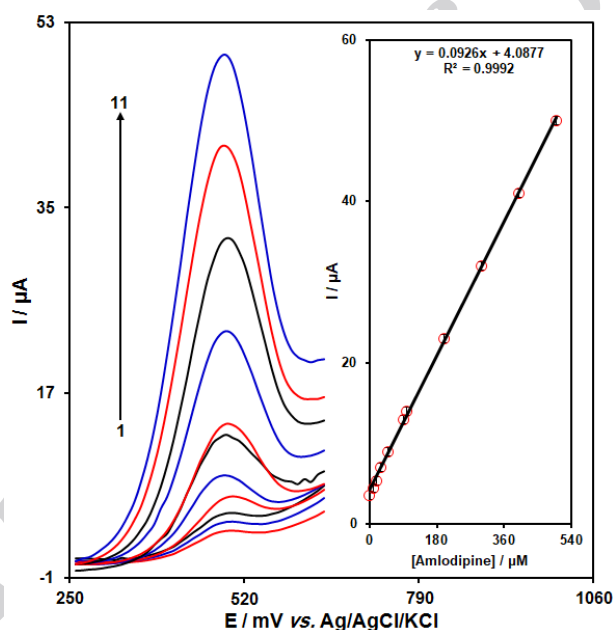
**Fig. 4.** Chronoamperograms obtained at In(III) nanocomplex/SPE in 0.1 M PBS (pH 7.0) for different concentration of amlodipine. The numbers 1–4 correspond to 0.05, 0.25, 0.5 and 1.0 mM of amlodipine. Insets: (A) Plots of  $I$  vs.  $t^{-1/2}$  obtained from chronoamperograms 1–4. (B) Plot of the slope of the straight lines against amlodipine concentration.

### 3.2. Chronoamperometric measurements

Chronoamperometric measurements of amlodipine at In(III) nanocomplex/SPE were carried out by setting the working electrode potential at 0.6 V for the various concentration of amlodipine in PBS (pH 7.0) (Fig. 4). For an electroactive material (amlodipine in this case) with a diffusion coefficient of  $D$ , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [59].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

where  $D$  and  $C_b$  are the diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ ) and the bulk concentration ( $\text{mol cm}^{-3}$ ), respectively. Experimental plots of  $I$  vs.  $t^{-1/2}$  were employed, with the best fits for different concentrations of amlodipine (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. amlodipine concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the  $D$  was found to be  $7.83 \times 10^{-5} \text{ cm}^2/\text{s}$ .



**Fig. 5.** DPVs of In(III) nanocomplex/SPE in 0.1 M PBS (pH 7.0) containing different concentrations of amlodipine. Numbers 1-11 correspond to 1.0, 10.0, 20.0, 30.0, 50.0, 90.0, 100.0, 200.0, 300.0, 400.0 and 500.0  $\mu\text{M}$  of amlodipine. Inset: shows the plot of the peak current as a function of amlodipine concentration in the range of 1.0-500.0  $\mu\text{M}$ .

### 3.3. Calibration plot and limit of detection

The peak current of amlodipine oxidation at the surface of the modified electrode can be used for determination of amlodipine in solution. Therefore, differential pulse voltammetry

(DPV) experiments were done (Initial potential=0.26 V, End potential=0.64 V, Step potential=0.1 V, Modulation amplitude=0.02505 V) for different concentrations of amlodipine (Fig. 5). The oxidation peak currents of amlodipine at the surface of a modified electrode were proportional to the concentration of the amlodipine within the range 1.0 to 500.0  $\mu\text{M}$ . The detection limit ( $3\sigma$ ) of amlodipine was found to be 0.2  $\mu\text{M}$ . Table 1. shows a comparison of the analytical figures of merit of the proposed method with electrochemical techniques for the determination of amlodipine.

**Table 1.** Comparison of the efficiency of some methods used in detection of amlodipine.

Method	Working Electrode	LOD	LDR	Ref.
Voltammetry	Boron-doped diamond electrode	0.0764 $\mu\text{M}$	0.497-28.0 $\mu\text{M}$	[1]
Voltammetry	Modified carbon paste electrode	0.015 $\mu\text{M}$	0.25-500.0 $\mu\text{M}$	[13]
Voltammetry	Pencil graphite electrode	0.04 $\mu\text{M}$	0.8-51.2 nM	[60]
Voltammetry	DNA-modified screen-printed electrodes	20.70 nM	0.066-2.0 $\mu\text{M}$	[61]
Voltammetry	Boron-doped diamond electrode	0.07 $\mu\text{M}$	0.2-38.0 $\mu\text{M}$	[62]
Voltammetry	Multi-walled carbon nanotubes paste electrode	0.049 $\mu\text{M}$	0.58-5.9 $\mu\text{M}$	[63]
Voltammetry	Glassy carbon electrode	0.014 $\mu\text{M}$	0.04-2.0 $\mu\text{M}$	[64]
Coulometric	Modified glassy carbon electrode	0.125 nM	0.001-0.2 $\mu\text{M}$	[65]
Voltammetry	Modified graphite screen printed electrodes	0.2 $\mu\text{M}$	1.0-500.0 $\mu\text{M}$	This work

### 3.4. Interference studies

The influence of various substances as compounds potentially interfering with the determination of amlodipine was studied under optimum conditions. The potentially interfering substances were chosen from the group of substances commonly found with amlodipine in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than  $\pm 5\%$  in the determination of amlodipine. According to the results, L-lysine, glucose, NADH, acetaminophen, uric acid, L-asparagine, L-serine, L-threonine, L-proline, L-histidine, L-glycine, L-tryptophan, L-phenylalanine, lactose, tyrosine, saccharose, fructose, benzoic acid, methanol, ethanol, urea, caffeine,  $\text{Mg}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{NH}_4^+$ ,  $\text{F}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{S}^{2-}$  and hydrochlorothiazide did not show interference in the determination of amlodipine.

### 3.5. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of amlodipine in amlodipine tablets and urine samples. The



results for determination of amlodipine in amlodipine tablets and urine samples are given in Table 2. Satisfactory recovery of the experimental results was found for amlodipine. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

**Table 2.** The application of In(III) nanocomplex/SPE for determination of amlodipine in amlodipine tablets and urine samples (n=5). All concentrations are in  $\mu\text{M}$

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Amlodipine tablets	0	7.0	-	3.4
	2.5	9.7	102.1	2.2
	7.5	14.1	97.2	3.3
	12.5	19.6	100.5	1.9
	17.5	24.3	99.2	2.4
Urine	0	-	-	-
	5.0	4.9	98.0	1.8
	10.0	10.1	101.0	2.9
	15.0	14.9	99.3	2.4
	20.0	20.5	102.5	2.8

#### 4. CONCLUSION

SPE modified with In(III) nanocomplex was developed and used as a novel sensor for the sensitive determination of amlodipine. The resulting device was found to act as a portable, inexpensive, and disposable tool for the determination of amlodipine. The unique properties of In(III) nanocomplex were found to present the sensor with considerable electrocatalytic activity in oxidizing amlodipine. The detection limit of the electrode for amlodipine was determined to be  $0.2 \mu\text{M}$ , and overall the electrode was considered as an excellent tool for clinical analyses where the determination of trace levels of amlodipine is required.

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