# Rapid Determination of Indapamide in Pharmaceuticals and Human Urine Using Square-wave Adsorptive Stripping Voltammetric Technique

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## ABSTRACT

Square–wave adsorptive stripping voltammetric method was selected for the investigation of the adsorbed Indapamide (INDP) drug in solutions of varying pH. A method has been developed for the ultra trace determination of INDP in presence Cu(II), due to the strong adsorption of the Cu(II) – INDP complex at the surface of the HMDE and subsequent reduction of the surface – bound complex. The observed data has been subjected to statistical analysis, which revealed high reliability and precision. A detection and quantitation limits of $2.9\times10^{-11}$ and $8.8\times10^{-11}$ mol/L$^{-1}$ respectively, were achieved in presence of $5.0 \times 10^{-7}$ mol/L$^{-1}$ Cu(II). The proposed procedure is much simple, fast, more sensitive, reproducible and the calibration graph is linear over range $2.0 \times 10^{-12} – 2.0 \times 10^{-7}$ mol/L$^{-1}$.

Moreover, this method was successfully applied to the direct determination of INDP in pharmaceuticals and spiked human urine. No prior extraction step is needed in case of urine. The effect of some interferences ($Mg^{+2}$, glycine, $Fe^{+3}$, uric acid, starch, glucose and $Na_2CO_3$) was considered.

**Keywords**: Indapamide; Square-wave adsorptive stripping voltammetry; Copper; Natrilix® tablets; Urine.

## 1. INTRODUCTION

Indapamide (INDP), (4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)- 3-sulfamoyl-benzamide), brand names: (lozol) (Fig. 1), is an oral, effective and a safe sulfonamide antihypertensive/diuretic drug developed in the early 1970s[1]. It is used for the treatment of high blood pressure and fluid retention caused by congestive heart failure. The medication can help with water retention and lower blood pressure by increasing the amount of salt and water that the kidneys remove from the blood. It is an antihypertensive agent administered to individuals with mild to moderate hypertension[2]. It is the first of the new class of antihypertensive diuretics, the indolines, and it is also prescribed to treat the salt and fluid retention associated with congestive heart failure[3]. INDP is rapidly and absolutely absorbed from gastrointestinal tract after oral administration[4].

Studies of the therapeutic effect of the drug required the use of sensitive methods for its determination at trace levels. Several analytical methods for quantitating INDP in pure forms, pharmaceutical dosage forms and in biological fluids have been developed; these include Colorimetric methods[5], fluorimetric methods[6, 7], Solid-phase extraction and high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS)[8,9], Spectrophotometric methods[10-15] and Electrochemical methods[16-18]. The clinical investigations of INDP in real samples still required the development of simple, sensitive, precise, selective and inexpensive analytical methods without the necessity for sample pretreatment or time-consuming extraction steps prior to the analysis[19]. Adsorptive stripping voltammetric analysis especially with the square-wave waveform is an extremely simple and sensitive technique that can be used for analysis of drugs without the necessity for extraction steps prior to the assay. Moreover, the square-wave

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voltammetry is a large-amplitude differential technique in which a waveform composed of a symmetrical square wave is applied to the working electrode[20]. Our knowledge of the previous found that INDP forms a complex with Cu(II) which is adsorbed at the surface of HMDE[16, 17]. Therefore, it was expected that adsorptive accumulation of compound or its copper complex could be useful as an effective preconcentration step prior to the voltammetric determination of INDP.

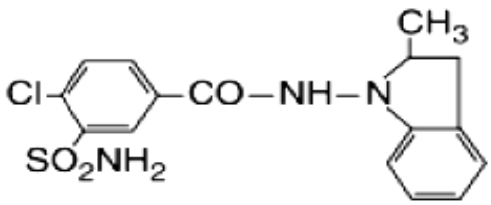


**Figure 1: The structural formula of Indapamide**

So, the present work was developed for the determination of INDP using Square–wave adsorptive stripping voltammetric method in aqueous solutions. This method is very simple, fast, more sensitive and accurate method. The optimum conditions for the INDP determination and the validity of the method have been described. The proposed method has been for determination of INDP in pharmaceutical formulation and urine with high recovery.

## 2. Experimental

### 2.1. *Apparatus*

Square-wave adsorptive stripping voltammetric studies were carried out using AMEL 433 TRACE ANALYSER involving three electrodes system consisted of a hanging mercury dropping electrode (HMDE) as a working electrode, an Ag/AgCl with saturated KCl as a reference electrode and a platinum wire as a counter electrode. A magnetic stirrer and stirring bar provided the convective transport during pre-concentration. The peak heights were automatically or manually measured using the 'tangent fit' capability of the instrument. Cyclic voltammograms were recorded with the same instrument (scan rate 100 $mVs^{-1}$). All measurements were performed at room temperature (25 ± 1°C). The pH measurements were made with Accumet® model 825 pH-meter.

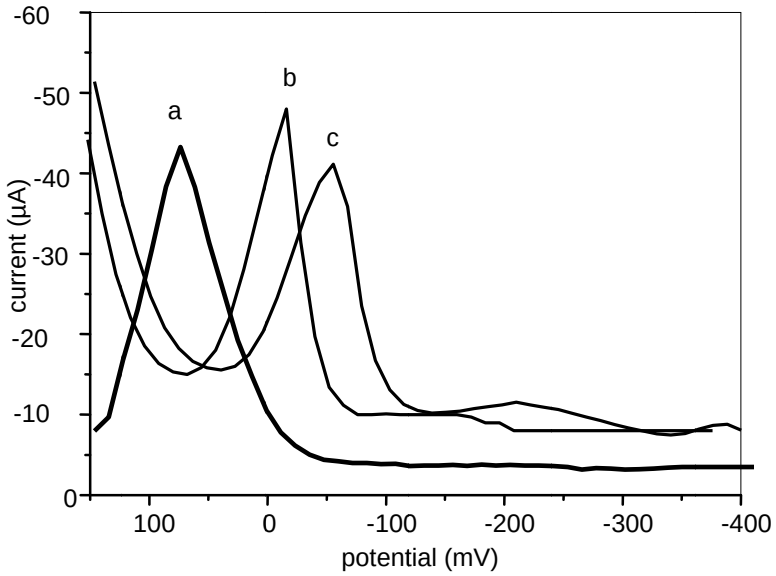


**Figure 2: Effect of different buffers at pH = 8.0 on the SWAdCS voltammetric peak current ($i_p$), for $5.0 \times 10^{-8}$ M Indapamide in the presence of $5.0 \times 10^{-7}$ M Cu(II). at $E_{acc.}$ = + 0.1 V, $t_{acc.}$ = 60 s, wave increment ΔE = 12 mV, wave amplitude $E_{sw}$ = 120 mV, rest time =5 s, wave period = 80 ms and sampling time = 8 ms (a)0.2 M phosphate, (b) B.R and (c) 0.1 M phosphate buffers**

### 2.2. *Reagents and solutions*

All chemicals used for preparation of buffers and supporting electrolytes were of analytical grade (Sigma). Bidistilled water was used throughout all experiments. Pure-grade powder of INDP and the pharmaceutical product, namely "Natrilix®" tablets were obtained from SERVIER EGYPT INDUSTRIES LIMITED. A stock solution of $1.0 \times 10^{-3}$ M INDP was prepared by dissolving the required amount of this drug in methanol. Various supporting electrolytes such as 0.1 M KCl, 0.1 M $KNO_3$ as well as Britton-Robinson, 0.2 M phosphate, 0.1 M phosphate and Boric acid/NaOH buffers were used. $1.0 \times 10^{-3}$ M solution of $Cu(NO_3)_2.3H_2O$ was prepared by dissolving the appropriate amount of salt in double distilled water.

### 2.3. *General Analytical Procedure*

10.0 mL of 0.2 M phosphate buffer pH 8.0 was taken to the electrochemical cell and the solution was purged with pure nitrogen for 15 min. The required accumulation potential ($E_{acc.}$ = +0.1 V) was applied to the working electrode for a selected accumulation time ($t_{acc.}$ = 60 s), while the solution was stirred continuously at 400 r.p.m. The stirring was stopped and after equilibrium time of 10 s, a negative-going potential scan was initiated using the following parameters: frequency ($f$) = 100 Hz; pulse amplitude ($E_{sw}$) = 120 mV; scan increment ($\Delta$E) = 12 mV. After the voltammogram of supporting electrolyte had been recorded, aliquots of the INDP in presence of $5.0\times10^{-7}$ M Cu(II) standard were introduced by micropipette and the square-wave voltammetric cycles were repeated using new mercury drop. The SWAdSV scan and cyclic voltammetry were conducted from +150 to +400 mV. All data were obtained at room temperature.

### 2.4. *Analytical Applications*

#### 2.4.1. *Assay of drug in tablets*

Ten tablets of Natrilix® (SERVIER EGYPT INDUSTRIES LIMITED) accurately weighted and finely powdered. An accurately weighed powder equivalent to 20.0 mg of INDP was transferred quantitatively into 50.0 mL measuring flask, dissolved it in a methanol – water mixture (1:1, v/v). The mixture was sonicated for 30 min. and then completed to the mark with the same solvent. The solution was then filtrated and the desired different sample for the drug was then obtained by accurate dilution with bidistilled water. Then suitable aliquot of the diluted solutions of INDP were transferred to a voltammetric cell in presence of $5.0\times10^{-7}$ M Cu(II) nitrate.

#### 2.4.2. *Assay of drug in human urine*

A urine sample (10.0 µL) taken from a healthy person was added to the voltammetric cell containing 10.0 mL of 0.2 M phosphate buffer at pH 8.0, i.e. the dilution factor of the urine sample in the cell was (1:1000). The voltammogram was recorded, then 10.0 µL spikes of the standard solution of INDP were introduced in the presence of $5.0 \times 10^{-7}$ M Cu(II) nitrate into the cell and the voltammograms were recorded after each addition.

## 3. Results and Discussion

### 3.1. *square-wave stripping voltammetry*

#### 3.1.1. *Effect of supporting electrolyte and pH*

The voltammetric response of drugs is mainly dependent on the pH of the buffer. Therefore, the electrochemical behavior of Cu(II) – INDP complex was evaluated over a pH range of 2.0–10.0 at HMDE using the SWAdSV method. The response was examined in the presence of various supporting electrolytes such as 0.1 M KCl, 0.1 M $KNO_3$ as well as Britton-Robinson, 0.2 M phosphate, 0.1 M phosphate and Boric acid/NaOH buffers (Fig. 2). The best result with respect to sensitivity (peak height), resolution (peak shape) and reproducibility were recorded in 0.2 M phosphate buffer solution at pH 8.0 as Cu(II) – INDP complex exhibited a cathodic peak (Fig. 3). After pH = 8.0 of 0.2 M phosphate the peak height decreased. Therefore, 0.2 M phosphate buffer at pH 8.0 was chosen as the supporting electrolyte for optimization of other variables and for analysis of Cu(II)–INDP complex.

#### 3.1.2. *Optimization of the operating parameters*

The dependence of adsorptive stripping peak current

of Cu(II) – INDP complex on accumulation time $t_{acc.}$ was tested at four concentrations $1.0 \times 10^{-7}$, $5.0 \times 10^{-8}$, $1.0 \times 10^{-8}$ and $5.0 \times 10^{-9}$ M of INDP in presence of $5.0 \times 10^{-7}$ M Cu(II) and also $1.0 \times 10^{-6}$, $5.0 \times 10^{-7}$, $1.0 \times 10^{-7}$ and $5.0 \times 10^{-8}$ M Cu(II) in the presence of $5.0 \times 10^{-8}$ M INDP in 0.2 M phosphate buffer pH equals 8.0 at accumulation potential $E_{acc.}$ = +0.1 V, (Fig. 4 and 5). These concentrations were examined over the accumulation time range 15 to 150 sec. the longer the accumulation time, the larger peak current and more drug adsorbed, for the concentration higher than $5.0 \times 10^{-9}$ M of INDP in presence of $5.0 \times 10^{-7}$ M Cu(II) the SWAdCS voltammetric peak height increased with increasing the accumulation time and the break observed at 60 sec means that surface coverage was attained. The influence of accumulation potential ($E_{acc.}$) on the monitored electrochemical response of $5.0 \times 10^{-8}$ M INDP in presence of $5.0 \times 10^{-7}$ M Cu(II) in 0.2 M phosphate buffer pH= 8.0 was examined over the potential range + 0.15 to – 0.4 V. As can be seen, the peak current increased in the negative direction until it reached to the maximum value at $E_{acc.}$ = + 0.1 V, where it decreased sharply after this potential. Thus, $E_{acc.}$ = +0.1 V will be adopted as optimum value for this work; (Fig. 6). Study of the effect of scan increment (ΔE) on square-wave stripping peak current of the drug in 0.2 M phosphate buffer at pH 8.0 revealed that, the peak current enhanced on the increase of scan increment (4–14 mV). A scan increment of 12 mV was preferable in the present study. At pulse amplitude ($E_{sw}$ of 120 mV), the peak was found to be much more sharp and defined. Hence, $E_{sw}$ = 120 mV was chosen for the determination of Cu(II) – INDP. At 12 mV scan increment and 120 mV pulse amplitude, wave period was varied from 50 to 180 ms. the highest peak current was found at 80 ms, which was used in the present study.

At constant INDP concentration the peak current increased with increasing Cu(II) concentration. (Fig. 7) showed the dependence of SWAdCS voltammetric peak current of $5.0 \times 10^{-8}$ M INDP on concentration of Cu(II) ions was tested at concentrations $5.0 \times 10^{-8}$, $1.0 \times 10^{-7}$, $5.0 \times 10^{-7}$, $1.0 \times 10^{-6}$ and $5.0 \times 10^{-6}$ M of Cu(II).

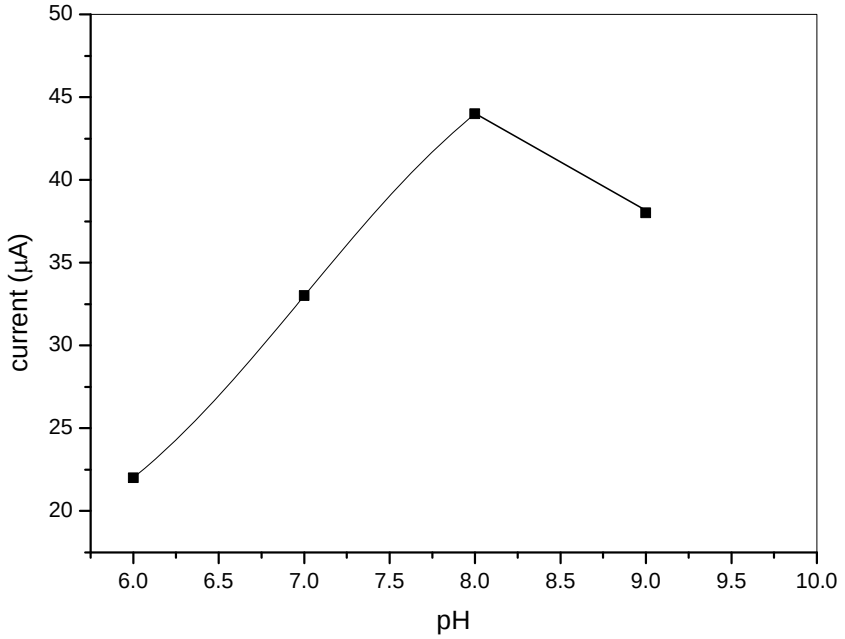


**Figure 3**: **The value of SWAdCS voltammetric peak current ($i_p$), for $5.0 \times 10^{-8}$ M Indapamide in the presence of $5.0 \times 10^{-7}$ M Cu(II) at different pH′s of 0.2 M phosphate buffer. The other conditions were as those indicated in Fig. 2.**

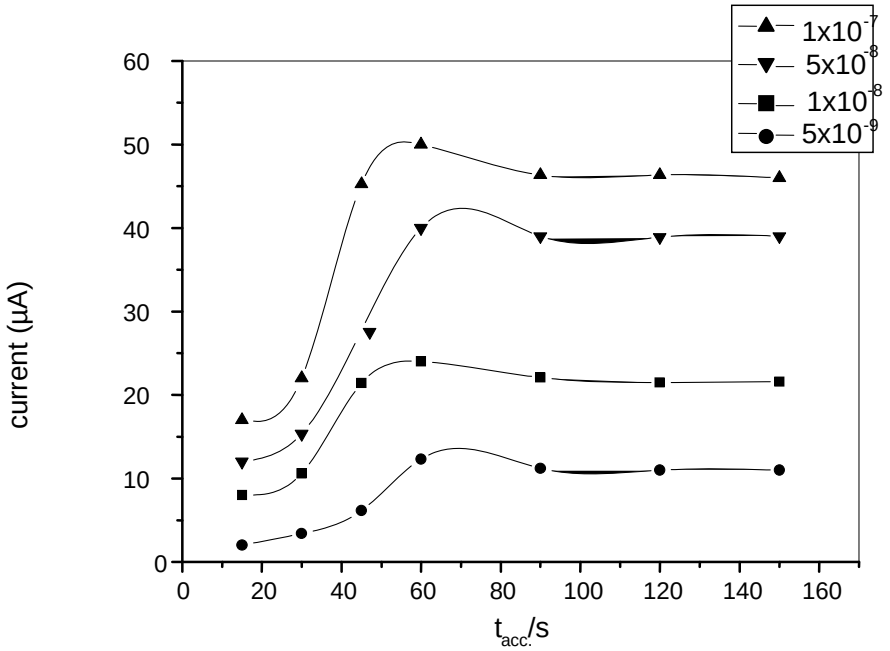


**Figure 4: Effect of the accumulation time $t_{acc.}$ on the SWAdCS voltammetric peak current ($i_p$), for $1.0\times 10^{-7}$, $5.0 \times 10^{-8}$, $1.0 \times 10^{-8}$ and $5.0 \times 10^{-9}$ M Indapamide in the presence of $5.0 \times 10^{-7}$ M Cu(II) in 0.2 M phosphate buffer pH = 8.0, and other operational parameters were as those indicated in Fig. 2**

In order to obtain the maximum development of the SWAdCS voltammetric peak current, optimization of such variables were attempted. Frequency was found to be 100 Hz using accumulation potential ($E_{acc.}$) of +0.1 V, scan increment (ΔE) of 12 mV, scan rate (ν) of 100 $mVs^{-1}$ and sampling time of 8 ms.

### 3.2. *Cyclic voltammetry*

Various supporting electrolytes such as 0.1 M KCl, 0.1 M $KNO_3$ as well as Britton-Robinson, 0.2 M phosphate, 0.1 M phosphate and Boric acid/NaOH buffers were tested by SWAdCS voltammetry, there is no voltammetric peaks corresponding to the reduction of INDP were observed.

The presence of metal ions, which are capable of forming complexes with the investigated drug or depositing at the Hg electrode, is interesting. Upon the addition of Cu(II) to INDP solution, the formation of Cu(I) –INDP complex is expected, due to stabilization of Cu(I) by adsorption at the electrode surface. It was reported that, in the presence of sulfonamide, Cu(II)–complex splits into Cu(II)/Cu(I) and Cu(I)/Cu(0)[21,22].

In analogy, we can suggest that the electrolysis of INDP in presence of copper is due to the Cu(I) –INDP complex that is adsorbed on the HMDE. During the subsequent negative potential sweep, this Cu(I) complex is reduced, allowing the drug to be determined.

Cu(II)-Indapamide complex $\xrightarrow{\text{reduction}}$ Cu(I)-Indapamide (Adsorbed complex)

Cu(I)-Indapamide (Adsorbed complex) $\longrightarrow$ Cu(I) + desorbed Indapamide (un stable)

2 Cu(I) $\longrightarrow$ Cu(II) + Cu(0) (un stable)

The adsorption of copper complexes mentioned above is stronger than the adsorption of INDP itself, and hence an enhancement of peak current is observed when Cu (II) is added.

Here, to investigate the adsorptive behavior of INDP at the hanging mercury drop electrode (HMDE), a cyclic voltammogram of $1.0 \times 10^{-6}$ M INDP in presence of $5.0 \times 10^{-7}$ M Cu(II) in 0.2 M phosphate buffer at pH 8.0 was

recorded after 60 s of stirring at +0.1V vs. Ag/AgCl. A single cathodic peak, corresponding to the reduction of the adsorbed drug, was observed at +0.06 V. There is a peak observed in the anodic branch, indicating that reduction is a reversible process. A maximum developed peak current ($i_p$) was achieved after accumulation of the drug onto the electrode surface for 60 s; this behavior confirmed the adsorptive character of the drug at the mercury surface, (Fig. 8).

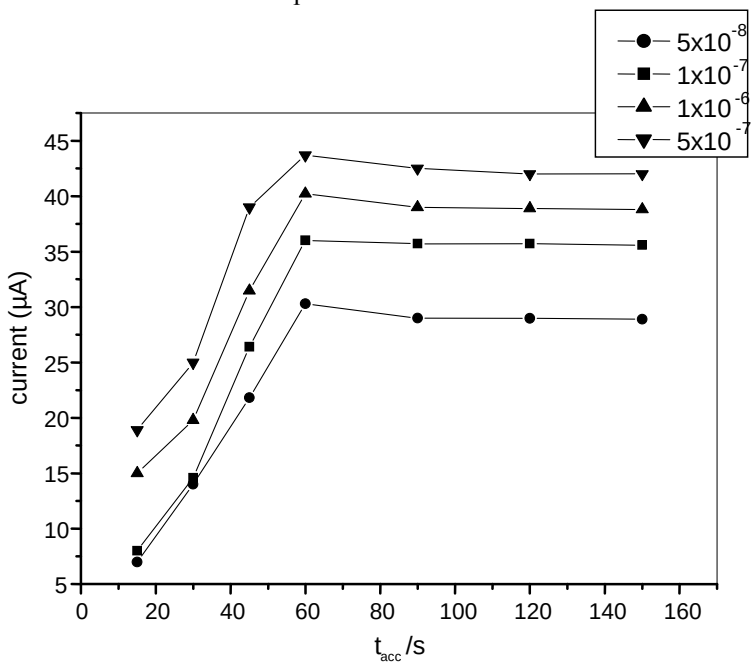


**Figure 5**: **Effect of the accumulation time $t_{acc.}$ on the SWAdCS voltammetric peak current ($i_p$), for $1.0 \times 10^{-6}$, $5.0 \times 10^{-7}$, $1.0 \times 10^{-7}$ and $5.0 \times 10^{-8}$ M Cu(II) in the presence of $5.0 \times 10^{-8}$ M Indapamide in 0.2 M phosphate buffer pH = 8.0, and other operational parameters were as those indicated in Fig. 2**

Also, the adsorptive stripping cycles carried out for increased values of scan rate (ν) gave rise to a reduction peak with intensities that showed a linear increase with scan rate between 40 and 200 $mVs^{-1}$, followed the relationship: Log $i_p$ (µA) = 1.299 + 0.9 log ν ($mVs^{-1}$) ; r = 0.9996, (Fig. 9). The slope value of 0.9 is close to the theoretical value of 1.0 that is expected for an ideal reaction of surface species[23].

The relation between $i_p$–$SR^{1/2}$ gives a straight line, that indicates that the reduction process depends predominantly on the adsorbed Hg (II)– INDP at the charged interface, followed the relation: $i_p$ (µA) = 128.2 $SR^{1/2}$ ($mVs^{-1}$) – 465.3; r = 0.9998

The repeatative cyclic voltammogram shows that peak current decreases sharply in the second and third cycles indicating the rapid desorption of drug species out of the mercury drop surface during the accumulations, (Fig. 10).

### 3.3. *Validation of the proposed method*

In the present work, quantification of INDP was based on the extend of the dependence of the peak current upon its concentration in the analyzed solution under the optimal procedural conditions. Validation of the proposed SWAdCS voltammetric procedure for trace assay of INDP in presence of $5.0 \times 10^{-7}$ M Cu(II) was examined via linearity and sensitivity, repeatability and intermediate precision, robustness, ruggedness, specificity and interference study.

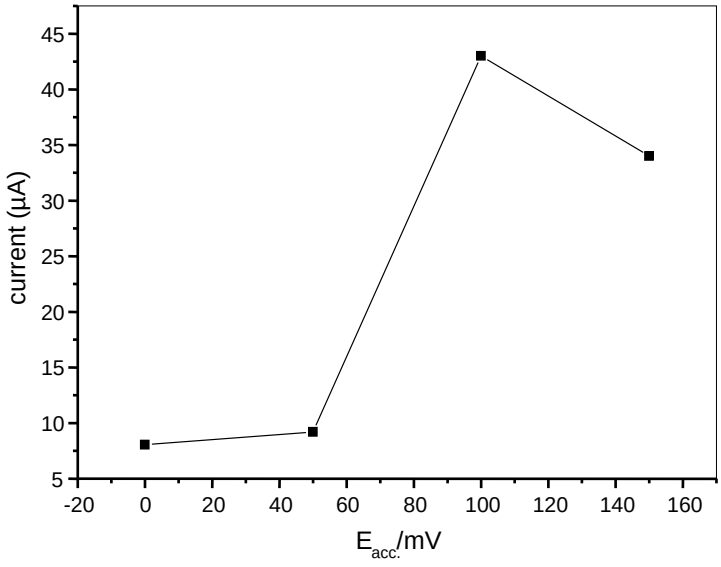


**Figure 6**: **Effect of the accumulation potential $E_{acc.}$ on the SWAdCS voltammetric peak current ($i_p$) of $5.0 \times 10^{-8}$ M Indapamide in the presence of $5.0 \times 10^{-7}$ M Cu(II) in 0.2 M phosphate buffer pH = 8.0, at $t_{acc.}$ = 60 s, rest time = 5 s, wave increment ΔE = 12 mV, wave amplitude $E_{sw}$ = 120 mV, wave period = 80 ms and sampling time = 8 ms**

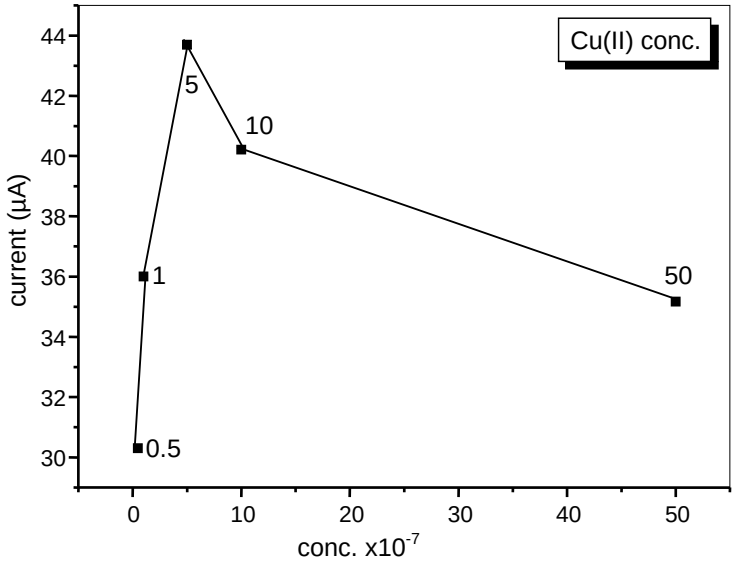


**Figure 7: Effect of Cu(II) concentration on the SWAdCS voltammetric peak current ($i_p$) of $5.0 \times 10^{-8}$ M Indapamide in 0.2 M phosphate buffer pH = 8.0, and other operational parameters were as those indicated in Fig. 2.**

### 3.3.1. *Linearity and sensitivity of the proposed method*

Calibration curves for INDP were attempted under the optimized procedure conditions followed different accumulation time periods at +0.1 V. The regression equation associated with the calibration curves, (Table 1) exhibited a good linearity that supported the proposed procedure. INDP limit of detection (LOD) and limit of quantification (LOQ) of bulk INDP were estimated from

the following equation[24,25]:

LOD = 3.3 SDa/ b LOQ = 10 SDa/b

where SDa is the standard deviation of the intercept, and b is the slope. Both LOD and LOQ values confirmed the sensitivity of the proposed method compared with those that calculated by using linear sweep adsorptive cathodic stripping voltammetry method[16, 17] and Solid-phase extraction and high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS)[9] (Table 2).

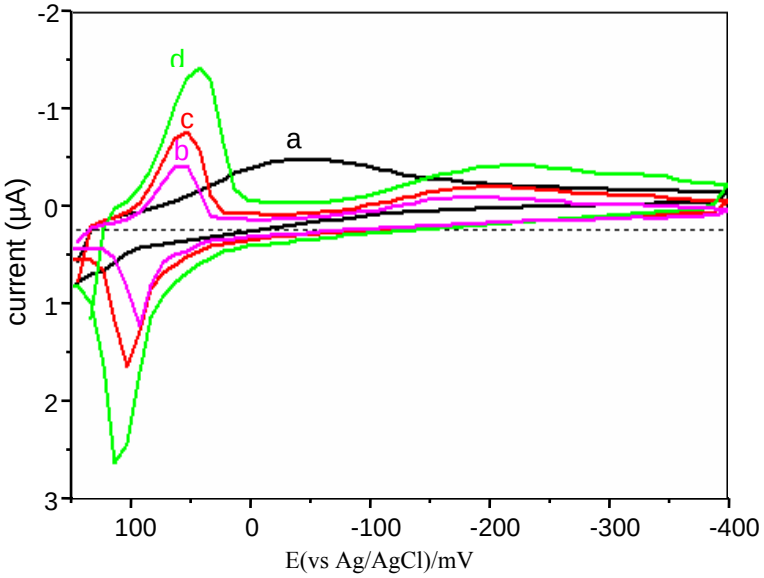


**Figure 8**: **Cyclic voltammogram for 1.0 × $10^{-6}$ M Indapamide in 0.2 M phosphate buffer pH 8.0 in the presence of 5.0 × $10^{-7}$ M Cu(II) and equilibrium time =5 s.**

**(a) without accumulation,**
**(b) after 60 sec accumulation at scan rate = 50 $mVs^{-1}$,**
**(c) after 60 sec accumulation at scan rate = 100 $mVs^{-1}$,**
**(d) after 60 sec accumulation at scan rate = 200 $mVs^{-1}$.**

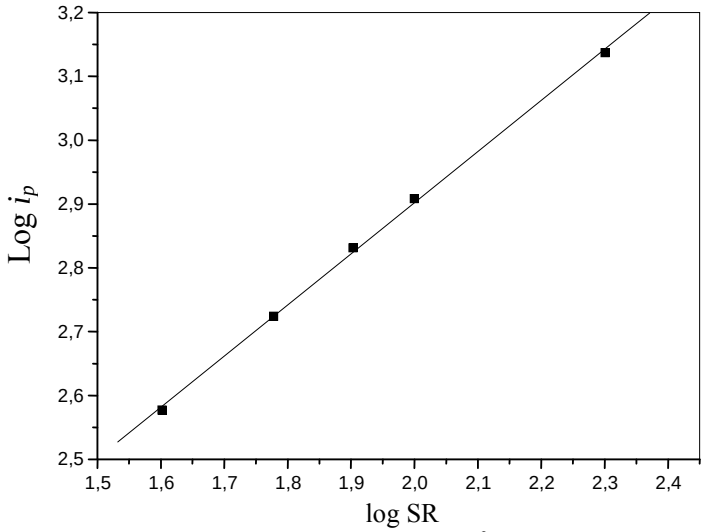


**Figure 9: log $i_p$ (peak current) vs. log ν (scan rate) for 1.0 × $10^{-6}$ M, Indapamide in the presence of 5.0 × $10^{-7}$ M Cu(II) in 0.2 M phosphate buffer pH 8.0 at equilibrium time = 5 s and $t_{acc.}$ = 60 s.**

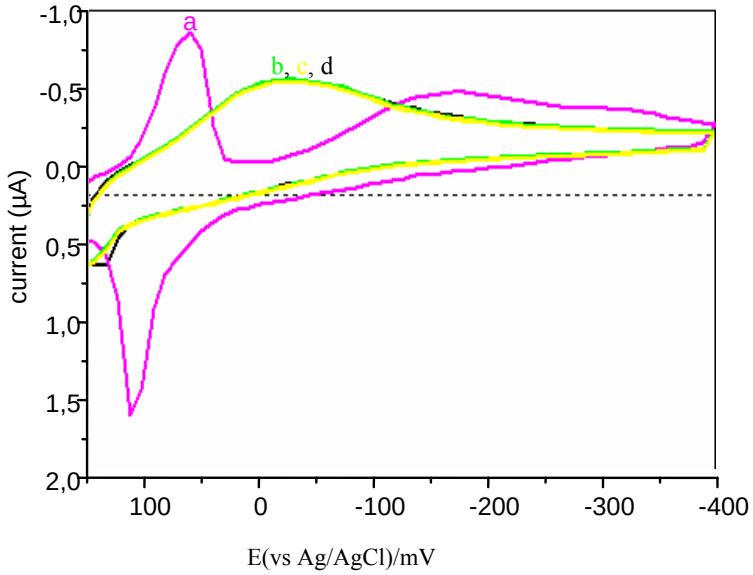


**Figure 10: Repeatative cyclic voltammograms for $1.0 \times 10^{-6}$ M Indapamide in the presence of $5.0 \times 10^{-7}$ M Cu(II) in 0.2 M phosphate buffer pH 8.0 at scan rate = 100 $mVs^{-1}$, equilibrium time = 5 s and $t_{acc.}$ = 60 s. (a) first cycle, (b) second cycle, (c) third cycle and (d) forth cycle**

**Table 1. Characteristic of the calibration plots of Indapamide in the presence of $5.0 \times 10^{-7}$ M Cu(II) at $E_{acc.}$ = +0.1V, $t_{acc.}$ = 60 s, rest time =5 s, wave amplitude $E_{sw}$ = 120 mV, wave increment ΔE = 12 mV, wave period = 80 ms and sampling time = 8 ms**

| Parameter | $t_{acc.}$ = 30 s | $t_{acc.}$ = 60 s |
|---|---|---|
| Linearity range (M) | $1.5 \times 10^{-10} - 2.0 \times 10^{-7}$ | $2.0 \times 10^{-12} - 2.0 \times 10^{-7}$ |
| Regression equation (slope in µA/nM) | $i_p$ = 5.54+0.154 c | $i_p$ = 6.17 + 0.13 c |
| Correlation coefficient (r) | 0.9988 | 0.9996 |
| Determination coefficient ($r^2$) | 0.9976 | 0.9992 |
| LOD (M) | $9.68 \times 10^{-9}$ | $2.9 \times 10^{-11}$ |
| LOQ (M) | $2.93 \times 10^{-8}$ | $8.8 \times 10^{-11}$ |

* Average of three determinations.

### 3.3.2. *Repeatability and intermediate precision*

Repeatability and intermediate precision were examined by performing six successive measurements for three concentrations $5.0 \times 10^{-7}$, $5.0 \times 10^{-8}$ and $5.0 \times 10^{-9}$ M of authentic INDP in the presence of $5.0 \times 10^{-7}$ M Cu(II) after 60s accumulation demonstrated the reproducibility of the result obtained by the proposed procedure. For intra-assay precision, recoveries were calculated from repeated analysis during one day and for inter-assay precision, recoveries were calculated from repeated analysis for five days over a period of one week. The RSD values of intra- and inter-day studies were illustrated in (Table 3). Accuracy of the result expressed as bias %:

[bias%= (measured concentration – concentration taken)× 100 / (concentration taken)].

Within and between days not more than 0.21 % at low and high concentrations.

**Table 2. Analytical precision and accuracy of the proposed SWAdCS voltammetric procedure for determination of Indapamide in bulk in the presence of $5.0 \times 10^{-7}$ M Cu(II) at $E_{acc.}$= + 0.1V, $t_{acc.}$ = 60 s, rest time = 5 s, wave amplitude $E_{sw}$ = 120 mV, wave increment ΔE = 12 mV, wave period = 80 ms and sampling time = 8 ms**

| Sample | Added amount mol $L^{-1}$ | Amount found mol $L^{-1}$ | R% | Precision (RSD %) | Accuracy (Bias%) |
|---|---|---|---|---|---|
| Intra-day precision | $5.0 \times 10^{-7}$ | $5.01 \times 10^{-7}$ | 99.76 | 0.539 | –0.002 |
| | $5.0 \times 10^{-8}$ | $4.95 \times 10^{-8}$ | 99.80 | 0.751 | –0.042 |
| | $5.0 \times 10^{-9}$ | $4.99 \times 10^{-9}$ | 99.57 | 0.6834 | –0.026 |
| Inter-day precision | $5.0 \times 10^{-7}$ | $4.99 \times 10^{-7}$ | 99.51 | 0.390 | –0.0124 |
| | $5.0 \times 10^{-8}$ | $4.89 \times 10^{-8}$ | 99.28 | 0.699 | –0.027 |
| | $5.0 \times 10^{-9}$ | $4.94 \times 10^{-9}$ | 99.09 | 0.559 | –0.025 |

* Average of five determinations.

**Table 3. LOD values of Indapamide by different methods**

| Method | value of LOD (mol/L) | References |
|---|---|---|
| Linear sweep adsorptive cathodic stripping voltammetry | $5.0 \times 10^{-9}$ | [16] |
| Adsorptive stripping method at carbon paste electrode modified with castor oil | $5.0 \times 10^{-9}$ | [17] |
| Solid-phase extraction and high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) | $5.47 \times 10^{-10}$ | [9] |
| SWAdCSV | $2.9 \times 10^{-11}$ | Present work |

### 3.3.3. *Robustness and Ruggedness*

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in procedural parameters and provides an indication of its reliability during normal usage. The most important procedure variables including pH (8.0 – 8.5), accumulation potential ($E_{acc.}$) (+100 mV to + 95 mV), accumulation time ($t_{acc.}$) (60 s – 65 s) and wave increment (ΔE) (12 mV – 13 mV). The results shown in (Table 4) indicated that none of these variables significantly affects the recovery of INDP. This provided an indication of the reliability of the proposed procedure for the assay of drug and it could be considered robust.

Ruggedness was examined by applying the developed procedure to assay of drug using potentiostats instruments (potentiostats model 263 A (PAR) and AMEL-433A) under the same optimized experimental conditions at different elapsed time. The results obtained

due lab-to-lab and even day-to-day variations were found reproducible since there was no significant difference in the recovery or the standard deviation values are obtained.

**Table 4. Results of the assay drug at various experimental conditions variables**

| Variables | Conditions | Recovery (%) R ± S.D.* |
|---|---|---|
| Change in pH of the medium at pH = 8.5 | $t_{acc.}$ = 60 s, $E_{acc.}$ = + 100 mV and ΔE = 12 mV. | 99.41 ± 0.76 |
| Accumulation time $t_{acc.}$ = 65 s | pH = 8.0, $E_{acc.}$ = + 100 mV and ΔE = 12 mV. | 100.12 ± 1.04 |
| Accumulation potential $E_{acc.}$ = + 95 mV | pH = 8.0, $t_{acc.}$ = 60 s, and ΔE = 12 mV. | 99.93 ± 0.84 |
| Wave increment ΔE = 13 mV | pH = 8.0, $E_{acc.}$ = + 100 mV and $t_{acc.}$ = 60 s. | 99.64 ± 0.24 |

*Average of five determinations.

**Table 5. Effect of interfering species on the SWAdCS voltammetric determination of Indapamide ($5.0 \times 10^{-8}$ M) in the presence of $5.0 \times 10^{-7}$ M Cu(II)**

| Interfering species | Concentration (M) | % R ± SD* |
|---|---|---|
| Glycine | $1.0 \times 10^{-8}$ | 100.36 ± 0.329 |
| | $1.0 \times 10^{-9}$ | 99.37 ± 0.292 |
| Mg $(ClO_4)_2$ | $1.0 \times 10^{-8}$ | 100.09 ± 0.648 |
| | $1.0 \times 10^{-9}$ | 99.70 ± 0.477 |
| Ferric nitrate | $1.0 \times 10^{-8}$ | 100.03 ± 0.039 |
| | $1.0 \times 10^{-9}$ | 99.79 ± 0.244 |

* Average of five determinations.

### 3.3.4. *Specificity and interference study*

Specificity of the optimized procedure for assay of $5.0 \times 10^{-8}$ M INDP in the presence of $5.0 \times 10^{-7}$ M Cu(II) was examined by addition of $1.0 \times 10^{-9}$ M up to $1.0 \times 10^{-8}$ M of Mg $(ClO_4)_2$, glycine and ferric nitrate as a common interference in pharmaceutical preparations, (Table 5) show that there is no change in the peak current of the drug was observed, this indicate that is no significant interference. Thus the procedure was able to assay INDP in the presence of interference and hence it can be consider specific. But by addition of $1.0 \times 10^{-9}$ M up to $1.0 \times 10^{-8}$ M of uric acid, starch, glucose and $Na_2CO_3$ there is change in the peak current of this drug was observed.

**Table 6. Recovery% results in dosage forms and urine sample**

| Samples | Added conc. (mol $L^{-1}$) | Found conc. (mol $L^{-1}$) | Mean recovery * (R%) | RSD% |
|---|---|---|---|---|
| Natrilix® 2.5 mg tablets | $0.545 \times 10^{-8}$ | $0.544 \times 10^{-8}$ | 99.82 | 0.151 |
| | $2.18 \times 10^{-8}$ | $2.179 \times 10^{-8}$ | 99.59 | 0.386 |
| Urine samples | $0.8 \times 10^{-9}$ | $0.798 \times 10^{-9}$ | 99.00 | 1.16 |
| | $5.0 \times 10^{-9}$ | $4.98 \times 10^{-9}$ | 99.65 | 0.443 |

* Average of five determinations.

### 3.3.5. *Assay a pharmaceutical formulation tablets*

The proposed SWAdCS voltammetric procedure was successfully applied to the direct determination of INDP in tablets pharmaceutical formulations and the validity was assessed by applying the standard addition methods. On plotting of peak height versus concentration of INDP, a straight line is obtained over a range $1.0 \times 10^{-9}$ to $1.0 \times 10^{-7}$ M for Natrilix® tablets. The average percentage recovery was 99.82 ± 0.151 and 99.59 ± 0.386 for Natrilix® tablets, (Table 6). The obtained mean percentage recovery (R%) and the relative standard deviation (RSD%) based on the average of two replicate measurements were recorded. Results obtained indicate good recoveries of the proposed method. The proposed method was also judged by comparing it with the linear sweep adsorptive cathodic stripping voltammetry method for determination of INDP in Natrilix® tablets[16].

### 3.3.6. *Assay of urine*

INDP was successfully determined in spiked human urine samples by applying the optimized procedure without any prior extraction steps displayed voltammograms of five standard additions of INDP in human urine samples; addition affecting a drug concentration of $1 \times 10^{-8}$ M, 60 s accumulation time was employed. The peak current versus drug concentration for samples a and b, respectively was presented by a straight line followed by the equation; $i_p$ (μA) =7.65 C($M/10^{-9}$) + 6, $i_p$ (μA) =2.75 C ($M/10^{-9}$) + 13.6 with a correlation coefficient of 0.9999 and 0.9995 and the collected data are illustrated in (Table 6). The percentage recoveries of INDP, based on average of five replicate measurements, were fond to for the two samples.

## 4. CONCLUSION

A simple, fast, sensitive and precise SWAdSV method was developed for the determination of INDP in pharmaceutical formulations and biological sample. This method was based on the reduction of Cu(II) – INDP at HMDE. The sensitivity of the method significantly enhanced adsorption of the drug on the electrode surface and after careful choice of the operating parameters; extremely low LOD and LOQ values could be reached.

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# تقدير سريع لدواء الاندابااميد فى عينات من المستحضرات الصيدلانية والبول باستخدام تقنية النزع الفولتامترى ذو الموجة الرباعية

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## ملخص

تم اختيار النزع الفولتامتري الكاثودي (المهبطي) بطريقة الموجة الرباعية للتحقيق من امتزاز دواء الاندبااميد في محاليل متنوعة من درجة الحموضة. وقد تم تطوير هذه الطريقة لتقدير أقل التركيزات من الاتدبااميد في وجود النحاس (II)، نظراً للامتزاز القوي للمتراكب (النحاس – الاندبااميد) على قطب الزئبق المعلق. وقد تعرضت البيانات إلى التحليل الإحصائي، والتي كشفت الموثوقية العالية والدقة. تم الحصول على قيمتي حدود الكشف والتقدير وهما $2.9\times10^{-11}$ و $8.8\times10^{-11}$ مول/لتر في وجود $5\times10^{-7}$ مول/لتر من النحاس (II). الإجراء المقترح هو بسيط جدا، سريع، أكثر حساسية، وقابل للتكرار ووجد أن منحنى المعايرة يكون خطي في المدى من $2.0\times10^{-11}$–$2.0\times10^{-7}$ مول/لتر.

وعلاوة على ذلك، هذه الطريقة تم تطبيقها بنجاح للتقدير المباشر للاندبااميد في عينات دوائية وعينات من البول البشري. ليس هناك حاجة إلى خطوة الاستخراج السابقة في حالة البولز وتم الأخذ في الاعتبار لتأثير بعض المدخلات (المغنيسيوم$^{+2}$، الجلايسين، الحديد$^{+3}$، وحمض اليوريك، والنشا والجلوكوز وكربونات الصوديوم).

**الكلمات الدالة:** الاندبااميد، المستحضرات الصيدلانية، تقنية النزع الفولتامتري.

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