

## ORIGINAL ARTICLE

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# Voltammetric determination of zoledronic acid in a pharmaceutical formulation

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### ABSTRACT

**Objectives:** To study the electroactivity of zoledronic acid (ZOL), optimize the parameters affecting voltammetric analysis of ZOL, and to make a comparison between voltammetric methods which used for ZOL assay.

**Materials and Methods:** Three voltammetric methods Cyclic, square wave and differential pulse voltammetry were used to determine ZOL solutions of (0.25- 1.2 mg.mL<sup>-1</sup>). Britton–Robinson universal buffer solutions (BRB) were used as supporting electrolytes and Glassy Carbon (GC) working electrode.

**Results:** The calibration plots were linear in the range from (0.20- 1.2 mg.mL<sup>-1</sup>) for DPV and CV and (0.09- 1.2 mg.mL<sup>-1</sup>) for SWV. DPV showed the highest correlation coefficient R<sup>2</sup> value of 0.993 and the lowest limit of detection LOD of 37.2 µg.mL<sup>-1</sup>. Furthermore, DPV exhibited the highest precision with the lowest RSD values. For commercial product of ZOL DPV has showed the best accuracy and precision of 102.32% recovery and 2.88% RSD respectively

**Conclusion:** ZOL is electroactive compound. pH of BRB supporting electrolyte is important for ZOL electroactivity. DPV is the recommended method for voltammetric analysis of ZOL because of its high performance regarding accuracy, precision and LOD compared to other studied methods.

**Keywords:** Zoledronic acid, electroanalytical methods, voltammetry, pharmaceutical compounds analysis

### INTRODUCTION

Zoledronic acid which has IUBAC name (1-hydroxy-2-imidazole-1-yl-phosphonoethyl) phosphonic acid (figure 1) is bone resorption inhibitor compound majorly used for treatment osteoporosis, bone Paget's disease and malignant hypercalcemia<sup>1-3</sup>. The bisphosphonate ZOL pharmacologic action is to inhibit bone resorption by actions on

osteoclasts or on osteoclast precursors; inhibit osteoclastic activity and skeletal calcium release induced by tumors<sup>2</sup>.



Figure 1. structure of zoledronic acid

Several methods were used for pharmaceutical compounds analysis such as chromatographic, spectroscopic and electrochemical methods. Most of standard methods rely basically on chromatography, especially high performance liquid chromatography (HPLC). On other hand chromatographic method has high operation and instrumentation cost, furthermore chromatographic method is not green analytical method because of large amount of organic solvents used in it.

Electroanalytical methods become better alternative to spectroscopic and chromatographic methods, because Electroanalytical methods have some advantages like low instrumentation and operation cost, short analysis time and simplicity.

Electroanalytical methods such as voltammetric, potentiometric and polarographic methods have been widely applied for pharmaceutical compounds analysis.<sup>4-8</sup> These methods showed reliable results according to accuracy, precision, sensitivity and selectivity.<sup>4,5</sup>

Several research studied worked on zoledronic acid determination. Most of these studies have used HPLC to study zoledronic acid in different media such as pharmaceutical products,<sup>9, 10</sup> in human urine and blood plasma,<sup>11</sup> in human bone<sup>12, 13</sup> and murine bone.<sup>14</sup> Legay et al. developed radioimmunoassay method for zoledronic acid determination in human serum, plasma, and urine.<sup>15</sup> Amin et al. developed switchable fluorescence probe for zoledronic acid determination in human serum.<sup>16</sup> Present work is studying the electroactivity of zoledronic acid, and the parameters affecting voltammetric analysis of ZOL such as working electrode and supporting electrolyte, in addition it established a comparison between voltammetric methods used for ZOL assay in pure pharmaceutical formulation and final product solution.

## **MATERIALS AND METHODS**

### **MATERIALS**

Commercial product of zoledronic acid (Zoledronic acid HIKMA<sup>®</sup> 4 mg/5mL concentrate for solution for infusion) and zoledronic acid standard material were provided by Hikma pharmaceuticals company (Jordan). Potassium nitrate (KNO<sub>3</sub>, ACS

reagent, Fluka), Sodium sulphate anhydrous ( $\text{Na}_2\text{SO}_4$  Janssen Chemica), citric acid anhydrous (Al-Saggaf pharma).

Britton–Robinson universal buffer solutions (BRB) pH (2.1-11.6) were prepared by mixing certain amounts of boric acid, acetic acid and phosphoric acid then sodium hydroxide (0.20 M) was used to adjust pH of the mixture.

Phosphate buffer pH 6.8 ( $\text{NaH}_2\text{PO}_4 / \text{H}_3\text{PO}_4$ ) supporting electrolyte was prepared by Dissolving 24 g of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  in 800 ml of deionized water, then pH 6.8 reached by adding 85%  $\text{H}_3\text{PO}_4$ , After that deionized water was used to complete volume to 1.0 L.

Sodium citrate buffer (pH 3) supporting electrolyte solution was prepared by mixing 42.5 g of citric acid with 800 ml of deionized water, then pH 3 was reached by adding NaOH 20% w/v to the solution.

#### **Standard ZOL solutions**

Standard Stock solutions of ZOL were prepared by dissolving certain quantity of standard ZOL powder in supporting electrolyte solutions. Working solutions of ZOL standard were prepared by dilution standard stock solution with supporting electrolyte. Zoledronic acid HIKMA<sup>®</sup> 4 mg/5mL concentrate for solution for infusion vial was diluted by BRB buffer solution pH 10.52 to reach the required concentration, each concentration of all voltammetric analysis methods has been done triplicate.

#### **Apparatus**

Potentiostat (Metrohm Autolab) PGSTAT 204 was used for voltammetric determination of ZOL samples. Glassy carbon (GC), gold and Platinum were used as working electrodes in this study, with platinum as counter electrode and Ag/AgCl (3M KCl) as reference electrode.

### **RESULTS AND DISCUSSIONS:**

#### **Optimization of voltammetric analysis parameters:**

Several supporting electrolytes have been studied for voltammetric assay of ZOL in a pharmaceutical formulation. Results of supporting electrolytes studies have shown the significance of this parameter, since ZOL didn't show electroactivity with several supporting electrolytes such as  $\text{KNO}_3$  1.0M, Phosphate buffer pH 6.8 and Sodium citrate buffer pH 3.0 even with different kinds of working electrodes such as GC, Pt and Au electrodes. ZOL exhibited electroactivity when BRB used as supporting electrolyte with pH 5.94-10.52 and GC as working electrode (Figure 2), where BRB buffer of pH 10.52 showed the highest performance. Cyclic voltammograms of ZOL in (Figure 3) indicated that ZOL is electroactive with irreversible anodic peak current at 1.48V.

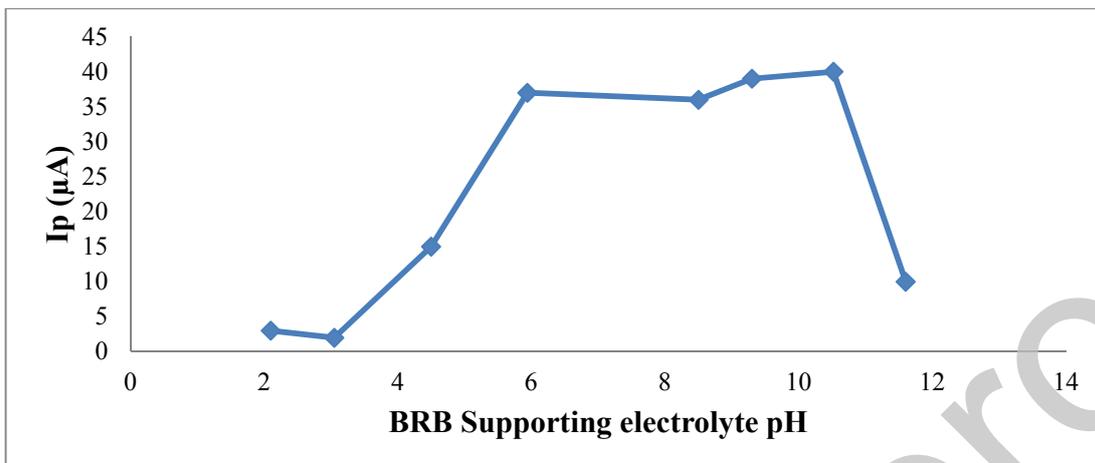


Figure 2. effect of BRB supporting electrolyte pH on anodic peak current of cyclic voltammetric analysis of ZOL ( $1.0 \text{ mg.ml}^{-1}$ ). scan rate  $0.1 \text{ V.s}^{-1}$ , GC working electrode

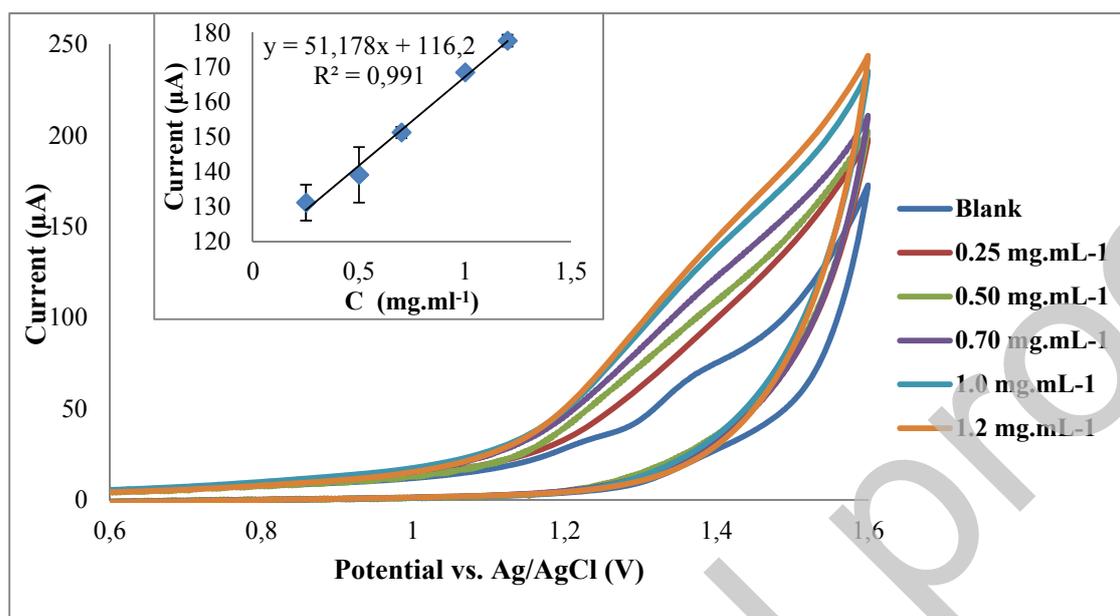


FIGURE 3. CV study of ZOL (0.25-1.2 mg.mL<sup>-1</sup>), GC working electrode, BRB pH 10.52 supporting electrolyte, scan rate 0.1V.s<sup>-1</sup>.

#### **Voltammetric methods study**

Three voltammetric methods were applied for ZOL assay in pharmaceutical formulation, CV, SWV and DPV. ZOL solutions of (0.25- 1.2 mg.mL<sup>-1</sup>) have been studied with BRB buffer of pH 10.52 supporting electrolyte and GC working electrode. Anodic current potential of 1.48V was selected for ZOL assay since it has shown the highest correlation between SOL concentration and current in all studied voltammetric methods (Table 1) (Figures 3-5).

#### **Linearity and range**

The linearity of studied voltammetric methods has been evaluated form ZOL standards regression line according to ICH guidelines. Each concentration of ZOL has been done triplicate. According to Table 1 DPV has the highest R<sup>2</sup> value of 0.993 compared to other studied method. Plateau of current values are showing up at concentrations higher than (1.2 mg.mL<sup>-1</sup>) for all studied methods, these results make the dynamic range of DPV and CV (0.20- 1.2 mg.mL<sup>-1</sup>) and (0.09- 1.2 mg.mL<sup>-1</sup>) for SWV.

#### **Detection and quantitation limits**

Limiting of detection and quantitation for voltammetric analysis of ZOL were evaluated based on the standard deviation of the blank (BRB buffer of pH 10.52). where;

$$\text{Limit of detection} = y_B + 3S_B$$

$$\text{Limit of quantitation} = y_B + 10S_B$$

S<sub>B</sub>: Standard deviation of the blank

y<sub>B</sub>: Anodic current response of the blank

According to the results in Table 1 DPV shows the lowest limit of detection (LOD) of 37.2 µg.mL<sup>-1</sup> and SWV the lowest limit of quantitation (LOQ) of 87.5 µg.mL<sup>-1</sup>.

Slopes of linear regression calibration curves indicate that CV has the highest sensitivity compared to other methods (Table 1).

### precision

Each concentration of ZOL has been done triplicate, standard deviation of each point at calibration curves indicated higher precision at high ZOL concentrations in all studied methods. Repeatability and reproducibility of voltammetric analysis of ZOL are shown in (Table 2). All studied voltammetric methods have shown low intraday and inter day relative standard deviations (RSD) which reflected high precision, where DPV exhibited the highest precision with the lowest RSD values (Table 2).

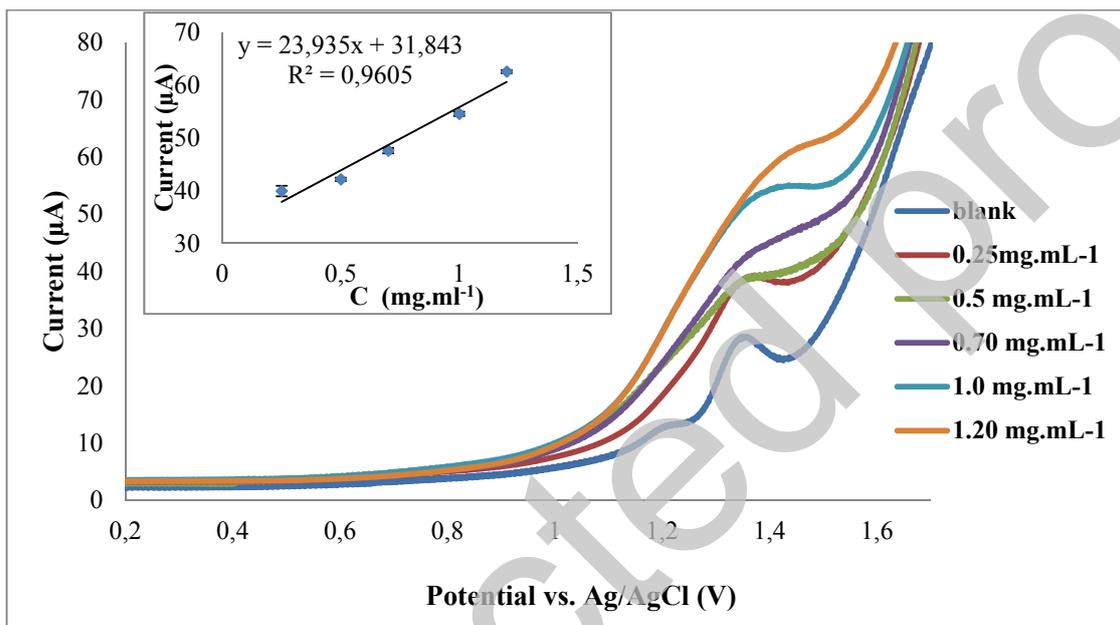


FIGURE 4. SWV study of ZOL (0.25-1.2 mg.ml<sup>-1</sup>), GC working electrode, BRB pH 10.52 supporting electrolyte.

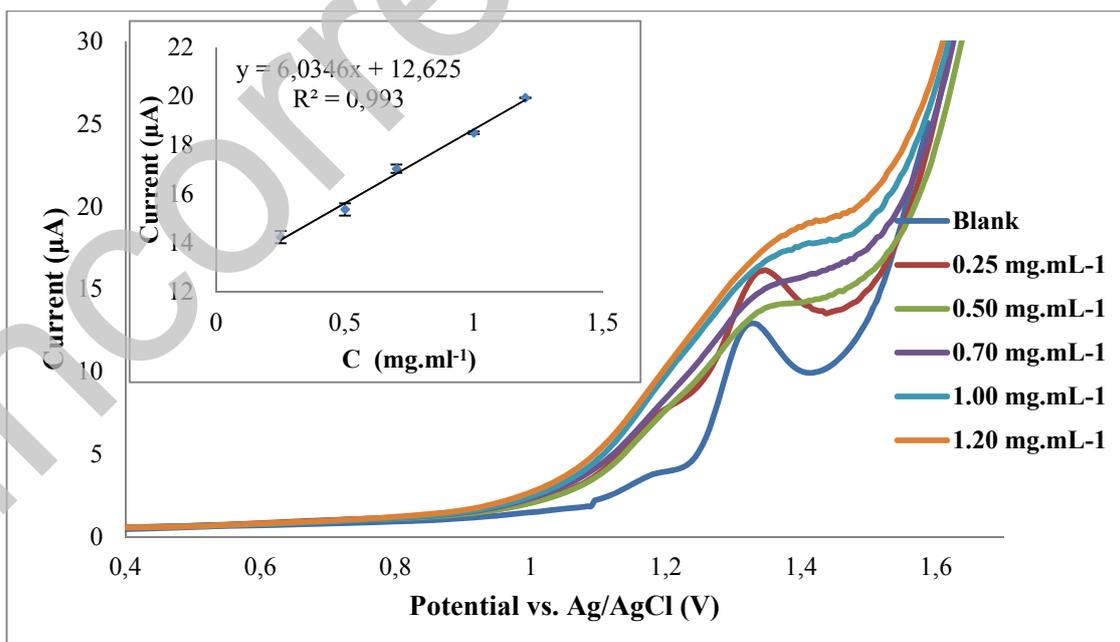


FIGURE 5. DPV study of ZOL (0.25-1.2 mg.ml<sup>-1</sup>), GC working electrode, BRB pH 10.52 supporting electrolyte.

**Table 1. Linearity of ZOL (0.25-1.20 mg.mL<sup>-1</sup>, BRB pH 10.53) GC electrode**

Method	Linear regression	R <sup>2</sup>	LOD ( μg.mL <sup>-1</sup> )	LOQ( μg.mL <sup>-1</sup> )
CV	y = 51.178x + 116.2	0.991	84.0	196
DPV	y = 6.0346x + 12.625	0.993	37.2	202
SWV	y = 23.935x + 31.843	0.961	41.7	87.5

**Table 2. Precision of ZOL (1.00 mg.mL<sup>-1</sup>, BRB pH 10.53) GC electrode**

Method	Intraday RSD%	Inter day RSD%
CV	0.476	0.652
DPV	0.286	0.344
SWV	0.733	0.854

**Intraday relative RSD%:** standard deviation on the same day of triplicate measurements (**repeatability**). **Inter day RSD%:** three consecutive days' measurements relative standard deviation. (**reproducibility**).

#### Accuracy

Commercial preparation of ZOL (Zoledronic acid HIKMA<sup>®</sup> 4 mg/5mL) concentrate for solution for infusion was used to study the accuracy of voltammetric methods. Table 3 showed the recovery and RSD of 0.40 mg.mL<sup>-1</sup> ZOL using voltammetric methods. DPV showed the best accuracy and precession of 102.32% recovery and 2.88% RSD respectively.

**Table 3. Recovery and Precision of Commercial preparation of ZOL (Zoledronic acid HIKMA<sup>®</sup> 4 mg/5mL) GC electrode (BRB pH 10.53)**

Method		0.40 mg.mL <sup>-1</sup>
CV	Found ±SD	0.3874±0.0158
	Recovery%	96.86
	RSD%	4.08
DPV	Found ±SD	0.4092±0.0117
	Recovery%	102.32
	RSD%	2.88
SWV	Found ±SD	0.4162±0.0144
	Recovery%	104.06
	RSD%	3.46

**Recovery**= found ZOL/added ZOL \*100%.

A comparison has been established between different methods used for ZOL determination and present method according to precession, accuracy and LOD (Table 4). It's clear from table 4 that most chromatographic methods have better detection limits compared with present study. But on other hand present study shows comparable recovery and RSD values.

Table 4. Comparison of detection limit, precision and recovery for determination of zoledronic acid between present work and other used methods

method	LOD	Precession	Recovery
RP-HPLC UV detector <sup>9</sup>	0.04 $\mu\text{g.mL}^{-1}$	2.5%	101%
RP-HPLC evaporative light scattering detection <sup>10</sup>	0.9 $\mu\text{g.mL}^{-1}$	0.4-0.8%	98-102%
HPLC-tandem mass spectrometry <sup>12</sup>	3.4 $\text{ng.mL}^{-1}$	0.52-8.7%	97-101.9%
RP-HPLC Hydroxy apatite-based Nanoparticles <sup>13</sup>	200 $\mu\text{g.mL}^{-1}$	0.32–1.15%.	99.01- 100.8%
switchable fluorescence probe <sup>16</sup>	0.011 $\mu\text{g.mL}^{-1}$	2.70%	92.2-104.0%
DPV, GC electrode (Present work)	37.2 $\mu\text{g.mL}^{-1}$	0.286-0.344%	102.32%

## CONCLUSION

Voltammetric analysis indicated that zoledronic acid is electroactive compound. CV of ZOL exhibited irreversible anodic peak current at 1.48V. Optimization of voltammetric analysis parameters indicated the significance of supporting electrolyte pH and the type of working electrode. CV, SWV and DPV have been applied for voltammetric analysis of ZOL, But DPV is the recommended method for voltammetric analysis of ZOL because of its high performance regarding accuracy, precession and LOD compared to other studied methods.

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