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# Square Wave Anodic Voltammetric Determination of Antidiabetic Drug Linagliptin In The Dosage Form And Biological Fluids By Microparticles Copper Pencil Graphite Electrode

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## Abstract

A new simple, cheap, and rapid method to increase the sensitivity of pencil graphite electrode (PGE) to determine Linagliptin (LNG) was developed by modified PGE with copper microparticles (Cu-PGE). The preparation of the disposable modified sensor electrode depended on the electrodeposition of Cu-microparticles on the GPE that can be applied for determined LNG by complexed it with $Cu^{+2}$ ions and measure the LNG-Cu complex onto the Cu-PGE improved. The preparation of this modified electrode is easy, simple, and cheap and has high sensitivity. The modified electrode detected the LNG in the LNG-Cu complex in the range from 47.25 - 283.53 ng $mL^{-1}$ with detection and quantitative limits of 6.23 and 20.77 ng $mL^{-1}$, respectively, with correlation coefficient 0.9995. The proposed method was applied with success to determined LNG in the dosage form, spiked urine, and spiked plasma samples; additionally, the obtained modified electrode was put in comparison with the reported electrodes.

*Keyword:* Square Wave Anodic Voltammetric; Linagliptin; Copper Microparticles Pencil Graphite Electrode; Dosage Form and Some Biological Fluids

## 1. Introduction

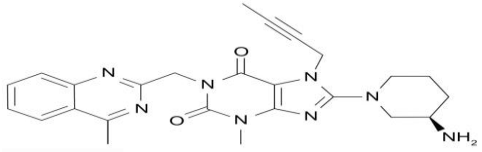
Diabetes, which is a metabolic disease, is the 21st century's most chronic disease. This disease characterizes by an inability of the body to produce or use insulin[1]. Dipeptidyl peptidase-4 (DPP-4) inhibitors, which is one of the most novel anti-diabetic drugs, are used in the management of type (II) diabetes for about ten years and have provided a promising glucose-lowering treatment more than most other anti-diabetic agents [2].

LNG scheme 1 (Trajenta$^{®}$) is an oral, consider as a highly selective (DPP-4) inhibitor[3]. There are many methods reported for determination of LNG, spectrophotometric[4-10], spectrofluorometric[11, 12], high-performance thin-layer chromatographic[13, 14], high-performance liquid chromatographic[4, 15-36], conductometric[37] and voltammetric methods[38-40] either alone or in the mixtures with other antidiabetic agents.

The squire wave voltammetric technique used in the proposed method has an excellent considerable sensitivity up to $10^{-12}$ M, fast analysis times (seconds), the ability to determine kinetic and mechanistic parameters, and small currents measured[41].

Pencil graphite electrode recently has excellent electroanalysis attention for many advantages: being disposable, availability, low cost, good electrochemical reactivity, simplicity, mechanical rigidity, and ease of modification[42].

The cited method is our team's continuous effort to presented differences and more sensitive ways to determine LNG[12, 39]. The mentioned electrode is more sensitive than the other reported ones; it has a detection limit of 6.0 ng $mL^{-1}$, while the previous electrodes have a detection limit of 5340.0, 100.0, and 8.0 ng $mL^{-1}$ [38, 39], respectively.



Scheme.1: Chemical structure of LNG

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## 2. EXPERIMENTAL

### 2.1. Apparatus

All voltammetric procedures were performed by using a potentiostat (Model 273 A), made by EG&G Princeton Applied Research (PAR Princeton, NJ, USA), depended on the electrochemical software (Model 270/250, version 4.30), connected to a three-electrode cell which used for the measurements. Cu-PGE, a working electrode, was fabricated by electrodeposition of microparticles Cu on the PGE (ATI, H B 2.0 mm x 127L made in Taiwan and were purchased from a local Bookstore), an Ag/AgCl (saturated KCl) reference electrode, and the counter electrode was a platinum wire. Mass transport was achieved with a Teflon-coated bar at approximately 400 rpm using a magnetic stirrer (KIKA Labortechinik, Germany). Tabletop high-speed universal harmonic centrifuge 12000 RPM model PLC-012 made in Taiwan. IMeshbean PH-108 IA Pocket Pen Type LCD screen pH Meter was used. All experiments were performed at room temperature without removing the dissolved oxygen.

### 2.2. Reagents and materials

The chemicals used were of analytical grade. They were purchased from Al-Nasr Company, Cairo, Egypt. *Britton–Robinson buffer (*BR) (0.04 M)[43] solutions of pH (2.5 - 8.5), which composed of boric acid, citric acid, glacial acetic acid, and orthophosphoric acid, was adjusted to the required pH with 0.1 M sodium hydroxide were prepared using double distilled water.

LNG was obtained as a gift from EVA pharm lot. No: 24529.Trajenta® (LNG) contains 5 mg per tablet, was purchased from the local market.

### 2.3. Electrode preparation

The electrodeposition of Cu microparticles on the surface of PGE was studied. Many parameters affected the deposition of Cu atoms on the PGE surface. These parameters included the Cu concentration, the used technique, the applied current, the accumulation potential, and the accumulation time. The study indicated a $5.0 \times 10^{-4}$ M solution was performed in the galvanostatic technique using direct current at - 0.6 V as an accumulation potential and 60 s as an accumulation time [44, 45]. Finally, the modified produced electrode was dried in air for 3 min. For each run, 10 mm of new Cu-PGE was immersed in the electrochemical cell containing 15.0 mL of the sample solution.

### 2.4. Preparation of the Standard Solutions

A stock solution of LNG (4.73 mg $mL^{-1}$) was freshly prepared in methanol. The stock solution was stable for at least three months if stored in the refrigerator. The required concentration of LNG was prepared by diluting the stock standard solution with double distilled water.

### 2.5. Voltammetric Procedure

The analytical procedure was performed by immersing Cu-PGE in a stirred 15.0 mL BR buffer (pH 4.5) containing 1.0 mL of free $Cu^{+2}$ ions ($1.0 \times 10^{-4}$ M $CuSO_4$) and different concentrations of LNG solution at a potential range from + 0.9 to + 1.5 V. The stirring was then stopped after 10 s to settle the solution and decrease the background current. A square wave voltammogram was recorded in a positive potential direction. A new Cu-PGE was used for each measurement.

### 2.6. Preparation of tablets sample

Ten tablets of Trajenta® (5 mg LNG/ tablet) were weighed and powdered. A weighed tablet powders were prepared to obtain a solution of 4.73 mg $ml^{-1}$ of LNG. It was put into a 10-mL calibrated flask and added about 7.0 mL methanol, sonicated for 20 min to ensure complete dissolution, completed to 10.0 mL by methanol, and then filtrated. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and diluting with double distilled water, and the voltammetric process was routine as before.

### 2.7. Spiked urine sample preparation

Urine and plasma samples were supplied from three healthy volunteers according to the certify for research study code number 42/ 2019, which was approved by The Commission on the Ethics of Scientific Research, Faculty of Pharmacy, Minia University, Egypt. 5 % of the drug excretes in urine [46]. The collected urine sample was centrifuged for 20 minutes at 5000 RPM, and then the supernatant was taken. 1.5 mL of the resulting urine supernatant solution was added into the electrochemical cell, and then the voltammetric measurement was conducted.

### 2.8. Spiked plasma sample preparation

After the centrifuge of plasma for 30 minutes at 5000 RPM. 0.5 ml of supernatant spiked plasma, 0.5 ml (4.73 mg $ml^{-1}$) LNG, and 1.0 ml acetonitrile were taken and complete to 5.0 ml by water. This content was centrifuged for 20 minutes at 5000 RPM. After the centrifuge was finished, an aliquot of produced plasma solution was put into the electrochemical cell, and then the voltammetric procedure was conducted.

## 3. RESULTS AND DISCUSSION

LNG contains carbonyl groups and several nitrogen atoms. This structure will give the compound the ability to form a metal complex with several cations. The complex could be built between the carbonyl group in the purine-2,6-dione with the nitrogen atom in the neighbor quinazoline moiety and the $Cu^{2+}$. The surface of PGE was modified by electrodeposition with Cu micro-particles. The micro-particles mediated the electro-reduction of the copper ion of the Cu-LNG complex present in the solution. LNG was reported to react with $Cu^{2+}$ to form the Cu-LNG complex in the ratio 1:1[37].

The Cu-LNG complex's electrochemical behavior was investigated by recording a cyclic voltammogram of 94.51 ng $mL^{-1}$ LNG at various scan rates values from 100 to 600 mV/sec. (**Fig. 1**).

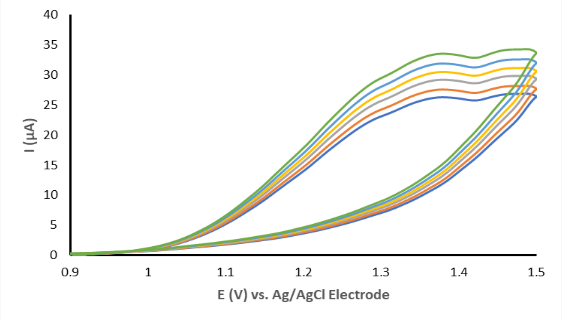


**Fig. 1**: Typical cyclic voltammograms for 94.50 ng $mL^{-1}$ LNG of LNG-Cu complex using Cu-PGE at scan rate values (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 V) of 60 s accumulation time

The Cu-LNG complex showed only one well–defined oxidation peak at about + 1.37 V. No peaks appeared in the reverse scan. This means that the Cu-LNG complex formed process is irreversible[39].

The peaks of separated solutions of Cu, LNG, and LNG + Cu at Cu-PGE are illustrated in **Fig. 2.** As shown in the figure, the potential for Cu-LNG solution (1.37 V) is different from those of either Cu ion or LNG alone, which indicates the successful occurrence of the complex between LNG and $Cu^{2+}$.

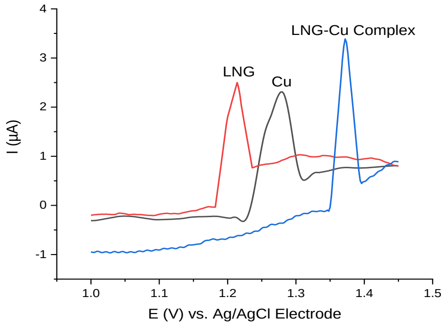


**Fig.2:** Square wave voltammograms of Cu (5.32 μg $mL^{-1}$), LNG (94.50 ng $mL^{-1}$), and LNG-Cu complex (47.25 ng $mL^{-1}$) on Cu-PGE

### *3.1. Surface morphology study*

Scanning *Electron Microscopy (SEM)* was used to examine the surface morphology of the modified Cu-PGE. The electrode surface was investigated at different stages.

**Fig. 3** shows the SEM micrographs for the surface of PGE, PGE coated with microparticles Cu and Cu PGE after adsorption of the LNG-Cu complex on the surface of Cu-PGE. There are apparent differences in the PGE surface at the different stages from the micrographs, representing that the LNG-Cu complex was successful formed on the electrode's surface.

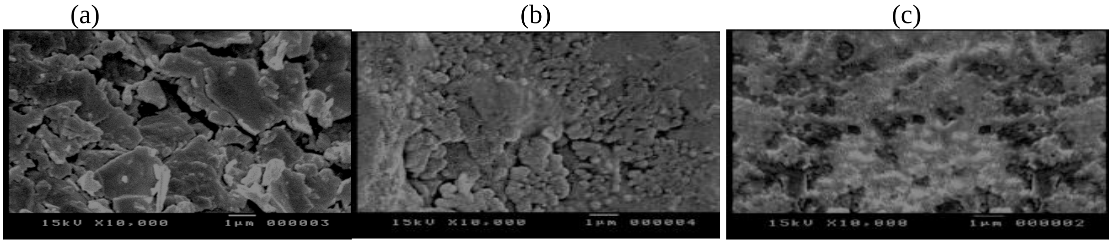

(a) (b) (c)

**Fig.3:** Micrograph SEM (a) free PGE, (b) PGE coated with copper microparticles, and (c) PGE coated with copper microparticles in the presence of an adsorbed layer of LNG-Cu complex

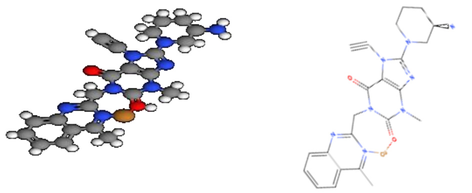
### *3.2. Suggested mechanism*

To increase the sensitivity of PGE toward LNG determination, microparticles Cu were electrodeposited onto the surface of PGE. The deposited Cu acts as a mediator and catalyst between the Cu-LNG complex and the PGE surface.

In the electrolyte solution, the free $Cu^{+2}$ ions ($1.0 \times 10^{-4}$ M) were complexed with LNG to form the Cu-LNG complex. The formed complex in solution was then adsorbed onto the Cu-PGE electrode surface.

At the Cu-PGE electrode surface, two electrons were transferred to reduce the $Cu^{+2}$ of the Cu-LNG complex to form metallic Cu. Thus, Cu microparticles mediate and catalyze the electroreduction of $Cu^{2+}$ ions present in the Cu-LNG complex. Based on this process, an indirect method was constructed to determine LNG concentrations [44, 47].

A proposal for the structure of the LNG-Cu complex is shown in **Scheme 2.**



Scheme 2**:** Suggested LNG-Cu complex structure

### *3.3. Method development*

#### 3.3.1. *Effect of pH, buffer types, and Ionic Strength*

The influence of buffer type on peak current ($I_p$) of the LNG-Cu complex was examined using different kinds of buffer solutions **Fig. 4 (a)**, e.g., Teorell-Steinhagen, acetate, citrate, succinate, and *BR buffers*. However, the best analytical signal ($I_p$) was obtained with *BR* buffer and therefore was chosen as the preferred buffer for LNG's determination**.**

**Fig. 4 (b)** illustrated the influence of the BR buffer solution's pH on the peak current of the reduction of the LNG-Cu complex, which was tested in the pH range 2.5 - 8.5. The highest value of $I_p$ was observed at pH 4.5. Therefore, the solution pH was adjusted at pH 4.5 using *BR* buffer in the subsequent experiments. This result is due to Lowering or increasing the buffer solution's pH, lowering the solution's conductivity, and decreasing the complexes' sufficient stability [48].

The effect of the concentration of the *BR* buffer solution (pH 4.5) on the square wave stripping voltammetry of LNG-Cu was examined. *BR buffer* having different ionic strength from 0.02 - 0.1 M were considered, and their effect on the peak current is illustrated in **Fig. 4 (c)**. Among the studied ionic strengths of pH 4.5, 0.04 M *BR buffer gave the best result.*

The study concluded that the best medium used for studying the stripping analysis of LNG is the 0.04 M *BR* buffer (pH 4.5).

#### 3.3.2. *Effect of accumulation potential*

The effect of accumulation potential ($E_{ads}$) on stripping peak current (Ip) for LNG in the LNG-Cu complex (LNG: 94.51 ng mL$^{-1}$, pH 4.5, $t_{ads}$ 60 s.) was investigated in the potential range from + 0.2 to + 1.5 V. The peak current of stripping signal increased by increasing the $E_{ads}$ in the range from + 0.2 to + 0.9 V, then decrease by a further increase of the potential from + 0.9 to + 1.5 V. The peak current has its maximum value at accumulation potential of + 0.9 V, which was used in the subsequent examinations.

The plot of the peak current versus the accumulation potential is shown in **Fig. 5.**

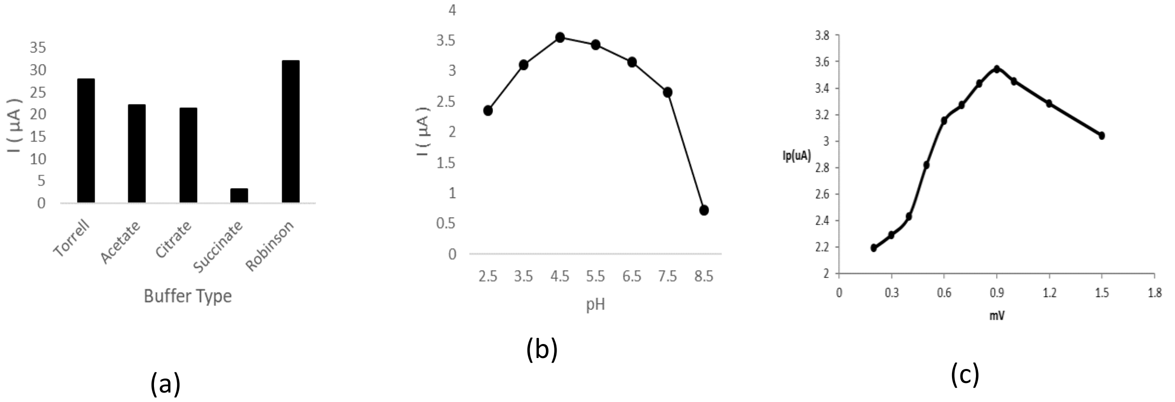


**Fig.4**: (a) Effect of type of buffer, (b)Effect of pH, and (c) Effect of buffer concentration in presence 94.51 ng ml$^{-1}$ of LNG in LNG-Cu complex on electrode surface at 60 s accumulation time

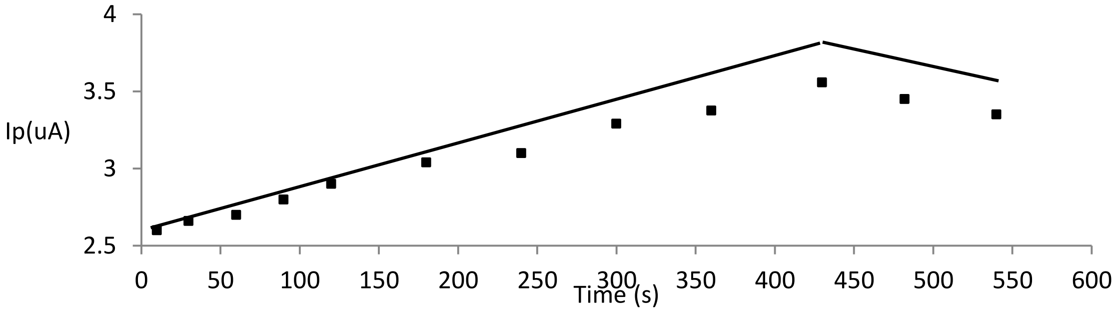


**Fig.5:** The plot of the peak potential ($I_p$) versus different accumulation potentials (94.51 ng mL$^{-1}$ of LNG, 0.04M BR Buffer, pH 4.5, and 60 s accumulation time)

### 3.3.3. *The effect of accumulation time*

The accumulation time ($t_{ads}$) was examined in the range of 10 – 540 s (94.51 ng mL$^{-1}$ LNG, pH 4.5, $E_{ads}$ + 0.9 V). The effect of $t_{ads}$ on the measured peak current at about 1.37 V is shown in **Fig. 6.** Ip's value was increased linearly by increasing the accumulation time up to 430 s; after that, it decreased. The phenomenon of drop-in peak current at a longer adsorption time is characteristic of adsorptive stripping with stirred solutions due to the electrode surface's saturation [49].

Hence, to increase the method's sensitivity, the accumulation time of 430 s was adopted in the subsequent work.The optimum operational parameters selected for determining the LNG-Cu complex by AdSWV at Cu-GPE were shown in **Table 1**. A well-defined complex peak was observed with the stirring at about 1.37 V of the LNG-Cu complex.

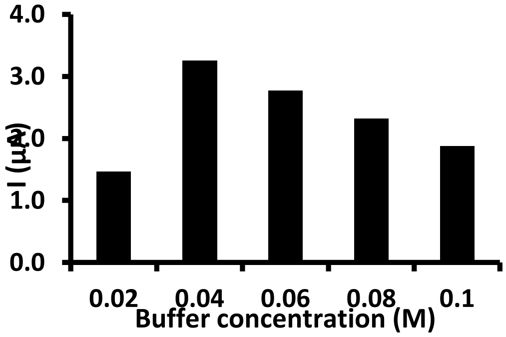


**Fig.6**: The relationship between peak current and accumulation time in the presence of 94.51 ng mL$^{-1}$ (LNG) at $E_{ads}$ +0.9, and 0.04M BR Buffer at pH 4.5

**Table 1:** The optimum operational parameters for the determination of LNG by AdSWV at Cu-PGE

| PARAMETER | SELECTED VALUE |
|---|---|
| Accumulation potential | + 0.9 v |
| Final potential | + 1.5 v |
| Modulation time | 10 s |
| Frequency | 50 HZ |
| Scan increment | 2 mv |
| Accumulation time | various |
| pH | 4.5 |
| Buffer type | 0.04 M *Britton–Robinson* |

### 3.4. *VALIDATION OF THE METHOD*

The methodology of the proposed voltammetric technique was validated according to the International Council for Harmonization (ICH) guidelines [50]. The validation included; linearity, range, detection limit, the limit of quantification, selectivity, accuracy, and precision.

### 3.4.1. ***Linear range***

The LNG-Cu complex's peak current yields a defined concentration dependence using square wave anodic stripping voltammetry (SWASV).

The increase in the anodic peak current of the complex is denoted as follows:

$$\Delta \boldsymbol{I} = \boldsymbol{I_{LNG-Cu}} - \boldsymbol{I_{Cu^{+2}}}$$

Where $\boldsymbol{I_{Cu^{+2}}}$ is the current response of $Cu^{2+}$ alone, $\boldsymbol{I_{LNG-Cu}}$ is the current response of the LNG-Cu complex, and **ΔI** is the difference between them.

Under the optimum condition, the AdSWV technique was applied five times to determine six LNG concentrations. The calibration plot between the measured current peak versus different LNG concentrations is shown in **Fig. 7**. The proposed method has excellent linearity over the concentration range of 47- 284 ng mL$^{-1}$.

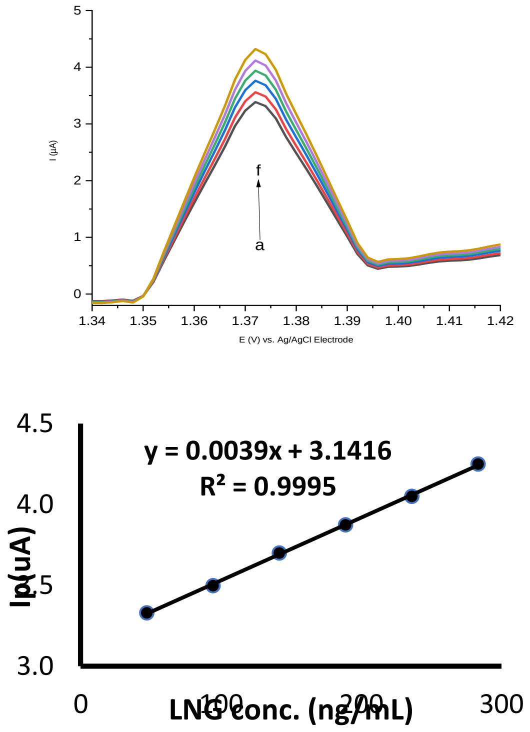


**Fig.7**: Calibration curve of LNG-Cu complex at six concentration (a)47.25, (b)94.51, (c)141.76, (d)189.02, (e)236.27, and (f)283.52 ng ml$^{-1}$ of LNG in LNG-Cu complex on electrode surface at IP +0.9, BR Buffer pH 4.5 0.04M, and 430 s accumulation time.

Other statistical parameters for analysis of LNG by the AdSWV method are shown in **Table 2.**

**Table 2:** Analytical parameters for the voltammetric determination of LNG

| **PARAMETER** | **SELECTED VALUE** |
|---|---|
| Linearity range ng mL$^{-1}$ | 47.25 - 283.52 |
| Slope | $3.88 \times 10^{-3}$ |
| The standard deviation of slope (sb) | $4.38 \times 10^{-5}$ |
| Intercept (µa) | 3.1416 |
| The standard deviation of the intercept (sa) | $8.067 \times 10^{-3}$ |
| Correlation coefficient (r) | 0.9995 |
| The standard deviation of the residuals (sy,x) | 0.59 |
| LOD ng ml$^{-1}$ | 6.0 |
| LOQ ng mL$^{-1}$ | 20.0 |

Where n is the number of experiments n= 5.

### 3.4.2. *limits of detection, and quantitation*

The equations for calculating the limit of detection and the limit of quantitation: were; (LOD =3SD/b) and (LOQ =10SD/b), where (SD) is the standard deviation of intercept and (b) is the slope of the regression line [51-53].

The calculated detection and quantitation limits were 6.0 and 20.0 ng mL$^{-1}$, respectively.

**Table 3** is shown a comparison of the used electrode in the proposed method and other reported methods. As seen in **Table 3**, the proposed method is more sensitive than the reported methods.

**Table 3:** Comparison of the sensitivity of the proposed and reported square wave stripping voltammetric methods using different electrodes

| **Electrode** | **LOD (µg mL$^{-1}$)** | **Ref.** |
|---|---|---|
| **Cu-PGE** | **0.006** | **This work** |
| CPE modified by $Fe_2O_3$ | 0.008 | **[54]** |
| PGE Without modification | 0.10 | **[38]** |
| CPE modified by $Co_3O_4$ NPs and MWCNTs | 5.34 | **[39]** |

### 3.4.3. *Accuracy and precision*

The proposed method's accuracy was tested by analyzing three different LNG concentrations, and the results were presented as % recovery (**Table 4**). The proposed method's high accuracy was revealed from the recovery percentage's closeness to 100 % with a low value of relative standard deviations.

**Table 4:** Accuracy of the proposed method by determination of the LNG-Cu complex in three deference concentration of LNG

| **Conc. Taken from LNG (ng mL$^{-1}$)** | **Recovery* % ± SD** | **RSD** |
|---|---|---|
| **47.25** | 96.09 ± 0.96 | 1.00 |
| **94.51** | 97.93 ± 1.46 | 1.49 |
| **141.76** | 95.72 ± 1.01 | 1.05 |

*Mean of five determinations

Precision was examined at inter-day and intraday precision levels. Three replicated measurements were carried out at three concentration levels. The replicated study was performed within one day for intra-day precision and at three different days for inter-day precision.

The results summarized in **Table 5** show that the calculated RSD was below 2.0 % in both cases, indicating the method's high precision at both repeatability and intermediate levels.

**Table 5:** Evaluation of the intra- and inter-day precisions of the proposed method for the determination of the LNG at three concentrations

| **Precision level** | **Conc. Level (ng mL$^{-1}$)** | **% Recovery* ± SD** | **RSD** |
|---|---|---|---|
| **Intra-day** | 47.25 | 98.25 ± 1.07 | 1.09 |
| | 94.51 | 97.75 ± 1.18 | 1.21 |
| | 141.76 | 96.57 ± 1.75 | 1.81 |
| **Inter-day** | 47.25 | 96.65 ± 0.92 | 0.95 |
| | 94.51 | 97.35 ± 1.91 | 1.96 |
| | 141.76 | 97.85 ± 1.13 | 1.14 |

*Mean of five determinations

### 3.4.4. *Selectivity*

The efficiency and selectivity of the proposed method were evaluated by the determination of a fixed concentration of LNG in the presence of an excessive amount of some common interfering species (1:10), which present in the contents of the tablets, such as $Na_2CO_3$, KCl, thiourea, ascorbic acid, starch, oxalic acid, citric acid, and dextrose, as well as the co-formulated drug, metformin.

The results obtained in **Table 6** indicated that the common interfering species did not significantly affect LNG's determination by the proposed method.

**Table 6:** Effect of the interfering substances on the determination of 94.51 ng mL$^{-1}$ of LNG by using the proposed method

| **Interferent** | **Signal change** |
|---|---|
| **$Na_2CO_3$** | + 2.59 % |
| **KCl** | + 3.63 % |
| **Thiourea** | - 2.56 % |
| **Ascorbic acid** | - 2.19 % |
| **Starch** | - 1.83% |
| **oxalic acid** | + 1.72 % |
| **Citric acid** | - 1.14 % |
| **Dextrose** | - 1.51 % |
| **metformin** | - 5.24 % |

### 3.4.5. *Determination of LNG in the Pharmaceutical dosage form*

The developed voltammetric stripping method was applied for the analysis of LNG in Trajenta® 5 mg tablets. The main % recovery was 99.99, with an SD of 1.43.

These results were compared statistically with the reported method [4]. **Tables 7** illustrate a comparison of the proposed method with the reported method, which indicated that there are no noticeable differences in % recovery and SD between the proposed and reported method as the calculated values of student's t- and F- tests did not exceed the tabulated values at a 95 % confidence level. Thus, the method has an acceptable level of accuracy and precision.

**Table 7:** Application of the proposed method for the analysis of Trajenta® tablets (5 mg of Linagliptin per tablet) included in the LNG-Cu complex by both the proposed and reported methods [4].

| **Parameters** | **Reported method [55]** | **Proposed method** |
|---|---|---|
| % Recovery | 100.07 | 99.99 |
| ± SD | 1.09 | 1.43 |
| t-value[a] | -- | 0.08 |
| F-value[b] | -- | 1.72 |

[a] The value is the mean of five determinations for both methods

[b] Tabulated values at 95% confidence limit are $t$ = 2.306, $F$ = 6.388

### 3.4.6. *Determination of LNG in Spiked plasma and urine*

To examine the effect of plasma and urine components on the proposed voltammetric method's results, aliquots of plasma or urine samples free from the drug after its centrifugation were added to the electrochemical cell 0.04 M BP buffer pH 4.5, and the voltammetric measurement was carried out. After that, plasma and urine samples were spiked with LNG standard solution at three different concentrations and analyzed by the proposed method.

The results of the determination of LNG in spiked plasma and urine are presented in **Tables 8**. The results indicated a functional recovery and an excellent SD for both spiked plasma and urine application.

**Table 8**: Results of using the proposed method to determine the LNG-Cu complex in spiked plasma and urine

| **LNG conc. (ng mL$^{-1}$)** | **spiked plasma Recovery* % ± SD** | **RSD** | **spiked urine Recovery* % ± SD** | **RSD** |
|---|---|---|---|---|
| 47.25 | 90.29 ± 1.00 | 1.10 | 92.70 ± 1.82 | 1.97 |
| 94.51 | 92.37 ± 1.45 | 1.57 | 94.08 ± 1.70 | 1.81 |
| 141.76 | 90.01 ± 1.78 | 1.97 | 93.57 ±1.33 | 1.42 |

* The value is the mean of three measurements

## 5. CONCLUSION

A new, highly sensitive, rapid, selective, and reproducible square wave voltammetric method to determine Linagliptin in pure form, tablets, and biological fluids is described. The proposed method has the highest linearity, detection limit, and quantitation limit over most of the reported methods, and the modified electrode has the most heightened sensitivity overall reported electrodes. Moreover, the proposed method could be applied in clinical laboratories because of its simplicity and low detection and quantitation limits.

## 6. Conflicts of interest

The authors declare that there is no conflict of interest

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