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A facile nano-iron oxide sensor for the electrochemical detection of the anti-diabetic drug linagliptin in the presence of glucose and metformin

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Abstract

Background: A highly sensitive sensor for the electrochemical determination of an antidiabetic drug Linagliptin (LG) was constructed using carbon paste electrode (CPE) modified with iron oxide nanoparticles ($\text{Fe}_2\text{O}_3\text{NPs}$). The electrochemical performance of LG was examined analytically, and some dynamics were considered for the first time.

Results: This work indicates that the oxidation reaction of LG on CPE/ $\text{Fe}_2\text{O}_3\text{NPs}$ is a one electron and one proton process, which is controlled by both diffusion and adsorption. The simultaneous determination of LG with glucose and metformin (MET) was also considered by square wave voltammetry in universal buffer pH 7.4. Experimental results specify a linear relation between LG peak current and its concentration in the range of 0.03 to 86 $\mu\text{g/ml}$, leading to a detection limit of 8.0 ng/ml.

Conclusion: This novel sensor was successfully used to determine LG in commercialized tablets and in spiked urine samples.

Keywords: Diabetes, Iron oxide nanoparticles, Linagliptin, Carbon paste electrode

Background

Finding new methods for the problems in the medical field is the center of researchers everywhere. Diabetes mellitus is a worldwide public health problem. People who have diabetes can develop serious or life-threatening complications, so taking medication(s) may help to manage diabetes and improve health (Association AD 2014). Insulin deficiency and hyperglycemia cause the concentration of blood glucose to increase or decrease than the normal range of 80–120 mg/dL (4.4–6.6 mM), which leads to death (Waldhäusl et al. 1979). The complications of fighting diabetes, include heart disease, kidney failure, or blindness, which can be reduced through personal control of blood glucose (Group UPDS 1998). Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor

developed by [Boehringer Ingelheim](#) for treatment of type II diabetes in patients who cannot control blood sugar levels alone. Linagliptin works by increasing the amount of insulin released and decreasing the amount of sugar made by the body by slowing the breakdown of insulinotropic hormone glucagon-like peptide (GLP)-1 for better glycemic control.

Combination therapy has a pivotal role in type II diabetes mellitus management in patients unable to maintain normal glycemic level using metformin alone. Addition of linagliptin, dipeptidyl peptidase-IV inhibitor, to metformin improves glycemic control. Various analytical techniques are applied for simultaneous determination of binary diabetes drugs mixture such as LG and MET mixture (Haak 2015) including HPLC with UV detector (Vemula et al. 2015), LC-MS/MS (Abbas Moussa et al. 2019), and UV-Vis diffuse reflectance spectroscopy (El-Bagary et al. 2013). No electrochemical

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reported work applied for the determination of either the LG or our studied mixture, only voltammetric determination for MET (Brocks et al. 2010).

Magnetic nanoparticles (NPs) are in the focus of much interest especially iron oxide nanoparticles due to their enormous and unique physical and chemical properties. The most considered materials with promising properties are those with magnetic properties due to their enormous and unique physical and chemical properties and applications such as drug delivery, biomedical uses, and tumor labeling (Jalilian et al. 2009; Polyak and Friedman 2009). These applications require that the nanoparticles have high magnetization values, a size smaller than 100 nm, and a narrow particle size distribution. The nano-size of iron oxide nanoparticles and its biocompatibility make them perfect for surface engineering and functionalization (Laurent et al. 2011; Prodan et al. 2013). Also, Fe₂O₃ NPs provide a path to a new generation of chemical and electrochemical sensors for glucose biosensor applications with some materials such as Pt nanoparticles and multi-walled carbon nanotubes (Li et al. 2010; Rong et al. 2007), Ni oxide nanoparticles (Salimi et al. 2007), and silicon dioxide (Baby and Ramaprabhu 2010). The goal of the present work is to carry out a study on the effect of different parameters such as pH, scan rate, and the effect of changing the percentage of Fe₂O₃Nps added, on the determination of LG raw material, on pharmaceutical formulation, and in human urine samples.

Experimental

Materials

Linagliptin (LG) was supplied from the European Egyptian Pharmaceutical Industries, Egypt, iron nanoparticles. Graphite powder and paraffin oil were purchased from sigma Aldrich. Universal buffer (B–R buffer) 0.04 M of pH 2–11 (CH₃COOH + H₃BO₃ + H₃PO₄), was used as the supporting electrolyte, then 0.2 M NaOH was used to gain the desired pH value.

Apparatus

All voltammetric measurements were performed by stationary electrode using 797VA Computrace software (1.0) from Metrohm, Switzerland, electrochemical analyzer. A three-electrode cell system incorporating the carbon paste electrode as a working electrode and the impedance measurements were done by the three-electrode electrochemical workstation EC-Lab SP-150 potentiostat. A platinum wire as an auxiliary electrode was used with respect to calomel reference electrode. Energy-dispersive X-ray measurements were performed by EDX spectroscopy unit of Model Quanta 250 FEG (Field Emission Gun) with accelerating voltage 30 KV.

Methods

Preparation of CPE/Fe₂O₃NP sensor

Carbon paste electrode (CPE) was prepared by mixing graphite powder (0.5 g) with drops of nujol oil in a glass mortar. CPE/Fe₂O₃NP-modified electrode was prepared by mixing ratios of 5, 10, 12, 15, and 20% modified paste containing 95, 90, 88, 85, and 80 mg of graphite powder and 5, 10, 12, 15, and 20 mg of Fe₂O₃NP modifier mixed, respectively, with suitable amount of paraffin oil. The components were stirred for 10 min in a glass mortar at room temperature to achieve homogenous paste that was used for voltammetric measurements without preconditioning.

Validation in pharmaceutical samples

Five tablets of the commercial pharmaceutical Trajenta (5 mg LG/Tablet) were powdered and dissolved in deionized water. After supersonic treatment for 30 min, it was filtered and the concentration of LG in the working range was achieved, and then, SWV were recorded using the standard addition method.

Analysis of spiked urine samples

Urine was supplied from healthy volunteers; the spiked urine sample was centrifuged for 10 min at 13,000 rpm to get rid of protein residues, and then, the supernatant was taken carefully. An aliquot (1 mL) of the clear solution was added to a mixture of 9 mL B–R buffer to achieve a final concentration of 1.0 mM, at an optimum pH value to perform a calibration curve.

Result

Surface characterization

Figure 1 shows SEM images of the bare CPE and CPE/Fe₂O₃NP-fabricated electrodes. The surface of the CPE is dominated with microstructure with a heterogeneous grain growth, (Fig. 1a). In Fig. 1b, CPE/Fe₂O₃NP surface (Shehata et al. 2016) showed small iron oxide nanoparticles placed at different sites over the CPE surface with a cloudy aggregates and uniform distribution, displaying a larger surface area.

Electrochemical impedance spectroscopy scans were measured at peak potential (1050 mV) for bare CPE, and CPE modified with 10%, 12%, 15%, and 20% Fe₂O₃NPs are shown in (Fig. 2) as Nyquist plots. It shows a semicircle at high frequency indicating a charge transfer process connected to a linear line at low frequency indicating a diffusion process (Azab and Fekry 2017a, 2017b). Thus, the mechanism of the reaction depends on both charge transfer and diffusion processes. The diameter of the semicircle increases in the following order: bare CPE > 10% Fe₂O₃NPs > 12% Fe₂O₃NPs > 20% Fe₂O₃NPs > 15% Fe₂O₃NPs. The Nyquist plot data were simulated with equivalent circuit (inset) with 2% error.

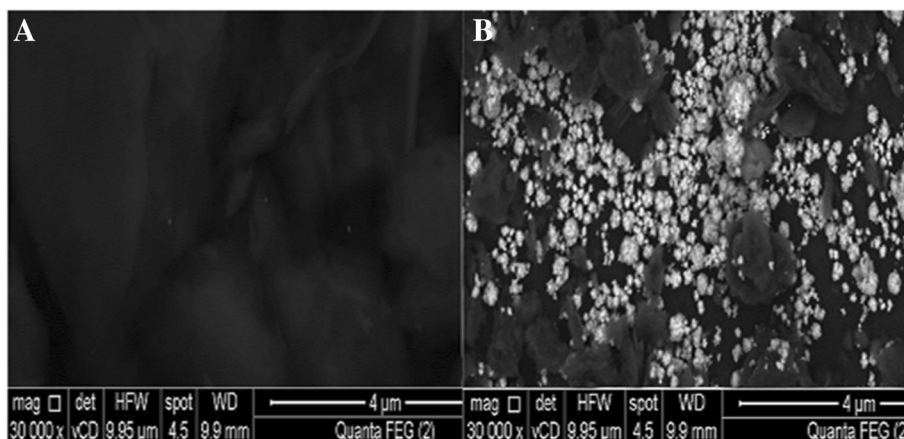


Fig. 1 The SEM image of **a** bare CPE and **b** CPE/Fe₂O₃NPs

Analysis was performed by Thales software provided with the Zahner-Electric workstation (Polyak and Friedman 2009). The model consists of $R_4Z_wC_3$, R_3C_2 , and R_2C_1 in series with the solution resistance (R_1). C refers to the capacitance; R is the resistance, and Z_w is the Warburg component related to diffusion (Bard et al. 1980). Generally, the data shows that upon the addition of 15% Fe₂O₃NPs the oxidation of LG gives lower impedance value (highest conductivity) and highest diffusion process ($W = 41.7 \Omega \text{ cm}^2 \text{ s}^{-1/2}$) compared to $W = 37.1 \Omega \text{ cm}^2 \text{ s}^{-1/2}$ for the bare CPE, due to large surface area (Azab and Fekry 2017a, 2017b; Mohamed et al. 2017) that NPs provide giving a nano-environment with

high electron communication features for the electrochemical detection of LG with fast and good capacity which progress the direct electron movement between the active sites of NPs and LG. The same result was obtained in the SWV of 1.0 mM LG (inset), in which it was clear that the highest anodic peak current was achieved when 15% Fe₂O₃NPs was used.

The cyclic voltammograms of hexacyanoferrate (II) (Fig. 3) showed well-defined redox peaks for the modified CPE/Fe₂O₃NPs, with mid-point potential at ca. +0.450 V vs Ag/AgCl (slightly higher than for the bare CPE at ca. +0.400 V). The shapes of the CVs were almost unaffected by the scan rate, and both cathodic and anodic peak currents depend linearly on the square root of the scan rate over the entire range of scan rates considered, indicating a diffusion-controlled electrochemical process, with no adsorption on the Fe₂O₃NP. When Fe₂O₃NP is combined to the graphite paste, larger differences between oxidation and reduction peaks

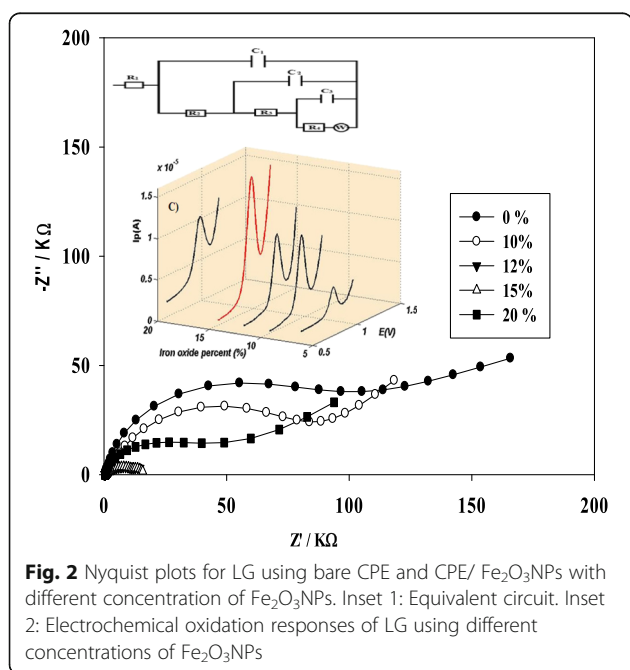


Fig. 2 Nyquist plots for LG using bare CPE and CPE/ Fe₂O₃NPs with different concentration of Fe₂O₃NPs. Inset 1: Equivalent circuit. Inset 2: Electrochemical oxidation responses of LG using different concentrations of Fe₂O₃NPs

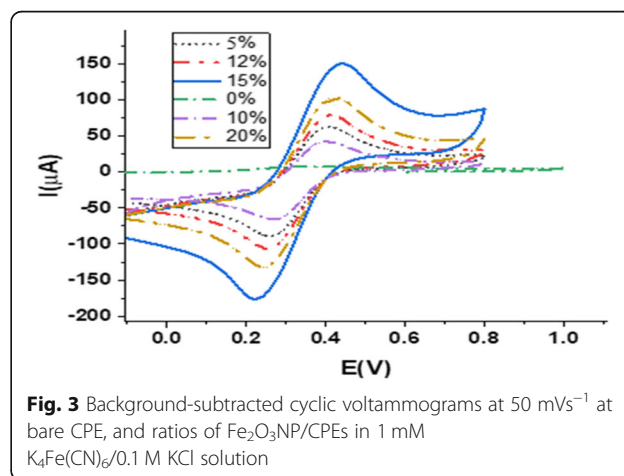


Fig. 3 Background-subtracted cyclic voltammograms at 50 mVs⁻¹ at bare CPE, and ratios of Fe₂O₃NP/CPEs in 1 mM K₄Fe(CN)₆/0.1 M KCl solution

were observed which slightly increased with increasing scan rate. This is common on carbon electrode surfaces (Yogeswaran and Chen 2007).

Parameters optimization

Effect of pH: The effect of solution pH on the electrochemical response of LG at CPE/Fe₂O₃NPs was recorded in B–R buffers and was found to be discerning over the pH range of 2–11. The peak potentials decrease gradually in solutions as pH is raised, manifesting that protons have taken part in the electrode reaction processes. The results show that pH7 and 9 gave the maximum anodic peak current response, because the surface of iron oxide atoms act as Lewis acids and coordinate with water, which dissociates giving amphoteric hydroxyl group. These hydroxyl groups carry negative charges (Lefebure et al. 1998). And since the pKa value of LG is 9.86, therefore, an electrostatic attraction force was induced between the positively charged LG at alkaline

pH values and the negatively charged iron oxide nanoparticles surface which give a high current response. So, the rest of the work will be performed at pH 7.4 (physiological pH of the body).

Effect of varying the scan rate: The effect of varying the scan rate over the range of 10–240 mV/s was considered to detect the diffusion and adsorption characters of 1.0 LG at CPE/Fe₂O₃NP surface in B–R buffer pH 7.4 using cyclic voltammetry (Fig. 4). The positive shift in the peak potential commends that the oxidation process is irreversible. The relation between the peak current versus square root of scan rate was constructed (inset A) giving a straight line with an equation of $I_p (\mu A) = 1.08\sqrt{v} - 0.153$; $R = 0.9994$, which indicates mass transfer-controlling process of oxidation via diffusion. Also, a plot of log peak current with log scan rate gives a linear relationship with a slope of 0.494 (inset B), suggesting that LG oxidation on CPE/Fe₂O₃NPs is mainly controlled by diffusion with some adsorption support.

The surface areas of CPE and CPE/Fe₂O₃NPs were calculated using 1.0 mM K₃ Fe (CN)₆ when $n = 1$ and

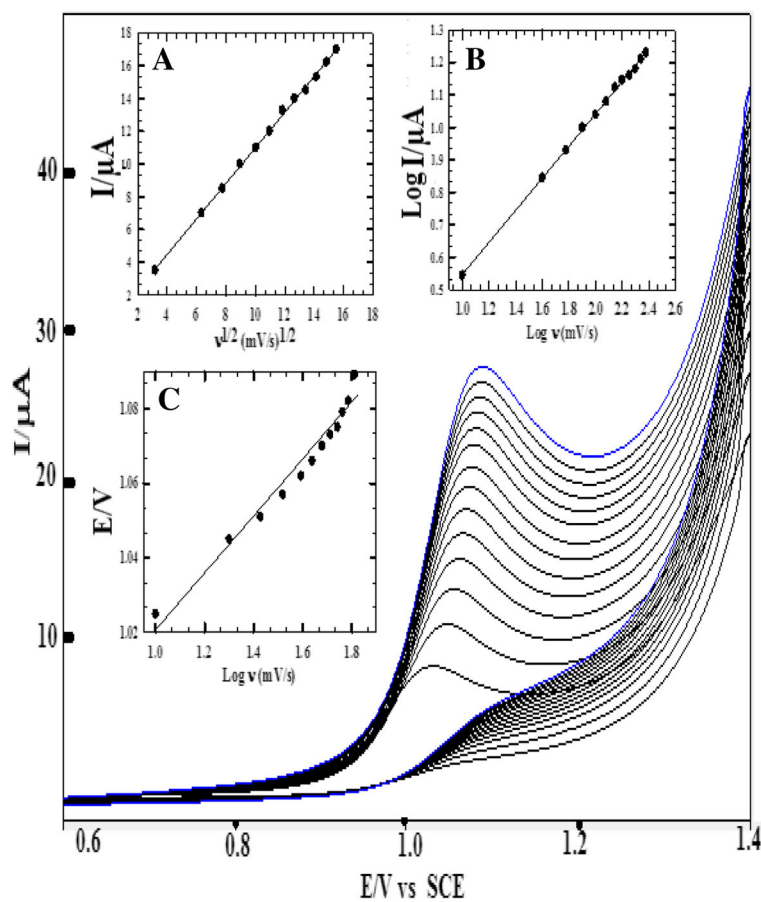


Fig. 4 Effect of varying the scan rate on 1.0 mM LG at CPE/Fe₂O₃NPs in B–R buffer pH 7.4 using cyclic voltammetry. Inset a: Plot peak current versus square root of scan rate. Inset b: Plot of log peak current versus log the scan rate. Inset c: Plot of peak potential versus log the scan rate

$D = 7.6 \times 10^{-6} \text{ cm}^2/\text{s}$ using the Randles–Sevcik equation (Compton and Banks 2007; El-Shal et al. 2019)

$$I_p = 2.69 \times 10^5 n^{3/2} AD^{1/2} \nu^{1/2} C \quad (1)$$

The surface areas were evaluated to be 0.072 cm^2 and 0.143 , for CPE and CPE/Fe₂O₃NPs, respectively, which indicates that the addition of iron oxide nanoparticles doubled the surface area of the sensor and therefore increases the electroactive sites that enhanced the current response of LG.

Also, the determination of the sensor dynamics parameters (α and n) is essential to determine the proposed mechanism of LG, Scheme 1. The relationship between E_p and $\log \nu$ (inset C) is given by the Laviron equation (Shen et al. 2009):

$$E_p = E_0 + [2.303RT/(anF)] \log [(RTk_s)/(anF)] + [2.303RT/(anF)] \log \nu \quad (2)$$

where α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction, and E_0 is the formal potential obtained from the relation between E_p and ν by extrapolating to the vertical axis at $\nu = 0$ and it was found to be 1.04 V , an can be calculated from the slope of E_p vs. $\log \nu$ (inset C). In this system, the slope is 0.0763 , an was calculated to be 0.777 . From the Bard and Faulkner equation (Bard et al. 1980) and the CV plot of LG.

$$\alpha = \frac{47.7}{E_p - E_p/2} \text{ mV} \quad (3)$$

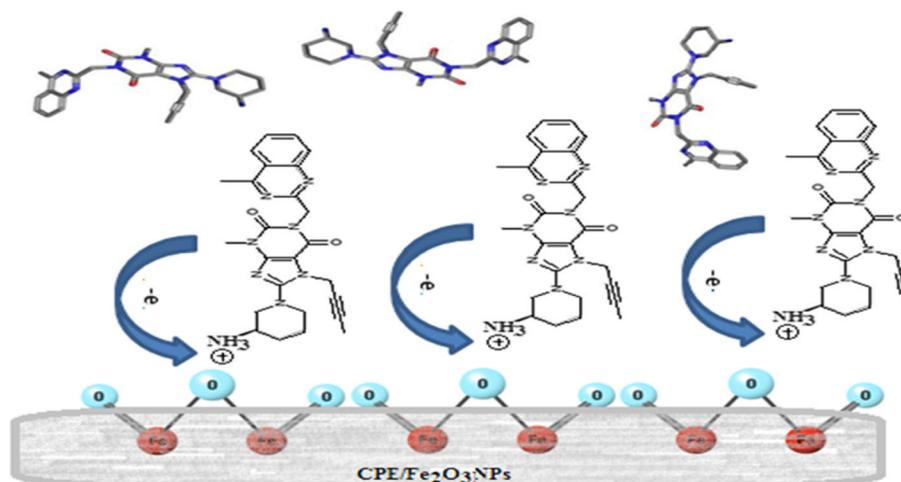
The electron transfer coefficient was found to be 0.53 , and then, n was calculated to be $1.2 \approx 1$ which

indicated that only one electron was involved in the oxidation of LG. The value of k_s can be calculated from the intercept of the Laviron equation and was found to be $1.06 \times 10^2 \text{ s}^{-1}$.

Discussion

The electrooxidation performance of LG was documented as CV, in the range of 800 to 1300 mV (Fig. 5), in B–R buffer pH 7.4, at a scan rate of 100 mV s^{-1} for the tested electrodes: (bare CPE) and (CPE/Fe₂O₃NPs). For bare CPE, the anodic peak oxidation of LG appeared at $E_p = 1050 \text{ mV}$ with a current value of $2.5 \mu\text{A}$. The peak current increases upon adding Fe₂O₃NPs; this designated that the Fe₂O₃NPs has increased the sensor surface area. Finally, for 15% modified CPE/Fe₂O₃NPs, the anodic peak current extended to a highest value of $6.9 \mu\text{A}$. This may be attributed to an electrostatic attraction among the cationic LG and the anionic Fe₂O₃NPs forming hydrogen bond that endorses faster electron transfer developing well the diffusion of LG among the surface. The schematic demonstration for this contact is explained in Fig. 5 inset.

Square wave voltammograms of LG with different concentrations from 0.03 to $86.2 \mu\text{g/ml}$ using CPE/Fe₂O₃NPs were presented in Fig. 6. The International Conference on Harmonization (ICH) guidelines (I. H. T. (Guideline 1997)) for method validation were followed for validation of the suggested method. The current response increases steadily as a function of LG concentration, giving a calibration curve with a correlation coefficient of 0.9993 (inset A). Also, the detection limit $\text{LOD} = 3s/m$ and the quantification limit $\text{LOQ} = 10s/m$ were calculated, where s is the standard deviation and m is the slope of the calibration curves which were found to be $0.008 \mu\text{g/ml}$ and $0.026 \mu\text{g/ml}$, respectively.



Scheme 1 The suggested mechanism for LG at CPE/Fe₂O₃NPs

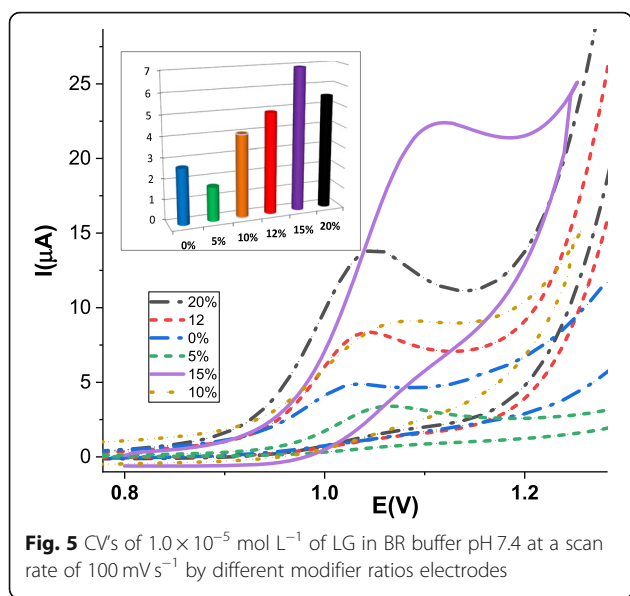


Fig. 5 CVs of 1.0×10^{-5} mol L⁻¹ of LG in BR buffer pH 7.4 at a scan rate of 100 mV s^{-1} by different modifier ratios electrodes

The high sensitivity of film can be attributed to the increase of the surface area with the iron oxide nanoparticles, the diffusion and adsorption mechanisms, high catalytic activity, and biocompatibility of the iron oxide NPs. The evaluated sensitivity of the fabricated CPE/Fe₂O₃NP sensor is relatively higher than that of previously reported techniques (Table 1).

This is because the oxidation of Fe₂O₃NPs in aqueous media, in accordance with the dissolved oxygen (O₂) surrounding the electrode surface, leads to an increase in the adsorbed oxygen on the Fe₂O₃NP surfaces, which therefore increases oxidizing capability of the analyte. According to the following mechanisms (Banik et al.

2015; El-Bagary et al. 2013; Padmaja and Veerabhadram 2015; Bailey and Turner 1996:



Also, the reduced LG donates electrons to Fe₂O₃NP surface. Therefore, resistance is decreased, or conductance is increased.

Influence of the square wave frequency (f)

The square wave frequency determines the intensity of the signal and the sensitivity of the technique. The relation between the anodic current and the square wave frequency shows a linear relation up to 140 s^{-1} (Fig. 6, inset B). For larger values of frequency, the effect is almost negligible (O’Dea et al. 1993). This behavior corresponds to a totally irreversible process controlled by adsorption of the analyte on the electrode surface. Also, the peak potential should vary linearly with the logarithm of the frequency following the relationship:

$$\frac{\Delta E_p}{\Delta \log f} = \frac{2.3 RT}{\alpha n F} \quad (6)$$

A value of 0.66 was determined for αn , and as the electron transfer coefficient was found to be 0.53, therefore, $n = 1.2$, indicating the transference of 1 electron per LG molecule, confirming the data obtained in the scan rate section. Thus, the suggested mechanism for LG is presented in Scheme 1.

Specificity test

Specificity of the method is monitored by measuring the current response of an analyte in the presence of other interferences. Glucose sensors account for approximately 85% of the biosensor industry. Non-enzymatic glucose electrodes give higher oxidation current response, higher stability, lower cost, and greater sensitivity. Electrocatalytic processes are important for glucose oxidation, since it is a kinetically very slow process with negligible faradaic current, showing a non-diffusion-controlled process (Vassilyev et al. 1985). The electrocatalysis occurs via the adsorption of analyte on the electrode surface, which involves the d-electrons to form bonds with the analyte (Pletcher 1984).

In this work, the specificity was checked by analyzing LG with MET and glucose in laboratory-prepared mixtures using bare CPE and CPE/Fe₂O₃NPs in Fig. 7. No peak was observed for glucose in case of bare CPE, and only a broad peak was observed for MET and LG, while CPE/Fe₂O₃NP sensor exhibits three good resolution anodic peaks: the first was for glucose which was oxidized to form glucolactone, which hydrolyses in H₂O to form

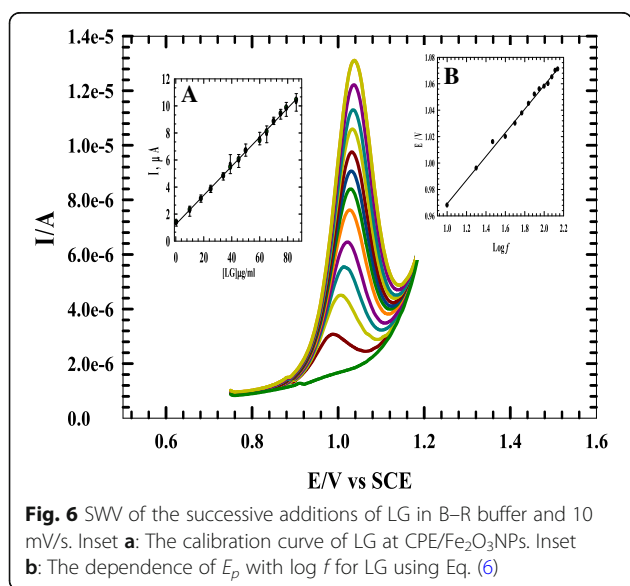


Fig. 6 SWV of the successive additions of LG in B-R buffer and 10 mV/s . Inset a: The calibration curve of LG at CPE/Fe₂O₃NPs. Inset b: The dependence of E_p with $\log f$ for LG using Eq. (6)

Table 1 Comparison of the proposed method with other methods

Method	Linear range (µg/ml)	Solvent	LOD (µg/ml)	Recovery	Reference
Spectrophotometric	8–32	H ₂ O	0.247	100.39%	(Shehata et al. 2016)
HPLC	0.125–4	Methanol	0.03	11.07%	(Shen et al. 2009)
Spectrophotometric	2–12	Methanol		99.86%	(Vassilyev et al. 1985)
RP-HPLC	10–50	Methanol and phosphate buffer	1.65	99.79%	(Vemula et al. 2015)
CPE/Fe ₂ O ₃ NPs	0.03–86	Universal buffer	0.008	100.1%	Proposed method

gluconic acid giving an anodic peak current at 260 mV, and the second peak at 760 is due to the oxidation of the imino group in guanidino group to a N-hydroxy imino group of metformin (Jerez et al. 2014), while the third one is due to LG oxidation at 960 mV. Also, linagliptin’s recovery was examined with some related substances that potentially present with LG in the dosage formulation under optimum conditions when the concentration of each constituent was 1.0×10^{-3} M and was found to be 99.66, 98.10, 99.93, 99.60, and 98.92 for glucose, metformin, talc, urea, and starch, respectively.

These results indicate that these substances did not interfere with LG, which confirms the specificity of the proposed method, since the tolerance limit was less than 2%.

Application of linagliptin

In pharmaceutical formulation: For the purpose of the practical applicability of the proposed method and the modified sensor, the SWV method was applied for the investigation of LG in five Trajenta (5 mg/tablet) tablets without any sample extraction to obtain 1.0 mM LG solution. The validity of the proposed method was evaluated by the standard addition technique, and the accuracy has

been determined giving satisfactory recoveries from 99.98 to 101.9%, and relative standard deviation from 0.04 to 0.63% reveals that the excipient presented in the tablet does not interfere with the active ingredient. The results suggested that CPE/Fe₂O₃NPs has high reproducibility and would be useful sensor for quantitative analysis of LG in pharmaceutical formulations.

In spiked urine sample: The validation of CPE/Fe₂O₃NPs for the quantitative assay of LG in urine was examined in B–R buffer pH 7.4. Six different concentrations on the calibration curve are chosen to be repeated for five times to evaluate the accuracy and precision of the proposed method, which shows a recovery values from 99.9–100.1%. Also, the statistical analysis of the results was obtained by applying the proposed and the reported methods, and the mean ± S.D. of LG in urine sample was found to be 100.05 ± 0.25 for the proposed method with *t* (2.776) value of 0.575 and *F* (19) value of 3.760 (*n* = 4), while the mean ± S.D. for the reported method was found to be 99.95 ± 0.13 when the values between parentheses was 85%.

Conclusion

This work describes a new and very simple CPE sensor for the electrochemical determination of linagliptin in pharmaceutical formulations, in urine samples, and in the presence of glucose and the co-administered drug MET using magnetic iron oxide nanoparticles. Some dynamic parameters are considered for the first time. The results showed that the method was sensitive, precise, and selective with no significant effects of the excipients background. A linear relation between LG peak current and its concentration in the range 0.03 to 86 µg/ml leads to a detection limit of 0.008 µg/ml.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

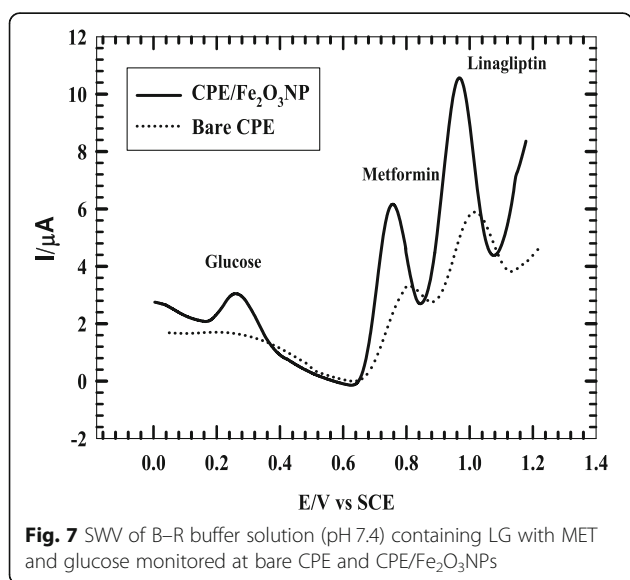


Fig. 7 SWV of B–R buffer solution (pH 7.4) containing LG with MET and glucose monitored at bare CPE and CPE/Fe₂O₃NPs

Authors' contributions

All authors contributed in this work and in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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