

Fabrication of a modified electrode based on multi-walled carbon nanotubes decorated with iron oxide nanoparticles for the determination of enrofloxacin

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Multi-walled carbon nanotubes decorated with Fe₃O₄ nanoparticles (Fe₃O₄NPs/MWCNTs) were prepared and used to fabricate an electrochemical sensor for the determination of enrofloxacin. The electrochemical response of the carbon paste electrode (CPE) modified with Fe₃O₄NPs/MWCNTs towards enrofloxacin was investigated by cyclic voltammetry and differential pulse voltammetry. The results indicated a remarkable growth in the oxidation peak current together with negative shift in the oxidation peak potential for enrofloxacin, in comparison to both the unmodified CPE and the CPE modified with MWCNTs (MWCNT/CPE). The prepared sensor displayed a rapid and sensitive response towards enrofloxacin, which was attributed to the synergetic electrocatalytic effect of Fe₃O₄NPs and MWCNTs. The sensitivity enhancement with nearly three orders of magnitude (0.3–100 µM) was gained, compared with the MWCNT/CPE, with a detection limit of 0.09 µM (S/N = 3). The proposed method was successfully applied for the highly sensitive determination of trace amounts of enrofloxacin in cow's milk, egg, honey and chicken samples.

1. Introduction: Enrofloxacin is a synthetic chemotherapeutic agent from the class of quinolone carboxylic acid derivatives. It has antibacterial activity against a broad spectrum of Gram negative and Gram positive bacteria. It is rapidly absorbed from the digestive tract, penetrating into all measured body tissues and fluids [1]. Enrofloxacin has a satisfactory effect in the treatment of dogs, chickens, turkeys, cattle and horses [2]. However, the long-term application of fluoroquinolone would lead to an increase of resistant bacteria species in poultry and also drug residues in animal muscle and tissue, which is a significant threat to human health through the food chain. The European Union (EU) has fixed a maximum residue limit in edible animal products for some quinolones, such as enrofloxacin. The maximum residue limit values of Enro in milk and muscle tissues are 100 µg l⁻¹ and 300 µg kg⁻¹, respectively [3]. Therefore, it is essential to establish a simple, sensitive and applicable method for detecting Enro residues in different matrices.

In recent years, many analytical methods have been developed for the measurement of Enro (Table 1), including chemiluminescence (CL) enzyme immunoassay [4], enzyme-linked immunosorbent immunoassay [5], the phosphorimetric method [6], high-performance liquid chromatography [7] and CL analysis [8]. All these techniques have their advantages and disadvantages. These methods have been proved to be sensitive and accurate, but they are not suitable for the high-throughput monitoring of Enro because they are expensive and time-consuming, require complex and tedious sample pretreatment, and suffer practical difficulties. However, electrochemical techniques have some advantages, such as low cost, easy application, and providing direct, sensitive and fast detection of lower concentrations. Ensafi *et al.* [9, 10] reported adsorptive cathodic stripping voltammetry (ADSV) using the complexing agent Cu(II) as a simple and sensitive electroanalytical method for the determination of enrofloxacin in plasma and some pharmaceutical samples with a 0.33 nM detection limit. However, the linear dynamic range of the method is narrow, and the toxicity of mercury (as an electrode) has caused a decline in its application in routine laboratories.

Multi-walled carbon nanotubes (MWCNTs) were discovered by Iijima [11]; since then, this material has attracted enormous interest

because of its unique structural, mechanical and electronic properties. Modification of the surface of carbon nanotubes (CNTs) with different materials, such as metals, metal oxides, complex metal oxides and polymers, can improve characteristics of CNTs. Decoration of CNTs by nanoparticles can improve the optical, magnetic and electrochemical properties of CNTs. The modification of the electrode with decorated MWCNTs for use in analytical sensing is well documented and results in low detection limits, increased sensitivities, reduced overpotentials and resistance to surface fouling [12–14].

Low toxicity, good biocompatibility, simple preparation, and strong superparamagnetic properties are some of the benefits of Fe₃O₄ nanoparticles, which make for increasing interest in the application of this nanomaterial in the construction of biosensors and chemical sensors. For example, the application of Fe₃O₄ magnetic nanoparticles as a modifier on the surface of a glassy carbon electrode has been reported by Yin *et al.* They used this modified electrode for the investigation of the oxidation of guanosine and for the determination of bisphenol A [15, 16].

In this Letter, the electrocatalytic activity of Fe₃O₄-MWCNTs towards the oxidation of enrofloxacin is demonstrated. The results show a considerable improvement in the corresponding anodic peak current, which is due to the catalytic effect of Fe₃O₄/MWCNT/CP in the electrochemical oxidation of Enro. This catalytic behaviour of Fe₃O₄-MWCNTs allows the development of a sensitive electrochemical sensor for the determination of Enro in different matrices, such as food and pharmaceutical samples. Long-term stability, excellent catalytic activity and high reproducibility are some of the remarkable properties of the developed sensor. The modified electrode is successfully applied for the determination of Enro in food samples such as milk, honey, egg and chicken meat.

2. Materials and method:

2.1. Chemicals and reagents: MWCNTs with 10–20 nm o.d., 5–10 nm i.d., 20–200 nm tube length and of purity more than 95% were purchased from the Research Institute of Petroleum Industry (RIPI, Tehran, Iran). Spectroscopic-grade mineral oil (Nujol) was obtained

Table 1 Comparison of some characteristics of different methods for the determination of enrofloxacin

Method	LOD	Linear dynamic range	Real sample	Reference
CLEIA ^a	0.03 ng/ml (8.3×10^{-11} M) ^c	0.35–100 ng/ml (9.7×10^{-10} – 2.8×10^{-5} M)	milk, egg, honey	[4]
ELISA ^b	0.07 $\mu\text{g l}^{-1}$ (1.9×10^{-10} M)	0.07–1.3 $\mu\text{g l}^{-1}$ (1.9×10^{-10} – 3.6×10^{-9} M)	chicken muscle	[5]
HPLC/fluorescence	—	10–50 $\mu\text{g l}^{-1}$ (2.8×10^{-8} – 1.4×10^{-7} M)	goat milk	[17]
Phosphorimetric method	1.1 ng (3.1×10^{-12} mol)	4.7–180 ng (1.3×10^{-11} – 5.0×10^{-7} mol)	urine and drugs samples	[6]
HPLC	0.013 $\mu\text{g ml}^{-1}$ (3.6×10^{-8} M)	4.0–108 $\mu\text{g ml}^{-1}$ (1.1×10^{-5} – 3.0×10^{-4} M)	injection and tablets containing drugs	[7]
MWCNT/GCE	0.18 $\mu\text{g l}^{-1}$ (5.0×10^{-10} M)	0.7–280.3 $\mu\text{g l}^{-1}$ (1.95×10^{-8} – 7.8×10^{-7} M)	plasma and pharmaceutical samples	[18]
ACSV ^c	0.33 nmol l ⁻¹ (3.3×10^{-10} M)	10.0–80.0 nmol l ⁻¹ (1.0×10^{-8} – 8.0×10^{-8} M)	plasma and pharmaceutical samples	[19]
TFC–MS/MS ^d	25 $\mu\text{g kg}^{-1}$	—	cattle, pig, turkey, rabbit	[20]
Fe ₃ O ₄ /MWCNT/CPE	9×10^{-8} M	3.0×10^{-7} – 1.0×10^{-4} M	plasma and pharmaceutical samples	this study

^aCL enzyme immunoassay.^bEnzyme-linked immunosorbent immunoassay.^cAdsorptive cathodic stripping voltammetry.^dTurbulent flow chromatography/tandem mass spectrometry. LOD, limit of detection.^eUnit has been changed to M for easy comparison.

from Aldrich and used as received. All the other chemicals were of analytical reagent grade from Merck (Darmstadt, Germany). All aqueous solutions were prepared with double-distilled water (DDW). A stock standard solution of Enro (0.01 M) was prepared in 0.1 M acetate buffer. The working solutions were prepared by serial diluting the stock solution with DDW.

2.2. Apparatus: All electrochemical experiments were carried out with a potentiostat-galvanostat (Computrace 797 VA) electrochemical system in conjunction with a 663VA Stand Metrohm (Swiss) electrochemical cell. A conventional three-electrode cell was used with a saturated Ag–AgCl reference electrode, a carbon-paste working electrode (with or without modifier) and a Pt wire as the counterelectrode. The buffer solutions deoxygenated by nitrogen (99.999%) purging were used for all voltammetric experiments. During the experiments, nitrogen gas was maintained over the surface of the test solutions. A digital pH meter (Metrohm model 827) was used to follow the pH values of the solutions. Fourier transform IR spectra were obtained using a JASCO FTIR spectrometer (model 680 plus) using KBr pellets. The transmission electron microscope (TEM) images were recorded using a PHILIPS TEM model CM30 by placing one drop of the dispersed sample in ethanol on a carbon-coated copper grid.

2.3. Preparation of Fe₃O₄/MWCNT and Fe₃O₄/MWCNT/CPE: To remove the impurities and obtain open-end MWCNTs with hydrophilic surfaces 1.00 g of MWCNTs was added to a 50 ml mixture solution of nitric acid and sulphuric acid (volume ratio of 1:3) in a 100 ml flask and refluxed at 120°C for 20 h. Subsequently, the mixture was cooled down to room temperature, diluted with DDW and filtrated. The functionalised MWCNTs (MWCNT-COOH) were washed with DDW and dried for 5 h at 50°C in a vacuum oven.

An in situ co-precipitation method was developed to obtain the Fe₃O₄NPs/MWCNTs composite. First, 0.5 g functionalised MWCNTs was added into 10 ml DDW and ultrasonicated for 10 min. Afterwards, the suspension was mixed with a 50 ml solution of analytical grade of 0.02 M FeCl₃·6H₂O and 0.01 M FeSO₄·7H₂O (the Fe^{II}:Fe^{III} molar ratio was maintained at 1:2) and was heated at 60°C for 2 h. Then, the mixture was cooled to room temperature and the pH value of the mixture reached 10.0 by dropping 1 M ammonium hydroxide solution. The entire process was done under N₂ atmosphere. The Fe₃O₄NPs/MWCNTs were separated with a magnet and washed with DDW

repeatedly. The resulting product was dried in an oven at 100°C overnight.

To prepare the modified Fe₃O₄NPs/MWCNT carbon paste electrode (CPE) (Fe₃O₄NPs/MWCNT/CPE), a 50.0 mg portion of Fe₃O₄NPs/MWCNTs was well mixed with 450.0 mg of graphite powder and then the mixture was dispersed in 2.0 ml of DMF and homogenised ultrasonically for 20 min. The DMF was evaporated at 100°C in an oven overnight. Graphite powder consisting of 10% w/w Fe₃O₄NPs/MWCNTs was mixed with Nujol mineral oil (30% w/w) using a mortar and pestle for 20 min to obtain a well-mixed paste. This composite mixture was packed into the end of a glass tube with a 3 mm inner diameter and 25 cm length. Electrical contact was made by forcing a copper wire down the glass tube into the paste. Unmodified electrodes (UMCPE) and a MWCNT-modified electrode (MWCNT/CPE) were prepared in the same way without the addition of Fe₃O₄NPs/MWCNTs, or with the addition of MWCNTs (10%w/w), respectively. The working surface of the electrodes was polished using a soft polishing tissue to obtain a shiny surface to ensure a clean, flat and repeatability of the electrodes surface. Then, the electrodes were rinsed with distilled water. Before differential pulse voltammetric measurements, the modified electrode was cycled five times between –0.5 and 1.5 V (scan rate 100 mVs⁻¹) in 0.1 M acetate buffer solution (pH 4.4).

2.4. Preparation of real samples: Several commercial food samples (egg, milk, chicken and honey) were purchased from local markets. A measure of 1.0 g aliquot of honey or egg (mixture of yolk and albumin) and 2.0 ml of cow's milk was transferred to a 25 ml Erlenmeyer flask (with a screw cap) and spiked with known variable amounts of enrofloxacin. Then, 1.0 ml of 0.1 M phosphate buffer pH 3.0 was added and shaken for 10 min. A measure of 1.0 ml of n-hexane and 2.0 ml of acetonitrile were added to the solution and shaken for 15 min. This mixture was transferred to a 10 ml centrifuge tube, and centrifuged at 6000 rpm for 8 min. The organic phase was transferred to a glass tube, evaporated to dryness using a stream of nitrogen at room temperature and then made up to 10 ml with acetate buffer. The samples were then measured by the procedure described above. A weighed aliquot of chicken (3.0 g) was homogenised in 3.0 ml of acetate buffer pH 5.0 using an SD-3 homogeniser (Pars Khazar Manufacturing, Iran) for 15 min. Afterwards, the sample was shaken with 3 ml-acetonitrile for 5 min. Then, it was centrifuged for 10 min at 5000 rpm and decanted into a polypropylene container. The residue of chicken was extracted with another 3.0 ml aliquot of acetonitrile. The combination of two extracts was

evaporated to dryness under a nitrogen stream. The dried extract was redissolved in 2.0 ml acetate buffer pH 5.0 and used for the analysis.

3. Results and discussion

3.1. Characterisation of Fe₃O₄NPs/MWCNT: TEM and FTIR spectroscopy were used to study the microscopic structure of Fe₃O₄NPs/MWCNTs. Fig. 1a shows that the well-distributed Fe₃O₄NPs have been deposited on the surface of the MWCNTs. It was also estimated that the size of the iron oxide nanoparticles immobilised on the MWCNTs was less than 10 nm. Fig. 1b illustrates the FTIR spectra of bare MWCNTs (MWCNT-COOH) and MWCNTs decorated with Fe₃O₄. The spectra clearly show an absorption band at around 3440 cm⁻¹, which is the characteristic stretching vibration of the hydroxyl functional group (O-H) on the surface of the CNTs or adsorbed water in the sample. For both MWCNT-COOH and Fe₃O₄-MWCNTs the spectra show C=C, C=O and C-O stretching vibrations at 1634, 1713 and 1150 cm⁻¹, respectively. Moreover, the peak at 588 cm⁻¹ is attributed to the characteristic Fe-O stretching vibration of iron oxides, indicating that the Fe₃O₄/MWCNT composite includes magnetite.

3.2. Voltammetric response of enrofloxacin: Cyclic voltammograms of 0.05 mM enrofloxacin in 0.1 M acetate buffer pH 5.0 at the surface of unmodified CPE, (UMCPE, curve a), CPE modified with MWCNT (MWCNT/CPE, curve b) and with Fe₃O₄ decorated MWCNTs (Fe₃O₄/MWCNT/CPE, curve c) are shown in Fig. 2. As shown, the electro-oxidation of Enro at

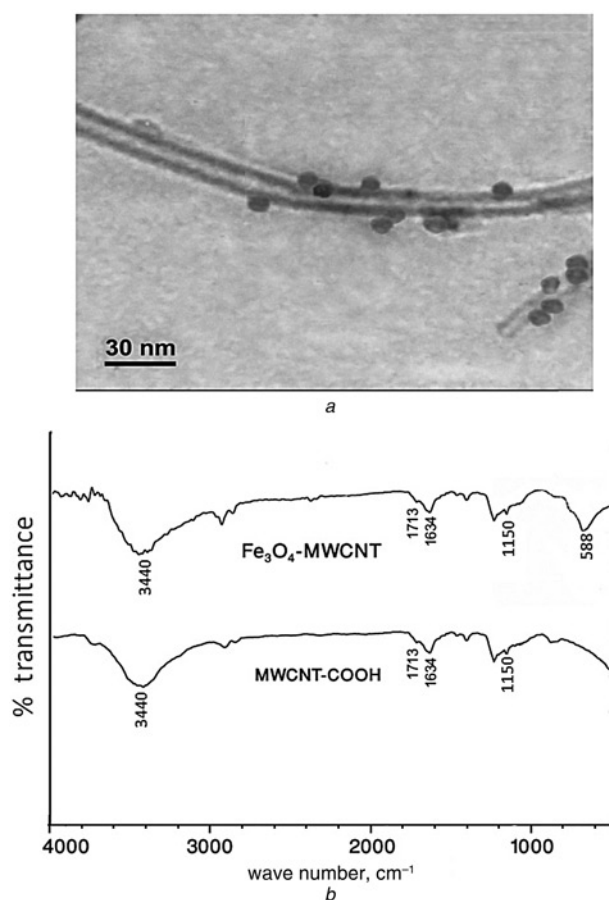


Figure 1 TEM image of Fe₃O₄/MWCNTs, and FTIR spectra of MWCNT-COOH and Fe₃O₄/MWCNTs
a TEM image
b FTIR spectra

UMCPE results in a wide anodic wave with an anodic peak potential of about 0.94 V. A considerable enhancement in the peak potential and peak current of Enro at Fe₃O₄/MWCNT/CPE was observed in comparison with MWCNT/CPE and UMCPE. Such a large enhancement of anodic current revealed that the kinetics of electron transfer for Enro improves remarkably at the Fe₃O₄/MWCNT/CPE. On the other hand, multi-walled carbon nanotubes and Fe₃O₄ nanoparticles have a synergetic effect on the catalytic oxidation of Enro. This behaviour could be due to the presence of edge sites in the structure of carbon nanotubes, electroactive oxygenated functional groups, high electrical conductivity and the specific electronic structure of MWCNTs.

Fig. 3a shows the voltammetric evaluation of Fe₃O₄/MWCNT/CPE at different scan rates ranging from 25 to 400 mVs⁻¹ in a solution containing 0.05 mM Enro (pH 5.0). The linear dependence of peak current (*I*_p, μA) against scan rate (*v*, mVs⁻¹) indicates adsorption-like cyclic voltammograms (Fig. 3b) [21]. A possible explanation for such a behaviour is due to the adsorption of Enro on the surface of the modified electrode because of the unique properties of CNTs, such as strong adsorption ability, high specific surface area and the presence of functional groups. The *I*_p-*v* plot was no longer linear at scan rates faster than 400 mVs⁻¹, suggesting that the rate-determining step was controlled by the electron transfer process. The shift of the peak potential to more positive values by increasing the scan rate confirmed this suggestion (Fig. 3c). The relationship between the oxidation peak potentials and the logarithm of (*v*) is also linear with the corresponding equation

$$E_p(\text{mV}) = 56.34 \log(v, \text{mVs}^{-1}) + 1021, (R^2 = 0.9905) \quad (1)$$

By comparing (1) with that obtained by Zhang and Wang [22] for an irreversible electrochemical processes

$$E_p = \left(\frac{1}{2}\right)b \log v + a \quad b = \frac{2.303RT}{(1-\alpha)n_a F} \quad (2)$$

where α , n_a , F , T and R are the transfer coefficient, the number of electrons involved in the rate-determining step, the Faraday constant, the absolute temperature and the gas constant, respectively. A value of 0.524 was estimated for the term $(1-\alpha)n_a$. By considering the two-electron involvement in the rate-determining step of the electrode process [23, 24], a value of 0.738 was obtained for the value of α , which indicates that the activation free energy curve

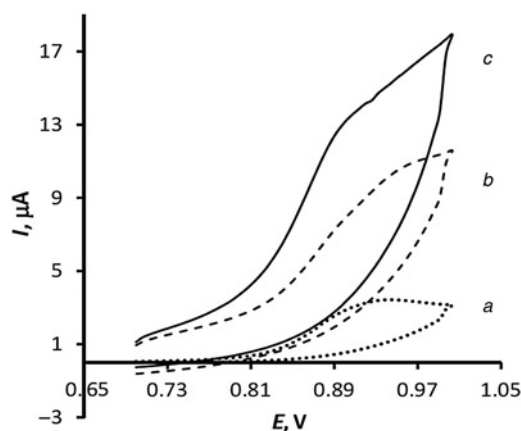


Figure 2 Cyclic voltammeteries of 0.05 mM Enro on the UMCPE, MWCNT/CPE, and Fe₃O₄NPs/MWCNT/CPE, in 0.1 M acetate buffer, pH 5.0, and scan rate 100 mVs⁻¹. Curve a: UMCPE; curve b: MWCNT/CPE; curve c: Fe₃O₄NPs/MWCNT/CPE

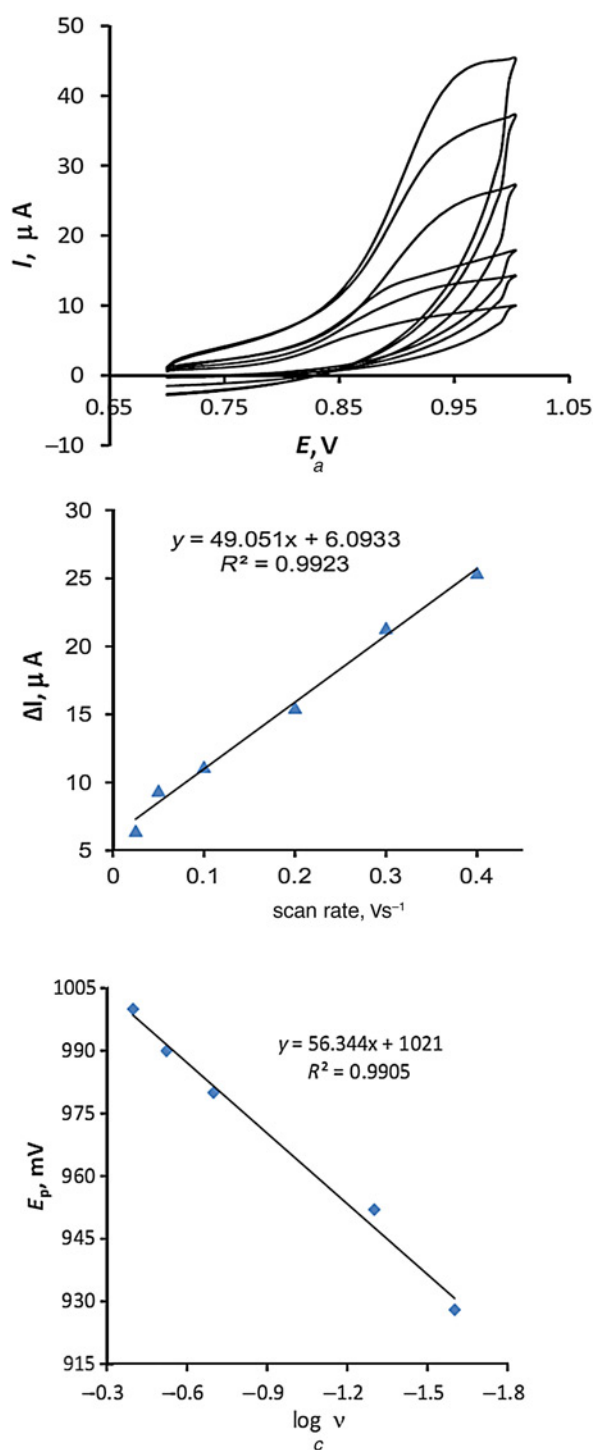


Figure 3 Cyclic voltammograms for oxidation of 5 mM Enro on surface of $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$ at different scan rates (25, 50, 100, 200, 300 and 400 mVs^{-1}) (Fig. 3a); corresponding peak currents against scan rate (Fig. 3b); corresponding peak potential against $\log(\text{scan rate})$ (Fig. 3c)

of oxidation of Enro is not symmetrical like an irreversible oxidation process.

3.3. Differential pulse voltammetric (DPV) measurements: The $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$ was first activated in an acetate buffer (0.1 M, pH 4.4) by cyclic voltammetric sweeps of between -0.5 and 1.5 V until stable cyclic voltammograms were obtained, and then transferred into another 10 ml of supporting electrolyte solution containing a certain concentration of Enro and voltammograms were recorded. Well-defined peaks were obtained

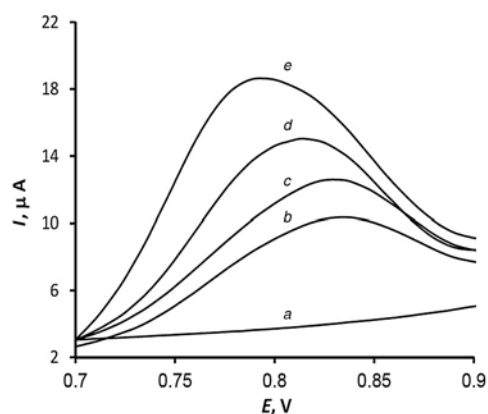


Figure 4 Differential pulse voltammograms of 0.05 mM Enro on the surface of activated (b) UMCPE, (c) MWCNT/CPE, (e) $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$, (d) $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$ without activation and (a) as (e) without Enro. Supporting electrolyte was 0.1 M acetate with pH 5.0 and potential sweep rate was 10 mVs^{-1}

within the potential range scanned (0.70–0.90 V against Ag–AgCl) with a current proportional to the concentration of Enro. Fig 4 illustrates the DPV response of 50 μM Enro on the surface of the UMCPE, MWCNT/CPE and $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$. The voltammogram of Enro on the activated UMCPE and MWCNT/CPE exhibits a well-defined peak at 0.84 V. However, a considerable enhancement in the peak potential and peak current at $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$ was observed. Compared with the unactivated $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$, considerable reduction in the overpotential and a remarkable enhancement in the peak current was observed. The electrochemical activation could increase active sites of the modified electrode, and accordingly improve the catalytic ability and electron transfer [25, 26]; hence, all DPV investigations were done using the activated modified electrode.

The differential pulse voltammetry response of the modified electrode towards Enro was optimised by measuring the peak current dependence on the pulse amplitude, pulse duration, measuring time, solution pH and potential scan rate. These parameters were optimised for obtaining maximum signal-to-noise ratio using a 0.01 mM Enro solution by the one-factor-at-a-time method. The calibration graph of Enro under optimum conditions is linear in the concentration ranges 0.1–10 μM with the following equation

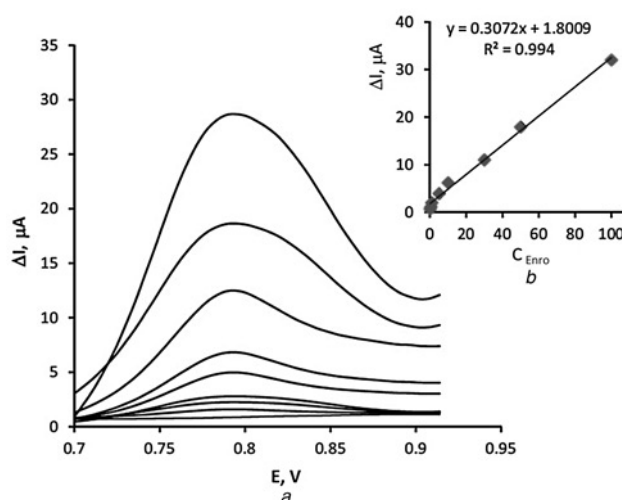


Figure 5 Differential pulse voltammograms for oxidation of different concentrations of Enro (0.1, 0.5, 1, 5, 10, 50 and 100 μM) on surface of $\text{Fe}_3\text{O}_4/\text{MWCNT}/\text{CPE}$ (Fig. 5a), and corresponding peak currents against Enro concentration

as shown in Fig. 5. A detection limit (3σ) of 0.08 μM was obtained

$$\Delta I_p(\mu A) = (304.3 \pm 0.35)C_{\text{Enro}}, \mu M + (5.47 \pm 0.35),$$

$$(R^2 = 0.9908, n = 7)$$

3.4. Repeatability, stability and reproducibility: The stability and repeatability of the sensors response are two important parameters for the evaluation of the performance of a sensor. Repeated voltammograms ($n=7$) show that the relative standard deviation for 0.5 and 10 μM of Enro was 4.5 and 3.3%, respectively. This indicates the good repeatability of the response of the modified electrode. Moreover, over a period of four months, the electrode retained 98.05% of its initial peak current response for a concentration of 1 μM of Enro. This shows the long-term stability of the modified electrode in the determination of Enro. The Enro concentration was determined with three independent electrodes. The relative standard deviation for 1 μM of Enro on these three electrodes was 4.8%. Then, the reproducibility of the modified electrode was acceptable. The results indicate that the modified electrode has good reproducibility and repeatability in both the voltammetric determinations and the preparation procedure with excellent long-term stability.

3.5. Interference study: To investigate the selectivity of the proposed method for the determination of Enro in the food matrix, various potentially interfering species on the determination of 0.05 mM Enro were investigated under optimum conditions. The tolerance limit was defined as the concentration that gave a relative error greater than ±5.0% in the determination. Some cations, anions and organic compounds that may exist in real food samples were selected here. The results are summarised in Table 2. It can be seen that the addition of some inorganic

Table 2 Influences of possible interferents on the peak cathodic current of 0.05 mM Enro

Interferents	Tolerance level, mM
K ⁺ , Na ⁺ , Mg ²⁺ , Al ³⁺ , Ca ²⁺	100
ascorbic acid, saccharose, fructose, lactose, glycine, glucose, benzoic acid and uric acid, carbonate, bicarbonate, Iron(III), Iron(II), Cd(II).	25.0
L-histidine	10.0
Zn(II), Co(II), Cu(II)	7.5
glutathione and phenylalanine	3.0
ciprofloxacin and levofloxacin	0.05

Table 3 Results of the recovery analysis of Enro spiked in food samples

Sample	Detection, μM	Spiked, μM	Founded, μM	%Recovery
chicken	5.63 ± 0.02	—	5.63 ± 0.025	—
	5.63 ± 0.02	20	25.71 ± 0.95	100.32
	5.63 ± 0.02	40	46.08 ± 0.73	101.01
milk	—	20	20.74 ± 0.98	103.70
	—	30	30.85 ± 0.85	102.80
	—	50	49.78 ± 0.77	99.56
egg	—	20	19.14 ± 1.02	95.70
	—	30	28.82 ± 0.95	96.07
	—	50	48.67 ± 0.78	97.35
honey	—	20	19.25 ± 0.65	96.25
	—	30	29.02 ± 0.66	96.73
	—	50	49.20 ± 0.56	98.40

SD: Standard deviation for four replicate measurements.

ions, such as, K⁺, Na⁺, Mg²⁺, Al³⁺, Ca²⁺ do not interfere with Enro detection. Some organic substances, such as 500-fold glucose, sucrose and glycine; fructose, lactose, glycine, glucose, benzoic acid and uric acid, carbonate, bicarbonate 200-fold L-histidine also do not interfere with the determination of 0.05 mM Enro. In addition, some antibiotics, such as ciprofloxacin and levofloxacin have no influence on the detection of Enro at the same concentration as Enro.

3.6. Analytical application: To study the ability of the proposed method in a practical analytical situation, the method was used for the determination of Enro in food samples (cow's milk, egg, honey and chicken). The standard addition method was utilised for the evaluation of the recovery of spiked samples at three different levels (20, 30 and 50 μM). The results of Enro determination that are summarised in Table 3 show an average recovery of 95.70–103.70%. The results indicate that the constituents of the samples do not significantly interfere with the determination of Enro. Therefore, the method seems to be promising for the determination of enrofloxacin in food samples.

4. Conclusion: In this work, we used MWCNTs decorated with iron oxide nanoparticles (Fe₃O₄/MWCNTs) to construct a highly sensitive sensor for the determination of Enro based on CPE. Enrofloxacin effectively adsorbs on the electrodes surface resulting in an increase in the electrooxidation currents with respect to both the MWCNT/CPE and the UMCPE. A remarkable improvement in the electron transfer kinetics of Enro oxidation was observed on the surface of Fe₃O₄/MWCNT/CPE, resulting in an increasing sharpness of the wave and anodic peak current and a lowering of the anodic overpotential. The differential pulse voltammetry using the modified electrode was used as a very selective and sensitive method with a sub-micromolar detection limit for the determination of Enro. Wide linear dynamic ranges (three orders of magnitude), low detection limits, very good reproducibility and repeatability, high sensitivity and stability can be presented as the advantages of the prepared sensor. The proposed method was utilised successfully for the determination of enrofloxacin residual in milk, egg, honey and chicken samples.

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6 References

- [1] Sereshti H., Izadmanesh Y., Samadi S.: 'Optimized ultrasonic assisted extraction–dispersive liquid–liquid microextraction coupled with gas chromatography for determination of essential oil of *Oliveria decumbens* Vent', *J. Chromatogr. A*, 2011, **1218**, (29), pp. 4593–4598
- [2] Martinez M., McDermott P., Walker R.: 'Pharmacology of the fluoroquinolones: a perspective for the if use in domestic animals', *Vet. J.*, 2006, **172**, (1), pp. 10–28
- [3] EMEA/MRL/574/99. The European Agency for the Evaluation of Medicinal Products Report, 2014; available at: <http://www.emea.eu.int>
- [4] Yu F., Yu S., Yu L., Li Y., Wu Y., Zhang H., ET AL.: 'Determination of residual enrofloxacin in food samples by a sensitive method of chemiluminescence enzyme immunoassay', *Food Chem.*, 2014, **149**, pp. 71–75
- [5] Wang Z., Zhang H., Ni H., Zhang S., Shen J.: 'Development of a highly sensitive and specific immunoassay for enrofloxacin based on heterologous coating haptens', *Anal. Chim. Acta*, 2014, **820**, pp. 152–158
- [6] de Souza C.F., Martins R.K.S., da Silva A.R., da Cunha A.L.M.C., Aucélio R.Q.: 'Determination of enrofloxacin by room-temperature phosphorimetry after solid phase extraction on an acrylic polymer sorbent', *Spectrochim. Acta A, Mol. Biomol. Spectrosc.*, 2013, **100**, pp. 51–58

- [7] Amin A.S., Dessouki H.A., Agwa I.A.: 'Ion-pairing and reversed phase liquid chromatography for the determination of three different quinolones: enrofloxacin, lomefloxacin and ofloxacin', *Arab. J. Chem.*, 2011, **4**, (3), pp. 249–257
- [8] Pulgarín J.A.M., Molina A.A., Muñoz S.R.: 'Rapid chemiluminescent determination of enrofloxacin in eggs and veterinary drugs', *Anal. Lett.*, 2011, **44**, (12), pp. 2194–2208
- [9] Ensafi A.A., Taei M., Khayamian T., Hasanpour F.: 'Simultaneous voltammetric determination of enrofloxacin and ciprofloxacin in urine and plasma using multiwall carbon nanotubes modified glassy carbon electrode by least-squares support vector machines', *Anal. Sci.*, 2010, **26**, pp. 803–808
- [10] Ensafi A., Khayamian T., Taei M.: 'Determination of ultra-trace amount of enrofloxacin by adsorptive cathodic stripping voltammetry using copper(II) as an intermediate', *Talanta*, 2009, **78**, pp. 942–948
- [11] Iijima S.: 'Helical microtubules of graphitic carbon', *Nature*, 1991, **354**, (6348), pp. 56–58
- [12] Li X., Chen Z., Zhong Y., Yang F., Pan J., Liang Y.: 'Cobalt hexacyanoferrate modified multi-walled carbon nanotubes/graphite composite electrode as electrochemical sensor on microfluidic chip', *Anal. Chim. Acta*, 2012, **710**, pp. 118–124
- [13] Ghalkhani M., Shahrokhian S., Ghorbani-Bidkorbeh F.: 'Voltammetric studies of sumatriptan on the surface of pyrolytic graphite electrode modified with multi-walled carbon nanotubes decorated with silver nanoparticles', *Talanta*, 2009, **80**, (1), pp. 31–38
- [14] Jain R., Sharma S.: 'Glassy carbon electrode modified with multi-walled carbon nanotubes sensor for the quantification of antihistamine drug pheniramine in solubilized systems', *J. Pharm. Anal.*, 2012, **2**, (1), pp. 56–61
- [15] Yin H., Cui L., Chen Q., Shi W., Ai S., Zhu L., *ET AL.*: 'Amperometric determination of bisphenol A in milk using PAMAM-Fe₃O₄ modified glassy carbon electrode', *Food Chem.*, 2011, **125**, pp. 1097–1103
- [16] Yin H., Zhou Y., Ma Q., Ai S., Chen Q., Zhu L.: 'Electrocatalytic oxidation behavior of guanosine at graphene, chitosan and Fe₃O₄ nanoparticles modified glassy carbon electrode and its determination', *Talanta*, 2010, **82**, pp. 1193–1199
- [17] Piñero M.-Y., Fuenmayor M., Arce L., Bauza R., Valcárcel M.: 'A simple sample treatment for the determination of enrofloxacin and ciprofloxacin in raw goat milk', *Microchem. J.*, 2013, **110**, pp. 533–537
- [18] Esafi A.A., Taei M., Khayamian T., Hasanpour F.: 'Simultaneous voltammetric determination of enrofloxacin and ciprofloxacin in urine and plasma using multiwall carbon nanotubes modified glassy carbon electrode by least-squares support vector machines', *Anal. Sci.*, 2010, **26**, pp. 83–88
- [19] Ensafi A.A., Khayamian T., Taei M.: 'Determination of ultra-trace amount of enrofloxacin by adsorptive cathodic stripping voltammetry using copper(II) as an intermediate', *Talanta*, 2009, **78**, (3), pp. 942–948
- [20] Krebber R., Hoffend F.-J., Ruttman F.: 'Simple and rapid determination of enrofloxacin and ciprofloxacin in edible tissues by turbulent flow chromatography/tandem mass spectrometry (TFC-MS/MS)', *Anal. Chim. Acta*, 2009, **637**, (1–2), pp. 208–213
- [21] Amini M.K., Khorasani J.H., Khaloo S.S., Tangestaninejad S.: 'Cobalt(II) salophen-modified carbon-paste electrode for potentiometric and voltammetric determination of cysteine', *Anal. Biochem.*, 2003, **320**, pp. 32–38
- [22] Zhang Z., Wang E.: 'Electrochemical principles and methods' (Science Press, Beijing, 2000)
- [23] Sereno S.d.C., Sereno L.n.E., Marioli J.M.: 'Electroanalytical determination of enrofloxacin in reversed phase HPLC', *Electroanalysis*, 2007, **19**, (24), pp. 2583–88
- [24] Huang K.-J., Liu X., Xie W.-Z., Yuan H.-X.: 'Electrochemical behavior and voltammetric determination of norfloxacin at glassy carbon electrode modified with multi walled carbon nanotubes/Nafion', *Colloids Surf. B, Biointerfaces*, 2008, **64**, (2), pp. 269–274
- [25] Lim C.X., Hoh H.Y., Ang P.K., Loh K.P.: 'Direct voltammetric detection of DNA and pH sensing on epitaxial graphene: an insight into the role of oxygenated defects', *Anal. Chem.*, 2010, **82**, p. 7387
- [26] Meng X., Xu Z., Wang M., Yin H., Ai S.: 'Direct determination of 5-methylcytosine based on electrochemical activation of surfactant functionalized graphene modified pyrolytic graphite electrode', *Electrochim. Acta*, 2013, **95**, pp. 200–204