Full Paper

Simultaneous Determination of Hydrochlorothiazide and Folic Acid in Pharmaceutical and Biological Samples using a New Modified Nanostructure Sensor

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Abstract- A sensor based on carbon paste electrode (CPE) modified with benzoylferrocene (BF) and NiO nanoparticls (NiO/NPs) was used for the highly sensitive voltammetric and electrocatalytic measurement of hydrochlorothiazide (HCT) and folic acid (FA). The NiO/NPs were synthesized via a direct chemical precipitation method and were characterized with X-ray diffraction (XRD), and scanning electron microscopy (SEM) techniques. The obtained sensor represented a suitable and powerful electron mediating behavior along with excellent-separated oxidation peaks of HCT and FA. The acquired peak currents from square wave voltammetry (SWV) technique were linearly dependent on HCT and FA concentrations in the ranges of 1.0–500.0 and 50.0–500.0 $\mu molL^{-1}$ with limits of detection equal to 0.14 and 4.3 $\mu molL^{-1}$, respectively. This mediator/nanoparticle modified electrode was applied for the detection and measurement of HCT and FA in pharmaceutical and biological samples.

1. INTRODUCTION

Hydrochlorothiazide (HCT) is one of the greatest of thiazide medicines often consumed for treating high-blood pressure. This medicine excretes the water and sodium ion from Urine by preventing of reabsorption the sodium ion by kidney tubules. Other uses include renal

tubular acidosis, diabetes insipidus, and to decline the risk of kidney stones in those with high calcium level in urine. Thus, measuring of this drug is significant. Numerous analytical procedures have been described for the measurement of hydrochlorothiazide in plasma, blood and medicinal preparations, including spectrophotometric [1], high performance liquid chromatography [2], thin-layer chromatography [3] capillary zone electrophoresis [4], chemiluminescence [5], liquid chromatographic [6], derivative spectroscopy [7], and electrochemical techniques [8-10]. In comparison to other procedures that have been used the electrochemical methods is important for because of their high dynamic range, lower cost, simplicity, sensitivity and accuracy [11-15].

Folic acid is a commonly distributed vitamin (called vitamin BC, vitamin B9 or vitamin M). It is complicated in single carbon transfer reactions in metabolism, and it is the precursor of the active tetrahydrofolic acid coenzyme [16]. Lack of FA is a common cause of anaemia and it is thought to growth the likelihood of heart attack and stroke. FA is a potential agent for cancer prevention [17] by free radical scavenging and antioxidant activity. Several techniques just like capillary electrophoresis (CE) [18], HPLC [19], enzyme-linked immunosorbent assays (ELISAs) [20], together with bioassay [21] have still existing for the determination of FA. Between the different approaches, electrochemical techniques are found to be very hopeful [22-24].

It has been detected that when pregnant persons use hydrochlorothiazide due to high-blood pressure, their body not only misses its essential liquids and sodium ions but the level of FA in their body also decreases due to repeated urination reaction. Hence, as it is possible that a number of problems, such as inborn malformations and neural tube defects, may be caused by low levels of FA in a pregnant person's body, they must to use 800 mg of FA, which is twice as much as the value used by a normal person (400 mg).

It is possible to recognize the best type of treatment for every specific patient whereas increasing the possible harms which are likely to happen as a result of side-effects caused by improper treatments by the simultaneous determination of HCT and FA [25].

Modification of various electrodes surface is important point in highly sensitive electrochemical measurement for electroactive materials [26-29]. According to this point that there are some limitations in using of un-modified electrode for electrochemical techniques just like low slow electron transfer reaction, selectivity and sensitivity, weak reproducibility and steadiness over a vast range of solution compositions and the high overvoltage where the electron transfer process happens [30,31]. Nanostructure materials namely metal-based nanoparticles with high surface area and unique properties used for different application [32,33] and especially modification electrochemical sensors in last year's.

A few papers are accessible regarding electrochemical dedication of only HCT in real samples using chemically modified electrodes. So far, as we know, there is a fewer papers that have been reported at the same time measurements of HCT and FA by means of

electrochemical techniques. In the present investigation, we explain fabrication of a sensor composed of NiO/NPs-carbon paste electrode modified with benzoylferrocene (BF) (BF/NiO/NPs/CPE) and research its application of the modified sensor for simultaneous determination of HCT and FA in pharmaceutical and biological samples.

2. EXPERIMENTAL SECTION

2.1. Apparatus and materials

The electrochemical signals were achieved with a Micro Autolab potentiostat/galvanostat type (III). The experimental conditions were organized with GPES and FRA softwares. A traditional three-electrode cell assembly containing of an Ag/AgCl/KCl electrode and a platinum wire as reference and auxiliary electrodes were used, respectively. The working electrode was either a modified carbon paste electrode (BF/NiO/NPs/CPE). An 827 pH meter (Metrohm) was used for pH adjustments.

All solutions were provided with double distilled water. All materials and reagents were of analytical grade and were attained from Merck Company. The pH range of 3.0-10.0 are tested by buffer solutions which were prepared from orthophosphic acid and its salts. Direct precipitation process was used for generation of NiO nanoparticles [34].

2.2. Fabrication of the modified electrode

The ratio of BF and NiO/NPs in modified electrode are optimized, for attaining the best mode in the preparation of modified sensor. The consequence displays that the maximum intensity is obtained in 1.0% w/w BF and 10.0% w/w NiO nanoparticles. Hence, using 1.0% w/w BF and 10.0% w/w NiO nanoparticles for preparation of modified sensor are selected. For this purpose, 0.010 g of BF was hand merged with 0.890 g of graphite powder and 0.100 g of NiO nanoparticles. Using a syringe, Then, 13 drops of liquid paraffin were added to the obtained mixture and blended well for 60 min until a homogenously pasted was attained. The provided paste was then pressed into a glass pipe. The electrical connection will be prepared by pressing Cu wire down at the behind of the paste in the glass tube. Whenever it was needed, a fresh surface was achieved by pushing a surplus of the mixture out of the pipe and polishing it on a soft paper. The unmodified carbon paste electrode was fabricated in the similar manner without adding NiO nanoparticles and mediator to the paste matrix to be tested for the comparative goals.

2.3. Production of real samples

Ten hydrochlorothiazide tablets (labeled 50 mg of Iran Daru Co.) were crushed and a HCT solution was achieved by dissolving 100 mg of the obtained powder in 200 mL water. Afterwards, various values of the HCT solution were added into a 10 mL volumetric

container and were diluted using phosphate buffer solution (PBS) (pH 5.0). The HCT content was explored by the suggested method using the standard addition manner. As well as, 10 tablets of FA (labeled 5.0 mg of Iran Daru Co.) were grinded and the FA solution was provided by dissolving 100 mg of the obtained powder in 200 mL water. Thereafter, various values of the achieved FA solution were moved into a 10 mL volumetric container and were diluted using PBS (pH 5.0). The FA content was also examined by the suggested method using the standard addition manner.

Urine sample of a normal person was kept in a refrigerator immediately after collection. 10 mL of the urine sample was centrifuged for 20 min at 1500 rpm. The supernatant was filtered out by a 0.45 μm filter. After, various values of the obtained solution were moved into a 50 mL volumetric container and diluted using PBS (pH 5.0) to the mark. The prepared urine samples were spiked with various volumes of HCT and FA.

3. RESULTS AND DISCUSSION

3.1. NiO nanoparticles characterization

Figure 1A shows the XRD patterns of the synthesized NiO nanoparticles. The peaks were appeared at the (111), (200), (220), (311) and (222) planes which correspond to NiO and the diffraction data are in good agreement with JPCD card of NiO (JPCD no.: 01-1239). An average crystal size of NiO nanoparticle was calculated about 18 nm (2θ=37.69) by using Scherer's equation (D=Kλ/(β cosθ). Figure 1B display a typical SEM image of the synthesized NiO nanoparticles. Results show that spherical NiO nanoparticles are well formed.
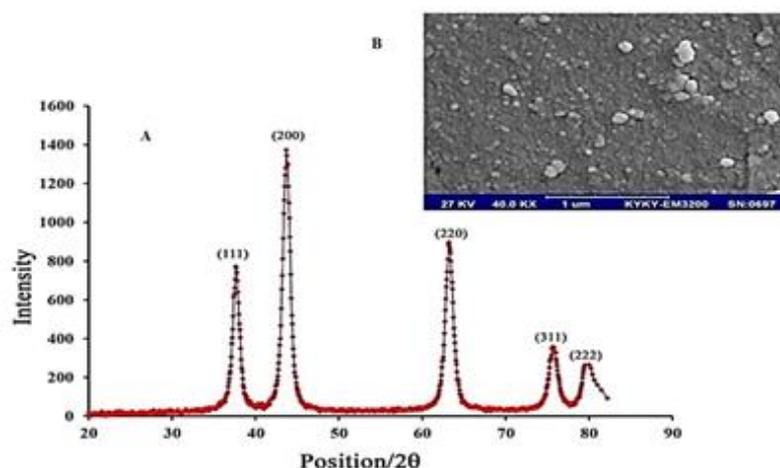

Fig. 1. A) XRD patterns of the as synthesized NiO nanoparticles; B) SEM image of NiO nanoparticles.

3.2. Voltammetric explorations

The combination of unsolvable benzoylferrocene with carbon paste produces a stable and chemically modified electrode without concern for its leaching from the surface of electrode. Firstly, the voltammetric behavior of BF/NiO/NPs/CPE was investigated. Results are indicated an anodic peak at the forward scan associated to the oxidation of the benzoeilferrocene to benzoeilferrocenium form (scheme1).
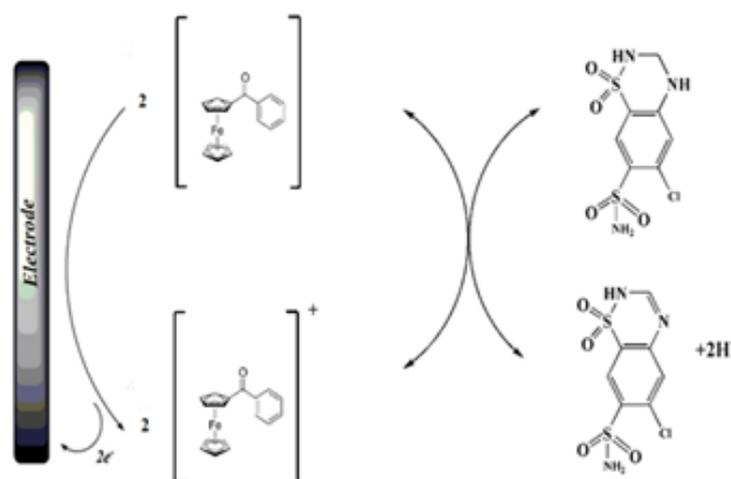
Scheme 1. Suggestion mechanism for electrocatalytic oxidation of hydrochlorothiazide at a surface of BF/NiO/NPs/CP
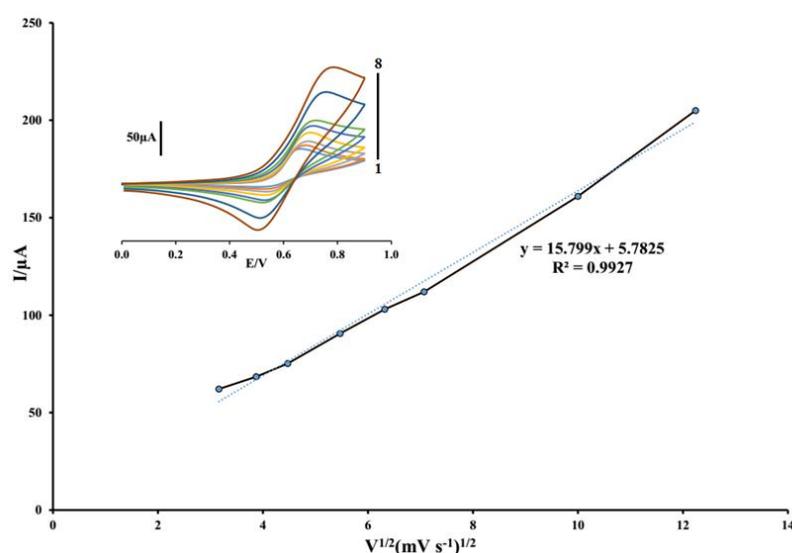

Fig. 2. Plot of I_{pa} versus $v^{1/2}$ for the electrooxidation of BF/NiO/NPs/CPE at various scan rates of 1) 10; 2) 15; 3) 20; 4) 30; 5) 40; 6) 50; 7) 100; 8) 150; mV s^{-1} in 0.1 molL^{-1} PBS (pH 5.0). Inset: Cyclic voltammograms of BF/ NiO/NPs/CPE at various scan rates

In the inverse scan of the potential, a cathodic peak observes associated to the reduction of benzoeilferrocenium to benzoeilferrocene form. A couple of quasi reversible signals are detected at E_{pa}=0.69 V and E_{pc}=0.57 V *vs*. Ag/AgCl/KCl (scan rate 0.15 V/s). The values of half wave potential ($E_{1/2}$) and ΔE_p (E_{pa}-E_{pc}) were 0.60 and 0.12 V *vs*. Ag/AgCl/KCl, respectively. Because of ΔE_p value more than the expected value (59/n mV) for a reversible process, the electrode behavior is quasi reversible. Figure 2 shows that the anodic peak current is linearly dependent on $v^{1/2}$ for all scan rates (I=1.0139 $v^{1/2}$+57.066; R^2=0.9927). This manner shows that the nature of the redox process is diffusion-controlled.

The cyclic voltammetric signals from the electrochemical oxidation of 1000 µmol L^{-1} HCT at BF/NiO/NPs/CPE (curve c), BF/CPE (curve b), NiO/NPs/CPE (curve d), and bare CPE (curve e) are presented in Figure 3. As seen, the potential of anodic peaks for oxidation process of HCT at BF/NiO/NPs/CPE (curve c) and at BF/CPE (curve b) were about 700 mV, while at the surfaces of NiO/NPs/CPE (curve d) and unmodified CPE (curve e), were about 980 and 1000 mV, respectively. According to these results, we can concluded that the good and suitable electrocatalytic effect for HCT oxidation was achieved using BF/NiO/NPs/CPE (curve c). For further explanation we can mention that the potential value of HCT oxidation process at surface of BF/NiO/NPs/CPE (curve c) shifted about 280 and 300 mV to fewer positive values compared to NiO/NPs/CPE (curve d) and bare CPE (curve e), respectively.
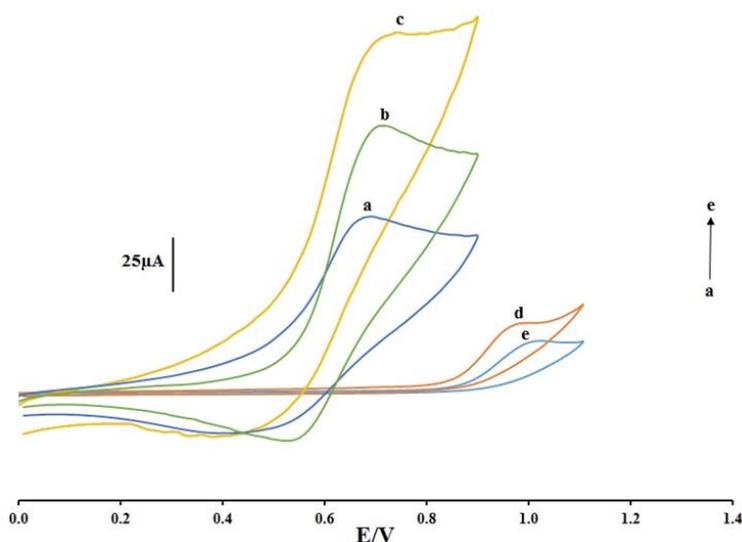

Fig. 3. Cyclic voltammograms of a) the buffer solution at BF/NiO/NPs/CPE, b)1000 µmolL^{-1} HCT at BF/CPE, c) 1000 µmolL^{-1} HCT at BF/NiO/NPs/CPE, d) 1000 µmolL^{-1} HCT at NiO/NPs/ CPE, and e) 1000 µmolL^{-1} HCT at CPE. Conditions: 0.1 molL^{-1} PBS (pH 5.0), scan rate of 20 mVs^{-1}

Furthermore, BF/NiO/NPs/CPE displays greater anodic peak current for the oxidation of HCT compared to BF/CPE, indicating that the simultaneous use of the mediator and NiO

nanoparticles (mediator/nanoparticle) has suggestively enhanced the performance of the applied sensor for HCT oxidation. Actually, BF/NiO/NPs/CPE in the buffer solution (pH 5.0) and the absence of HCT showed an expected behavior for redox reaction. However, there was a significant increase in the current of anodic peak using 1000 µmol L^{-1} HCT (curve c), which can be associated with the electrocatalytic effect of BF/NiO/NPs/CPE for oxidation of HCT.

Figure 4 presents the effect of scan rate (v) on potential and current of peak (E$_p$ and i$_p$) of 500 µmolL^{-1} HCT (pH 5.0) which was tested in the range of 5–20 mVs^{-1} at the surface of BF/NiO/NPs/CPE. The attained signals indicated a positive shift in E$_p$, which confirming the kinetic limitation in the under study electrochemical reaction. Moreover, there is a linear relationship between i$_p$ and v$^{1/2}$ (i$_p$ = 47.172 v$^{1/2}$ – 86.564, R^2 = 0.9945) that approves the diffusion controlled reaction for the electrooxidation of HCT on the BF/NiO/NPs/CPE surface in the considered range of scan rates.
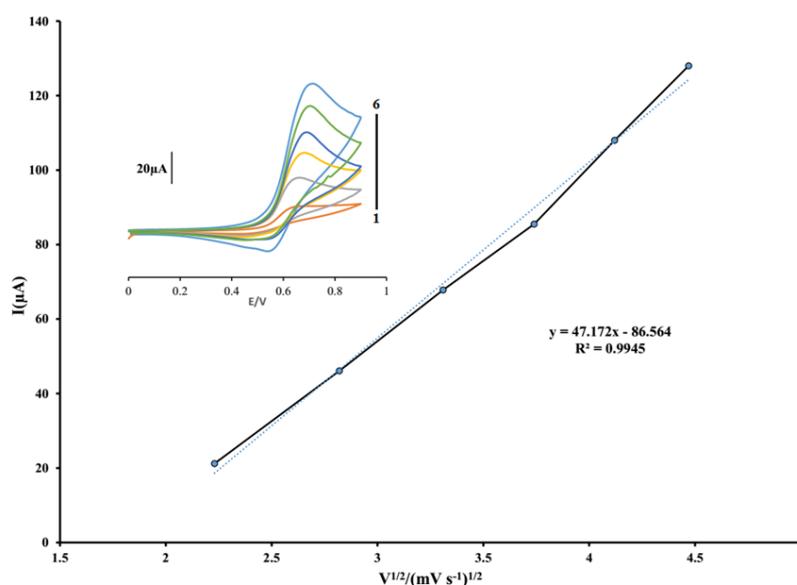

Fig. 4. Plot of I$_{pa}$ versus v$^{1/2}$ for the oxidation of 500.0 µmolL^{-1} HCT at various scan rates of a) 5.0, b) 8.0, c) 11.0, d) 14.0, e) 17.0 and f)20.0 mVs^{-1} in 0.1molL^{-1} PBS (pH 5.0) at BF/NiO/NPs/CPE. Inset: Cyclic voltammograms of 500.0 µmolL^{-1} HCT at various scan rates

The Tafel plot was illustrated to achieve evidence about the rate determining stage, as derived from details of the Tafel area of the CVs (Figure 5). The slope of the Tafel plot was equal to 0.128 V decade^{-1} for sweep rate 8 mVs^{-1}. So, we calculated the mean value of α equal to 0.53. Furthermore, the value of α was obtained for the oxidation of HCT for both the BF/NiO/NPs/CPE and unmodified CPE using the Equation (1):

$$\alpha n_\alpha = 0.048/ (E_p - E_{p/2}) \tag{1}$$

Where $E_{P/2}$ is the potential related to $I_{P/2}$. The αn_α values were obtained to be 0.6 and 0.3 at the surfaces of BF/NiO/NPs/CPE and the unmodified CPE, respectively. The obtained values indicate that the over voltage for HCT oxidation is reduced at BF/NiO/NPs/CPE surface, and as a result the speed of electron transfer reaction is greatly improved. This occurrence is approved by the higher I_{pa} values achieved during cyclic voltammetry experiments at BF/NiO/NPs/CPE.
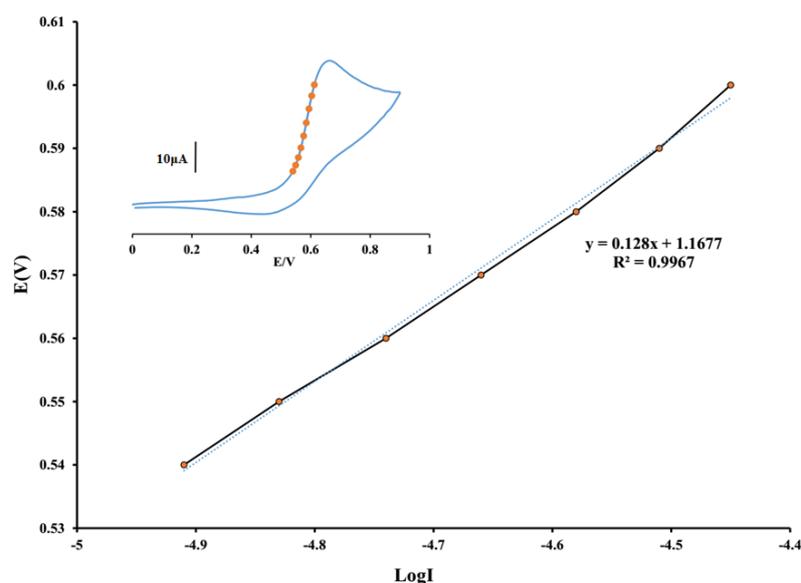

Fig. 5. Tafel plot for BF/NiO/NPs/CPE in 0.1 molL^{-1} PBS (pH 5.0) with a scan rate of 8 mVs^{-1} in the presence of 500.0 µmolL^{-1} HCT

Double potential step chronoamperometric studies of BF/NiO/NPs/CPE were done in the absence and the presence of HCT by adjustment the potential of working electrode at 0.1 and 0.9 V at the first and second steps, respectively (Figure 6A). As can be seen, in the existence of HCT when the potential is first applied (from 0.1 V to 0.9 V), there is not net cathodic current related to the reduction of the BF, however, the charge value associated with the forward stage is considerably more than that noticed for the backward stage. The linearity of the current *vs.* t$^{-1/2}$ shows that the current controlled by diffusion process (Figure 6B). Thus, the slope of this linear curve can be used for calculation of the diffusion coefficient, D, of HCT. The mean value of diffusion coefficient was obtained 5.68×10^{-5} cm^2 s^{-1}. These observations indicate that the mediator molecule at BF/NiO/NPs/CPE surface can catalyze the oxidation process of HCT. Based on method of Galus [35] using equation (2) and chronoamperometry data can also be calculated the catalytic rate constant, k_h, for the reaction between HCT and redox sites at the BF/NiO/NPs/CPE:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \tag{2}$$

Where, I_C is the electrocatalytic current of HCT at the surface of BF/NiO/NPs/CPE, I_L is the limited current in the absence of HCT, C_b is the bulk concentration of HCT, and t is the time elapsed. Based on the slope of I_C/I_L *vs.* $t^{1/2}$ plot, k_h can be obtained for a given HCT concentration (Figure 6C). Using the values of the slopes, the average value for k_h was found to be 5.11×10^2 $M^{-1}s^{-1}$.

Technique of electrochemical impedance spectroscopy (EIS) is one of the most operative and consistent methods to attain information about electrochemical characteristics of the surface modifications [36, 37]. Therefore, EIS was also employed to more investigations.
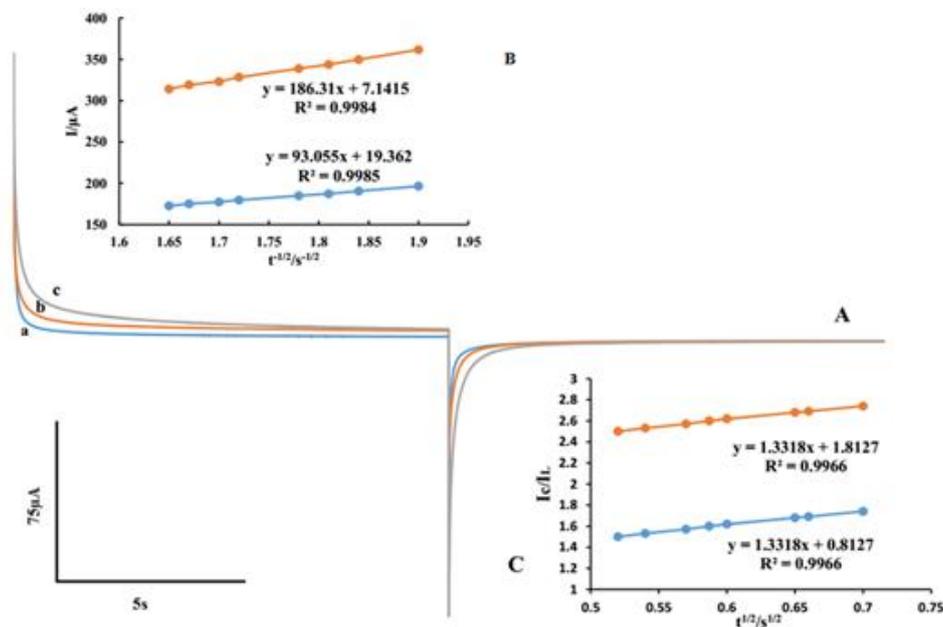

Fig. 6. A) Chronoamperograms obtained at BF/NiO/NPs/CPE in the absence (a) and in the presence of (b) 200 and (c) 350 µmolL^{-1} HCT in a buffer solution (pH 5.0); B) Cottrell's plot for the data from the chronoamperograms; C) Dependence of I_c/I_L on the $t^{1/2}$ derived from the chronoamperogram data

Figure 7 shows the Nyquist diagrams including the imaginary impedance (Z_{im}) versus the real impedance (Z_{re}) obtained at BF/NiO/NPs/CPE recorded at 0.64 V dc-offset in the absence and presence of 500 µmolL^{-1} HCT (pH 5.0). As can be seen from figure, the Nyquist diagrams comprise a depressed semicircle at high frequencies which may be related to the combination of charge transfer resistance (R_{ct}) related to electrooxidation of BF and the double-layer capacitance (Q), followed by a straight line with a slope of nearly 45° which it is due to the mass transport process through diffusion. The diameter of the semicircle decreases in the presence of HCT, approving the electrocatalytic capability of the mentioned system.

The used equivalent circuit for the Nyquist diagram recorded is depicted in Figure 7 (inset). In this circuit, Q, R_{ct}, and R_s show a constant phase element (CPE) corresponding to

the double-layer capacitance, the charge transfer resistance and solution resistance. W is Warburg term coupled to R_{ct}.

Square wave voltammetry (SWV) was used for the analysis of HCT in this research because this method has a much greater current sensitivity and superior resolution than cyclic voltammetry.
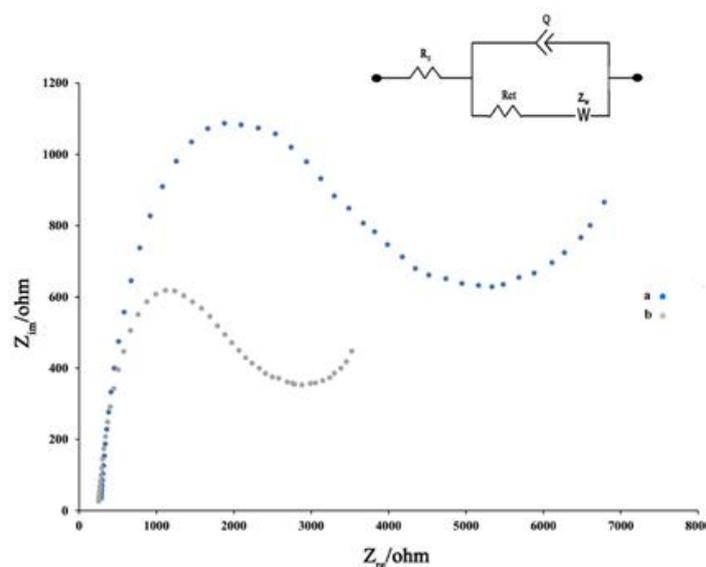

Fig. 7. Nyquist diagrams of BF/NiO/NPs/CPE (a) in the absence and (b) in the presence of 500 μmolL^{-1} HCT

Table 1. Comparison of the efficiency of some modified electrodes used in the determination of hydrochlorothiazide

Electrode	Method	LOD (molL^{-1})	LDR(molL^{-1})	Ref.
Born-doped diamond	voltammetry	1.2×10^{-6}	3.0×10^{-6}-7.4×10^{-5}	[15]
Born-doped diamond	voltammetry	6.39×10^{-7}	1.97×10^{-6}-8.81×10^{-5}	[38]
SRE/MWCNTs	voltammetry	2.6×10^{-6}	5.0×10^{-6}-7.0×10^{-5}	[39]
Carbon paste	voltammetry	1.4×10^{-7}	1.0×10^{-6}-5.0×10^{-4}	This work

The SW voltammograms obviously show that the relationship of peak current and HCT concentration is linear for 1–500 µmolL^{-1} with the regression equation of I_p (µA)= (0.2322 ± 0.027) C_{HCT} + (56.603 ± 0.9782) (R^2 = 0.9942, n=10. The limit of detection (LOD) was 0.14 µmolL^{-1} HX (LOD=3S$_b$/m where S$_b$ is the standard deviation of the blank signal and m is the slope of the calibration). The obtained LOD is comparable to the values described by other research groups which are presented in Table 1.

3.3. Determination of HCT in the presence of FA

After measuring of HCT alone, one of the main goals in this research was to analysis HCT and FA simultaneously using suggested sensor. It is done by simultaneously altering the concentrations of both of them, and then recording the SWVs. The SW voltammograms exhibited two distinct anodic peaks with a 300 mV separation of the peaks which are presented in figure 8 Inset. Attained sensitivities for HCT in the absence and presence of FA were found to be 0.2322±0.027 and 0.2188±0.051 µA/µM, respectively (Figure 8). As it turns out, the obtained sensitivities for the modified sensor towards HCT in the absence and presence of FA were nearly the same, which indicates that the electrooxidation processes of both HCT and FA at the suggested sensor are independent and simultaneous measurements of the two drugs are, therefore, possible with the least interference.
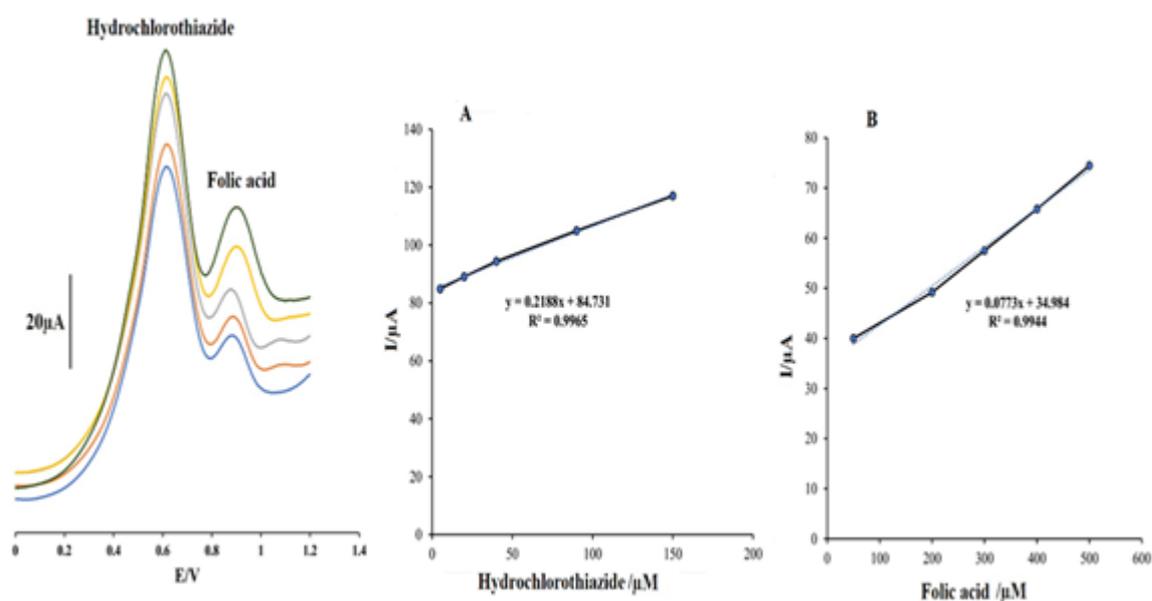

Fig. 8. SWVs of BF/NiO/NPs/CPE in 0.1 M PBS (pH 5.0) containing different concentrations of hydrochlorothiazide and folic acid in µM, from inner to outer: 5.0+50.0, 20.0+200.0, 40.0+300.0, 90.0+400.0 and 150.0+500.0, respectively. Insets (A) plots of I_p *vs*. hydrochlorothiazide concentration and (B) plot of I_p *vs*. folic acid concentrations

3.4. Study of repeatability and stability

The stability and repeatability of the proposed sensor were examined by SWV experiments of 20.0 μmolL^{-1} HCT. The relative standard deviation for 7 successive assays was 1.9%. The relative standard deviation for 4 measurements was 2.3% when using four different fabricated electrodes. When the electrode kept at room temperature in our laboratory, the achieved signals from the modified electrode retains 96% of its initial signals which has good stability and reproducibility.

3.5. Interference investigations

The influence of different materials as compounds potentially interfering with the analysis of HCT was investigated under obtained optimum conditions. The potentially interfering materials were selected from the class of materials generally found with HCT in biological and/or pharmaceuticals matrix. The limit of tolerance was defined as the maximum concentration of the interfering material that caused an error of fewer than ±5% in the analysis of HCT. According to the obtained results, neither a 800-fold excess of some ions namely Na^+, Al^{3+}, Mg^{2+}, Fe^{2+}, K^+, Cl^-, SO_4^{2-} and S^{2-}, nor a 500-fold excess of some compounds namely glucose, uric acid, ascorbic acid, methanol, ethanol, urea, caffeine and 10-fold of atenolol, propranolol, vitamin B2, vitamin B6 and cysteine don't show interference in the analysis of hydrochlorothiazide.

Table 2. The application of BF/NiO/NPs/CPE for simultaneous determination of hydrochlorothiazide and folic acid in their tablet samples (n=5)

Sample	Spiked (μM)		Found (μM)		Recovery (%)		R.S.D. (%)	
	HCT	FA	HCT	FA	HCT	FA	HCT	FA
HCT tablet	0	0	11	ND	-	-	2.3	-
	10.0	50.0	21.2	49.1	102.0	98	1.8	2.1
	20.0	75.5	30.5	76.2	97.5	101.6	1.1	2.3
	30.0	100.0	41.9	101.2	103.0	101.2	2.2	2.7
	40.0	125.0	50.6	123.6	99.0	98.8	1.5	3.1
FA tablet	0	0	ND	50.0	-	-	-	2.8
	25.0	50.0	25.3	98.7	101.2	97.4	1.5	2.4
	35.0	60.0	34.4	111.2	98.2	102	2.5	2.9
	45.0	70.0	45.8	118.7	101.7	98.1	1.6	1.8
	55.0	80.0	54.6	131.2	99.2	101.5	1.9	3.2

3.6. Study of real samples

For more evaluating the analytical applicability of the suggested sensor, it was also examined the determination of HCT and FA in their tablets and urine samples. The attained results are presented in tables 2 and 3. The reported results in the tables indicate the relative standard derivations and the recovery performances of the spiked samples are acceptable, that it indicates the applicability of the proposed sensor for determination of HCT and FA.

Table 3. The application of BF/NiO/NPs/CPE for simultaneous determination of hydrochlorothiazide and folic acid in urine samples (n=5)

Sample	Spiked (µM)		Found (µM)		Recovery (%)		R.S.D. (%)	
	HCT	FA	HCT	FA	HCT	FA	HCT	FA
Urine								
	10.0	100.0	9.8	102.3	98.0	102.3	1.9	2.3
	20.0	120.0	20.7	118.7	103.5	98.9	2.1	1.8
	30.0	140.0	29.3	141.4	97.6	101.0	1.7	2.7
	40.0	160.0	40.5	158.7	101.2	99.1	2.2	2.5

4. CONCLUSION

A mediator/nanoparticle platform for modified sensor was proposed for the simultaneous analysis of HCT and FA. The electrochemical measurements indicated effective electrocatalytic activity of the modified sensor in reducing the anodic overvoltage for the electrooxidation of HCT and complete resolution of its anodic wave from FA. High sensitivity and reproducibility of the voltammetric signals, and low LOD (0.14 µmolL^{-1}), together with the ease of fabrication and surface regeneration, creates the suggested modified sensor very beneficial and suitable for accurate measurement of HCT in the presence of FA in biological and pharmaceuticals samples.

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