Dysprosium Nanowire Modified Carbon Paste Electrode for the Simultaneous Determination of Naproxen and Paracetamol: Application in Pharmaceutical Formulation and Biological Fluid

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The electrochemical oxidation of naproxen and paracetamol was investigated using dysprosium nanowire modified carbon paste electrode (DyNW/CPE) by square wave voltammetry as a new rapid, convenient and sensitive method. The results indicated that Dy-NWCPE modified carbon paste electrode exhibited efficiently electrocatalytic oxidation for naproxen and paracetamol with relatively high sensitivity**,** stability and life time**.**The optimal operational conditions of the proposed procedure were: accumulation potential E_{acc} = 0.3 V, accumulation time t_{acc} = 20 s, scan increment = 3 mV, pulse-amplitude = 40 mV, frequency = 50 Hz using a phosphate buffer of pH 7 as a supporting electrolyte**.** This modified electrode exhibited a potent and persistent electron-mediating behavior followed by well-separated oxidation peaks toward naproxen and paracetamol at a scan rate of 100 mVs^{-1} with a potential difference of about 300 mV, which was large enough to determine naproxen and paracetamol individually and simultaneously**.** Linear calibration curves are obtained in the range 1.0×10^{-9}–5.0×10^{-4} mol L^{-1} and 1.0×10^{-8}–2.5×10^{-4} mol L^{-1} with a detection limit of 0.5 nmol L^{-1} and 0.3 nmol L^{-1} for naproxen and paracetamol, respectively. Determination in real samples has very good accuracy and precision.

1. INTRODUCTION

Naproxen [(+)-2-(6-metoxy-2naphthyl)propionic acid], is a non-steroidal anti-inflammatory drug that also presents analgesic and antipyretic properties often preferred to acetylsalicylic acid because of its better absorption following oral administration and fewer adverse effects. It is a member

of the 2-arylpropionic acid (profen) family of NSAIDs. It is an odorless, white to off-white crystalline substance.

Naproxen is extensively used in the treatment of many diseases like rheumatoid arthrits, degenerative joint disease, ankylosing spondylits, acute gout and primary dismenorrea.[1]

Paracetamol (N-acetyl-p-aminophenol) has been widely used as analgesic and antipyretic drug. Paracetamol is a weak acid (pKa=9.5) which is rapidly absorbed and distributed after oral administration. It is not a very effective anti-inflammatory agent. It is well tolerated, lacks many of the side-effects of aspirin. Side effect to the paracetomol (over usage) includes: liver disorders, skin rashes and inflammation of the pancreas. It is a one of the common drugs which is used very general alone or in combination of other drugs in whole the world.

Due to the critical roles of naproxen and paracetamol in the pharmaceutical industry, their determination is important in biological fluids and drug formulations. Several method was reported for determination of paracetamol; Spectrophotometry,[2,3] liquid chromatography,[4,5] capillary electrophoresis (CE),[6–8] enzyme based assay methods,[9] and for naproxen room temperature phosphorimetry,[10] voltametry,[11] high-performance liquid chromatography,[12-15] capillary electrophoresis,[16,17] coulometry[18] and oscilometric titration.[19]

In these recent years, nanomaterials have received considerable attention in the scientific and engineering fields owing to their unique advantages such as high conductivity, large surface/volume ratio, and extremely high mechanical strength and modulus.[20,21] In the electrochemistry and electroanalytical fields, a lot of nanomaterials have been used as electrode materials[22] to promote electron transfer reactions and to enhance electrode conductivity . Rare earth materials have unique optical, catalytic, and magnetic properties [23] and many kinds of rare earth nanostructures have been synthesized successfully.[24] However, none of them has been used in electroanalysis.

Using the fast Fourier transform method was found very sensitive system in combination by electrochemical method for trace detection of several compounds [25-30]. The present study relates to the preparation of a new Dysprosium nanowire modified carbon paste electrode (DyNW/CPE) to develop a sensor for selective and sensitive detection of naproxen and paracetamol individually. This ability to determine naproxen and paracetamol in a mixture has a significant attraction in biological and chemical researches.

2. EXPERIMENTAL PART

2.1. Apparatus

All of the electrochemical experiments were done using an Autolab potentiostat PGSTAT 30 (Eco Chemie B.V., Netherlands), equipped with the GPES 4.9 software.

A three-electrode configuration includes a carbon paste (CP, 2 mm in diameter) electrode or a home made DyNW/CPE as a working electrode, a platinum wire as a counter electrode, and an Ag│AgCl│KCl (sat.) electrode as a reference electrode.

2.2. Materials

Naproxen and Paracetamol (purity >99%) was a gift from the Center of Quality Control of Drug and Food (Tehran, Iran). A 1.0×10^{-3} mol L^{-1} Naproxen and Paracetamol stock standard solutions was prepared. The naproxen solution was prepared at 1/10 ratio of ethanol to phosphate buffer 0.05 mol L^{-1} at different pHs. Naproxen and paracetamol solutions were prepared just before use. Phosphate buffers of 0.05 mol L^{-1} with various pH values were prepared by mixing the stock solutions of 0.05 mol L^{-1} NaH_2PO_4 and Na_2HPO_4. All experiments were carried out at room temperature (25±2°C). The Dysprosium oxide (Dy_2O_3) was purchased from Merck (Germany), CAT No.1.12151.0010. DyNW prepared by the hydrothermal method which introduced by Li et al.[23, 24] Figure 1 is the transmission electron microscope (TEM) image of DyNW. All chemicals were of analytical-reagent grade, and doubly distilled water was used throughout.
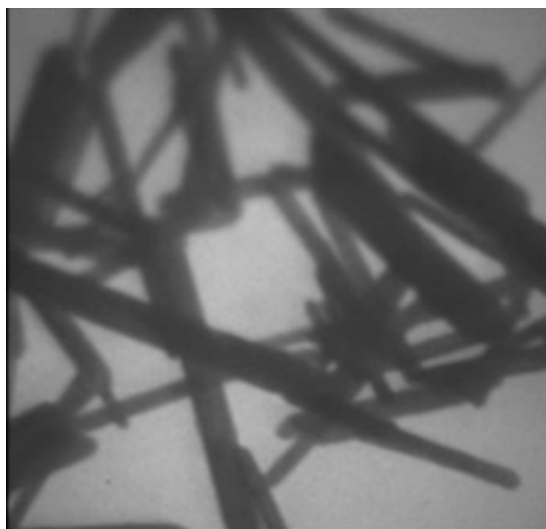

Figure 1. The TEM image of Dysprosium nanowires

The naproxen tablets (Naprosyn, Alhavi Co. Iran, 250 mg) and paracetamol (Acetaminiphen, Razi Co., Iran, 200 mg) was purchased from the local pharmacy. All chemicals were of analytical reagent grade. Twice distilled water was used throughout.

2.3. Electrode preparation

The DyNW/CPE was prepared by hand-mixing 0.97 g graphite powder, 0.03 g DyNW, and 0.34 mL paraffin oil adequately in agate mortar. A portion of the resulting paste was then packed firmly into the electrode cavity (2.0 mm diameter) of a polytetrafluorethylene (PTFE) sleeve. The unmodified CPE was prepared in a similar way using 1.25 g graphite powder and 0.45 mL paraffin oil. Electrical contact was established via a copper wire. The surfaces of all the modified and unmodified

CPEs were carefully smoothed on weighing paper and rinsed with twice distilled water prior to each measurement.
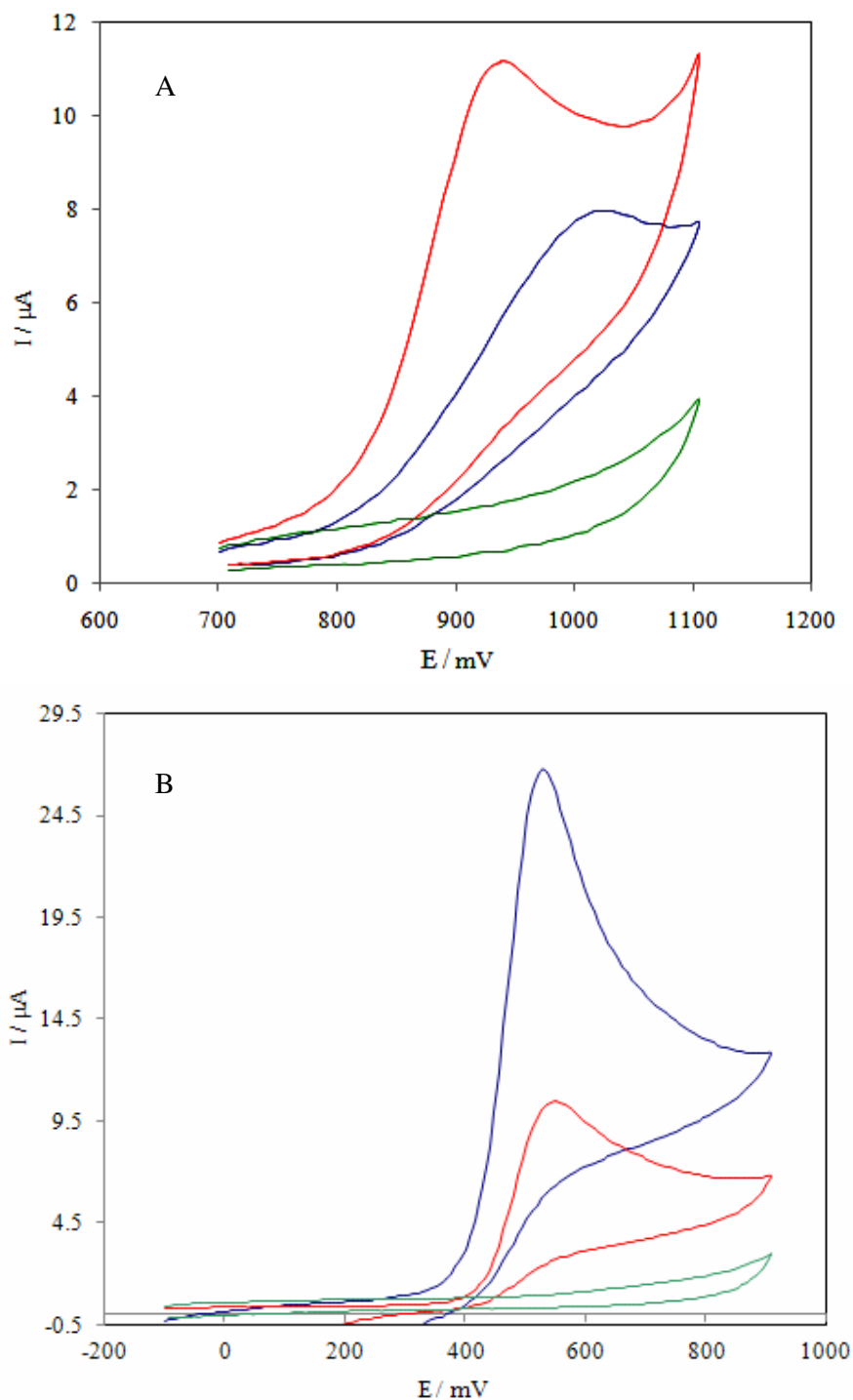

Figure 2. Cyclic voltammogram for oxidation (A) 1.0×10^{-4} mol L^{-1} naproxen at a) DyNW carbon paste electrode, b) at the bare carbon paste and c) buffer at pH 7 at carbon paste electrode (B) 1.0×10^{-4} mol L^{-1} paracetamol at a) DyNW carbon paste electrode, b) at the bare carbon paste electrode, c) buffer at pH 7 at carbon paste electrode
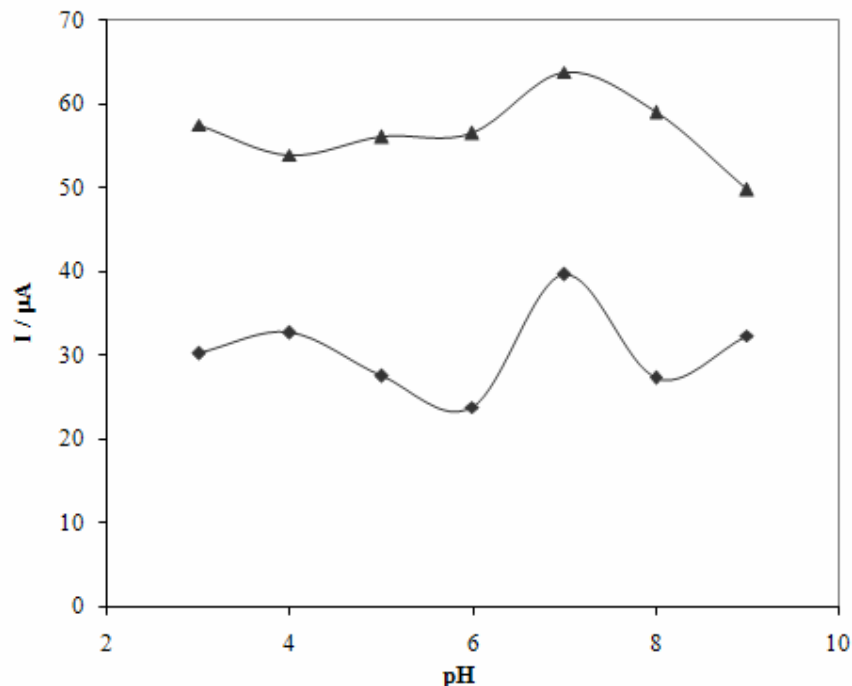
Figure 3. Effect of pH on the peak current of naproxen and paracetamol voltammograms at the Dy-NW carbone paste electrode. Solution containing of 0.05 mol L^{-1} phosphate buffer at different pH and 0.1 mmol L^{-1} of naproxen and 0.1 mmol L^{-1} of paracetamol. The frequency is 50 Hz, amplitude 40 mV, scan increment 3 mV.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of naproxen and paracetamol on Dy-NW carbon paste electrode

Initial studies of the voltammetric behavior of the drugs were performed using cyclic voltammetry. Figure 2(A) compares typical cyclic voltammograms of 0.1 mmol L^{-1} naproxen and paracetamol in phosphate buffer (pH 7.0) recorded at two different working electrodes (i.e. bare carbon paste (b), and Dy-NW carbon paste electrode (a)). At bare carbon paste electrode, a poorly defined oxidation peak was observed at 1000 mV. Under identical conditions, the oxidation of paracetamol Figure 2(B) occurred at 560 mV on the bare carbon paste electrode surface. At the Dy-NW carbon paste electrode, the anodic peak of naproxen and paracetamol shifted negatively to 880 mV and 540 mV respectively and the peak current increased significantly. The remarkable enhancement in current response followed by a drop in peak potential provide clear evidence of the catalytic effect of the modified carbon paste electrode which acts as a promoter to enhance the electrochemical reaction, considerably accelerating the rate of electron transfer. Carbon paste electrode accompanied with an increment of the peak current, indicating an improvement in the electrode kinetics when the Dy-NW carbon paste electrode is utilized.

3.2. Composition of the supporting electrolyte

Three buffer solutions including tris-HCl, phosphate buffer and acetate buffer were chosen for study. Stable signals for naproxen and paracetamol were obtained in the 0.05 mol L^{-1} phosphate buffer (pH=7.0). Therefore, the phosphate buffer was used in subsequent study.

3.3. Effect of pH

The influence of pH with 0.05 mol L^{-1} phosphate buffer was investigated for a solution containing 0.1 m mol L^{-1} naproxen, 0.1 mmol L^{-1} paracetamol. The results obtained are presented in Figure 3. It can be seen from Figure 3 that the naproxen peaks (curve a) increase as the pH increases to 7 and increase slightly to pH 9, but for paracetamol (curve b) the peak current remain unchanged to pH 7 and after that decrease by increase of pH. When the pH was below 7.0, the peak was thoroughly little. If the pH was higher than 7, the drugs inclined to decompose, resulting in the decrease of the response so the pH 7 was chosen as a best pH.

3.4. Effect of the deposition potential and deposition time

The effect of the accumulation potential on the peak current of naproxen and paracetamol at solution containing 1.0×10^{-4} mol L^{-1} naproxen, 1.0×10^{-4} mol L^{-1} paracetamol was studied in the potential range from -0.70 to 0.7 and the obtained results are shown in Figure 4 [31-34]. As it can be seen from Figure 4 that the peak currents of naproxen (curve a) increase till 0 mV and then remain constant except a little increase at 300 mV, and for paracetamol (curve b) it increase to 100 mV with a slight increase at 300 mV, but for simultaneous determination the 300 mV was selected as an accumulation potential because the current of both components increase a little bit rather than other potentials and the sensivity is enough. For optimization of the accumulation time, as can be seen at Figure 4B for naproxen (curve a) the current increase to 20 s and then decrease. For paracetamol (curve b) the current has a constant current till 20s and decrease when the accumulation time increases the best choice for accumulation was 20 s. So for further study the accumulation potential of 0.3V for 20 s was chosen as an optimized parameter.

3.5. Optimization of the SW parameters

Since the SWV method offers an improved sensitivity in electrochemical signal and detection limit, the response of the drugs was also investigated employing SWV mode [35]. The peak current obtained in SWV is dependent on various instrumental parameters such as SW frequency (f_{sw}), scan increment (Δs) and SW amplitude (E_{sw}). These parameters are interrelated having a combined influence on the peak current response. Hence, in order to establish the optimum conditions in the determination, the influence of the instrumental parameters on the peak current response of 0.1 m mol L^{-1} Naproxen in PBS (0.05 M, pH 7.0) was studied. The influence of the Esw on the peak current was

evaluated. When the SW amplitude was varied between 10-50 mV, the peak current initially increased with increasing amplitude and reached a maximum at around 40 mV. Hence, this value was fixed for the subsequent measurements. Similarly, the effect of f_{sw} was studied and 50 Hz was chosen as the optimum value. The effect of the Δs was investigated in the range $1-15$ mV. Interestingly, it showed little influence on the I_p. However, the response was more accurately recorded at a Δs of 3 mV. Overall, the optimized parameters can be summarized as follows: E_{sw} (40 mV); ΔS, 3 mV and f_{sw}, 50 Hz. Figure 5(a-c).
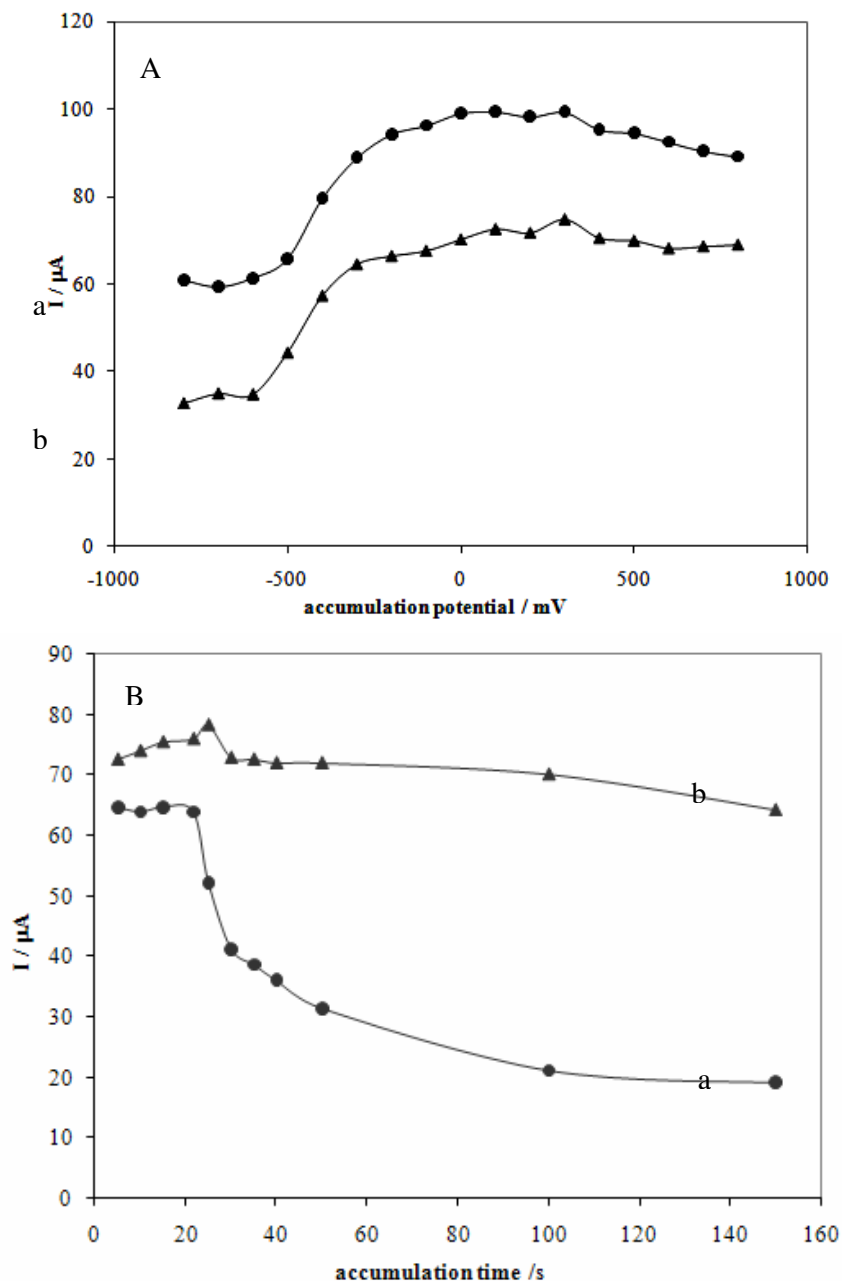

Figure 4. The effect of deposition time and potential on the peak height for a solution containing 0.05 mol L^{-1} of phosphate buffer (pH 7.0), 0.1 m mol L^{-1} of naproxen and 0.1 mmol L^{-1} of paracetamol. Pulse amplitude: 40 mV and frequency 50 Hz.
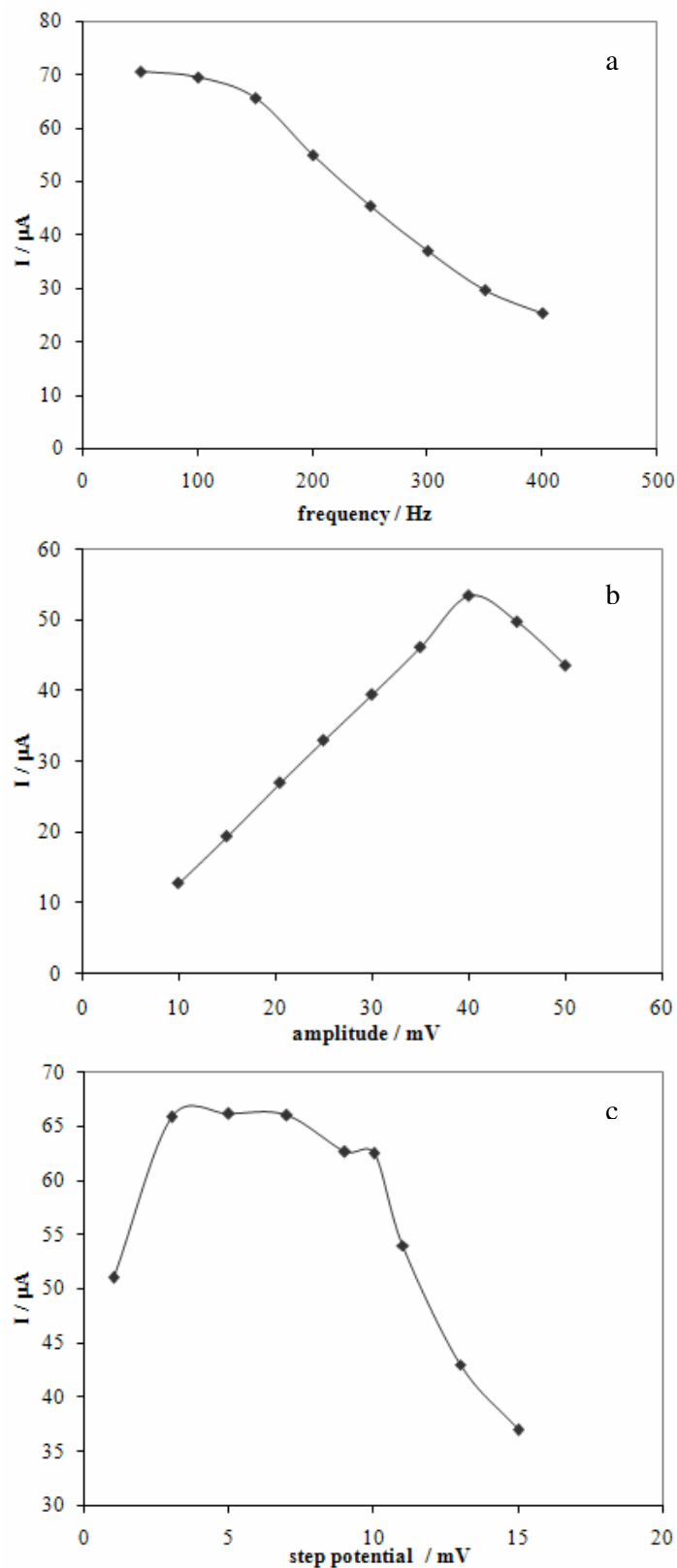
Figure 5. Optimization of square wave parameters for naproxen at 0.1 mmol L^{-1} in phosphate buffer pH 7, a) frequency, b) amplitude and c) step potential.

Figure 6. SW voltammograms of various concentration of A) naproxen at a fixed concentration of paracetamol.([paracetamol]=$1.0{\times}10^{-7}$ mol L^{-1} and [naproxen]=(a) 0.6, (b) 0.8, (c)1.0, (d) 1.2, (e) 1.4, (f) 1.6 μmol l^{-1}). (B) SWVs of various concentration of paracetamol at a fixed concentration of naproxen ([naproxen] = $1.0{\times}10^{-6}$ mol L^{-1}; [paracetamol]: (a) 0.06, (b) 0.08, (c) 0.1, (d) 0.12, (e) 0.14, f) 0.16 μmol L^{-1}.
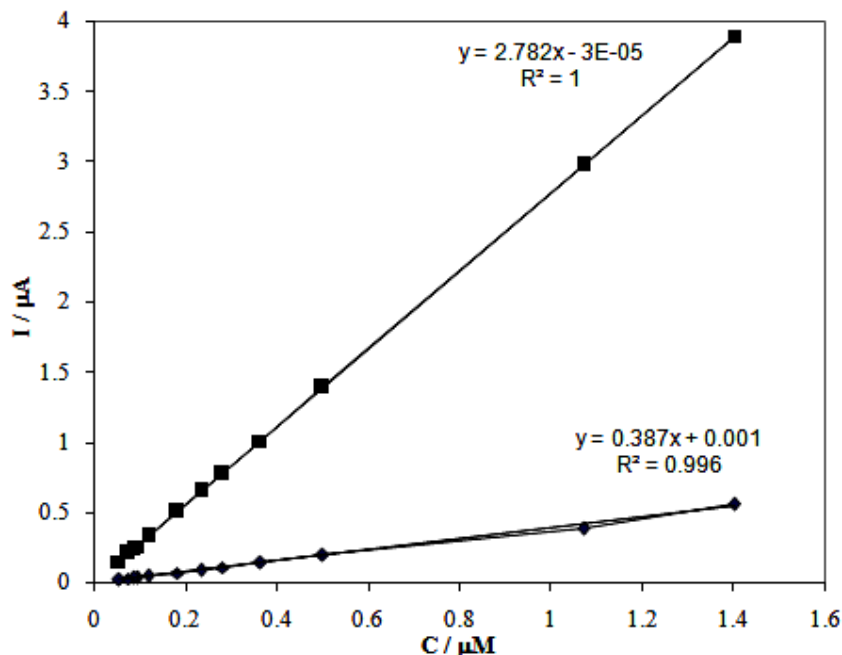
Figure 7. Calibration plot observed for naproxen (a) and paracetamol (b) at the modified DyNW/CPE at pH 7 using SWV technique which presented in Figure 6.

Table 1. Analytical precision and accuracy of paracetamol and naproxen determination by SWV (n=7)

	Concentration (ng ml^{-1})	Intraday					Interday				
		Measured Conc.	S.D	Accuracy (%)	Bias%	R.S.D %	Measured Conc.	S.D%	Accuracy (%)	Bias%	R.S.D%
Paracetamol	40	39.5	0.30	98.75	1.25	1.0	41.3	0.40	103.2	3.25	0.90
	50	48.6	0.29	97.5	2.80	1.1	49.5	0.38	99.0	1.0	0.88
	60	59.2	0.40	68.6	1.30	0.9	60.5	0.25	100.8	0.83	1.1
Naproxen	50	48.8	0.41	97.6	1.95	1.1	48.6	0.28	97.8	2.8	1.0
	60	59.7	0.38	99.5	0.5	0.8	61.2	0.32	99.5	0.33	0.97
	70	68.7	0.28	98.1	1.85	0.9	71.1	0.41	101.5	0.14	0.80

Bias %=[(Measures concentration- added concentration)]/added concentration] × 100; RSD= relative standard deviation; SD= standard deviation

3.6. Calibration curves

On the Dy-NW carbon paste electrode for the simultaneous determination of naproxen and paracetamol by SWASV using the following conditions: deposition potential, 0.1 V; deposition time, 20 s; pulse amplitude, 40 mV; frequency 50 Hz; the calibration curves of ΔI_p against concentrations of naproxen and paracetamol were constructed (Figure 6(a,b) and Figure 7). At Figure 6a, the constant amount of paracetamol and at Figure6b the constant amount of naproxen was used. The linear ranges

of naproxen and paracetamol determination were presented for both drugs from 1.0×10^{-5} - 1.0×10^{-8} mol L^{-1} as shown in Figure 7. The linear regression equation of naproxen was $Ip = 0.387X + 0.0016$ with a correlation coefficient of 0.9983, and the detection limit were 0.5 n mol L^{-1}. The linear regression equation of paracetamol was $I_p = 2.782X - 0.00003$ with a correlation coefficient of 1.0, and the detection limit is 0.3 n mol l^{-1}. Limit of detection (LOD) was calculated from the calibration curves as $3s_1/m$, where s_1 is the standard deviation (S.D.) of the intercept and m is the slope. Precision of the method was investigated by intra- and inter-day determination of paracetamol and naproxen at three different concentrations (n=7) in the linear range. Accuracy and precision of method was shown in Table 1.

3.7. Determination of naproxen and paracetamol in pharmaceutical sample

The Dy-NW carbon paste electrode was directly used to detect naproxen and paracetamol content in their tablets. For the determination of naproxen in pharmaceuticals 10 tablets of each brand were carefully weighed, powdered and the amount of one tablet corresponding to 250 mg of naproxen was transferred to a 1000 mL volumetric flask and dissolved in 10 ml of ethanol. It was necessary to use a small amount of methanol to dissolve sodium naproxen contained in Naprosyne®. The mixture was sonicated for 5 min in an ultrasonic bath and completed to the mark with 0.05 mol L^{-1} phosphate buffer pH 7. A suitable volume (50 µL) of the clear supernatant liquor was then added to a voltammetric cell containing 10 mL of supporting electrolyte. The square wave voltammogram was then recorded and the content of the drug in tablet was quantified by referring to the calibration graph. The content of naproxen was calculated and the result is shown in Table 2.

Table 2. Recovery results obtained for naproxen in naproxen tablets

Naproxen labeled concentration	Naproxen added Concentration(M)	Naproxen found (M)	Recovery%
250mg	0.0	0.0	100.1
	1.0×10^{-6}	1.1×10^{-6}	101.2
	5.0×10^{-6}	5.04×10^{-6}	100.8
	2.0×10^{-5}	1.017×10^{-5}	101.5

For the determination of paracetamol in pharmaceuticals 10 tablets of were carefully weighed, powdered and the amount of one tablet corresponding to 200 mg of paracetamol was transferred to a 100 mL volumetric flask and dissolved. The mixture was centrifuged for 15 min and 10 mL of the solution was completed to the mark with 0.05 mol L^{-1} phosphate buffer pH 7 at the 50 mL volumetric flask. A suitable volume (20 µl) of the clear supernatant liquor was then added to a voltammetric cell containing 10ml of supporting electrolyte. The content of paracetamol was calculated and the result is shown in Table 3.

The results achieved by the Dy-NW carbon paste electrode are in good agreement with the labeled naproxen content in the sample, thus indicating the feasibility of the method for naproxen determination in pharmaceutical formulation. Furthermore, in order to establish the suitability of the proposed method, known amounts of the standard naproxen solution were added into the analytical solution and the same procedure was applied. The recoveries indicate that the accuracy of the proposed voltammetric method is excellent. From the experimental results, it is clear that this novel electrode has great potential for the determination of naproxen in practical sample analysis.

Table 3. Recovery results obtained for paracetamol in its tablets

paracetamol Labeled concentration	paracetamol added Concentration(M)	paracetamol found (M)	Recovery%
200mg	0.0	0.0	100.1
	5.0×10^{-7}	5.5×10^{-7}	101.2
	1.0×10^{-6}	1.05×10^{-6}	105.0
	5.0×10^{-6}	4.95×10^{-6}	99.0

Table 4. Recovery results obtained for naproxen and paracetamol in urine samples at Dy-NW carbon paste electrode

	Added		Found		Recovery% (RSD)	
Sr. No.	naproxen	paracetamol	naproxen	paracetamol	naproxen	paracetamol
1	1.0×10^{-7}	1.0×10^{-7}	0.97×10^{-6}	0.98×10^{-7}	97(3.0)	98.5(2.0)
2	1.0×10^{-6}	1.0×10^{-6}	1.10×10^{-6}	1.04×10^{-6}	101(2.0)	104(3.0)
3	5.0×10^{-6}	5.0×10^{-6}	4.9×10^{-6}	4.82×10^{-6}	98(2.0)	98.5(2.0)

Mean value obtained for three individual replicates. RSD is the relative standard deviation (n=3).

3.8. Real sample analysis

The proposed method was applied to the determination of naproxen and paracetamol in urine sample of a volunteer. The volunteer patient was being treated with naproxen, 250 mg in a single daily dose; paracetamol 200 mg, three times per day. Urine samples were collected in polyethylene vessels at appropriate time intervals along 24 h, in order to study the drug elimination. Due to the complexity of the urine matrix, the standard addition method was applied to determine.

The sample was spiked with naproxen and paracetamol in concentration between 1.0×10^{-7}-5×10^{-6} mol L^{-1} and the recovery rates were determined in the range 97.0–101.0% for naproxen and 98.5–104.0% for paracetamol with RSD 2.0-3.0%. The results for the determination of naproxen and paracetamol in urine samples were summarized in Table 4. The results indicate the lack of

interferences. The recoveries indicate that the accuracy and repeatability of the proposed voltammetric method are very good.

4. CONCLUSIONS

Glassy carbon electrode modified with Dysprosium nanowire has been used successfully for electrocatalytic oxidation of naproxen and paracetamol in physiological buffer solution. The results indicate that the modified electrode facilitates determination of naproxen and paracetamol with good sensitivity and reproducibility compared to similar based electrodes or other instrumental methods. This sensor can be used for square wave determination of selected analytes as low as 0.5 and 0.3 nmol L^{-1} for naproxen and paracetamol with good reproducibility and little fouling effects of analytes and their reduction products. The modified electrode has been used for determination of naproxen and paracetamol in tablets. The proposed method offers the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the results obtained in the analysis of naproxen and paracetamol in serum sample demonstrates the applicability of the method for real sample analysis.

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