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Research Article

β-CYCLODEXTRIN MODIFIED CARBON PASTE ELECTRODE FOR THE DETERMINATION OF GEMIFLOXACIN AND NADIFLOXACIN

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ABSTRACT

The electrochemical behavior of Gemifloxacin (GF) and Nadifloxacin (NF) at β-Cyclodextrin modified carbon paste electrode has been studied. Compared with carbon paste electrode, the β-Cyclodextrin modified carbon paste electrode exhibited a marked enhancement of current response of Gemifloxacin and Nadifloxacin. A simple, precise, inexpensive and sensitive voltammetric method has been developed for the determination of the cited drugs. Cyclic Voltametry studies in Britton Robinson buffer indicate that the process was irreversible and diffusion controlled with some adsorption character. Differential pulse voltammetry (DPV) was used to determine Gemifloxacin and Nadifloxacin in the pure form. The adsorptive stripping response was evaluated as a function of some variables such as pH, the scan rate and accumulation time. The peak current varied linearly for GF and NF in the following range: $5.0x10^{-8}$ to $2.0x10^{-7}$ mol l^{-1}. The limits of detection (LOD) were $1.2x10^{-8}$, $1.0x10^{-8}$ mol l^{-1}, while the limits of quantification (LOQ) were $4.3x10^{-8}$, $3.3x10^{-8}$ mol l^{-1} for GF and NF, respectively. The percentage recoveries were found in the following ranges: 99.57-100.36% and 99.85-100.64% for GF and NF, respectively. The relative standard deviations were found in the following ranges: 0.426-0.828% and 0.316-0.937% in case of GF and NF, respectively.

Keywords: Gemifloxacin, Nadifloxacin, β- Cyclodextrin, modified carbon paste electrode, Differential pulse voltammetry.

1. INTRODUCTION

Cyclodextrins (CDs) are a class of cyclic oligosaccharides composed of six, seven or eight (α-, β-, γ-CD, respectively). β-Cyclodextrins (βCD) are cyclic carbohydrates with seven glucose units, which can capture the quiolone group. The mole ratio of the guest to host CDs is usually 1:1 and 2:1, due to these reasons there has been increasing interest in the use of CDs as a modifier of organic electrode reactions. It can bind a variety of guest molecules inside its torus-shaped cavities and serve as a model host site. The interaction of molecular cavities with reactive gust molecules has been widely investigated with the aim of bringing more light to the specific recognition effects and reaction selectivity. Recently, modified electrodes with CDs have attracted great interest due to their potential application as selective electrodes [1-4].

Modified electrodes acquired greater importance in the field of electrochemistry due to their advantages. Carbon and its derivatives, as the high performance material, occupy a special place in electrochemistry due to its extreme properties [5]. Carbon paste electrodes (CPEs) have found their place in modern electroanalysis due to their versatility, ease of preparation and composition modification, broad potential range availability and possibility to combine them with powerful separation methods [6-15].

Over the last twenty years, fluoroquinolones have emerged as one of the most important classes of antibiotics. Gemifloxacin (GF) is an oral broad spectrum antibacterial agent used in the treatment of acute bacterial exacerbation of chronic bronchitis and mild to moderate pneumonia. GF, a compound related to the fluoroquinolone class of antibiotics, is available as the mesylate salt [16, 17]. Nadifloxacin (NF), is a topical fluoroquinolone antibiotic for the treatment of acne vulgaris [18]. The structures of the investigated drugs are shown.

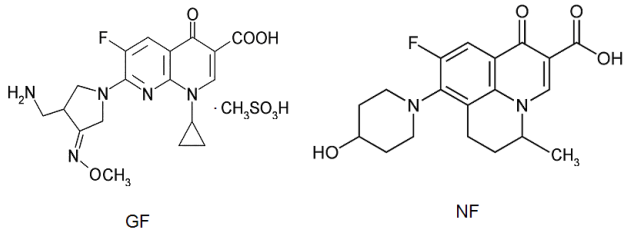

Fig. 1. The structures of GF and NF.

Fluorinated 4-quinolone derivatives have a broad-spectrum antibacterial activity against many gram positive and gram negative bacteria through inhibition of their DNA gyrase [19, 20]. A good guide to the work published for these

compounds can be found in the review written by Belal *et al*. [21].

Determination of fluoroquinolones in biological samples still require the development of simple, selective and inexpensive analytical methods without the necessity for sample pretreatment or time consuming extraction steps prior to analysis. Adsorptive stripping voltammetric analysis is an extremely simple and sensitive technique that can be used for the analysis of drugs without the necessity for extraction steps prior to the assay.

In continuation of previous work on the use of electrochemical methods for drug analysis [22-32] the present investigation deals with an electroanalytical method for determination of GF, and NF.

Limited number of methods has been reported for the separation and determination of GF and NF in pure form including HPLC [33, 34], LC [35], and electrophoresis [36]. Literature survey reveals that there is no comprehensive electrochemical study available concerning the redox behavior of NF. Only one communication reports determination of GF in pharmaceutical formulation [37].

We are interested in the adsorptive properties of βCD modified carbon paste electrodes because they can be utilized to establish a sensitive method to determine NF and GF in aqueous solution. The optimum experimental conditions such as Effect of pH, scan rate and accumulation characters were investigated.

2. MATERIAL AND METHODS

2.1. Apparatus

The electrochemical analyzer with 797 VA Computrace software (1.0) from Metrohm, Switzerland was used. The three-electrode electrochemical system comprising a carbon paste electrode (CPE) (BAS model MF-2010) was used as the working electrode, Ag/AgCl/3 mol l^{-1} KCl reference electrode, and a platinum wire counter electrode. The data were treated with Origin (Ver. 7) software to transform the initial signal. A mettler balance (Toledo-AB104) was used for weighing the solid materials. A cyberscan 500 digital (EUTECH Instruments, USA) pH meter with a glass combination electrode was served to carry out pH measurements. Deionized water used throughout the present study.

2.2. Reagent

The active ingredient pharmaceutical drugs, GF and NF were kindly provided from national organization for drug control and research. Gemique tablets containing 320 mg GF per tablet, manufactured by Obour Pharmaceutical Company, Egypt. In the present time there is no pharmaceutical form of NF in Egypt because NF is a new drug. Stock solutions of $1.0x10^{-3}$ mol l^{-1} of GF and NF were prepared by dissolving a calculated weight of the active ingredient drugs in deionized water and DMSO, respectively. More dilute solutions were prepared daily just before use. Britton-Robinson (BR) buffer solutions (pH 2-7) were used as supporting electrolytes. Britton-Robinson (BR) buffers were made in a usual way (i.e. by mixing a solution of 0.04 mol l^{-1} phosphoric acid, 0.04 mol l^{-1} acetic acid and 0.04 mol l^{-1} boric acid). Buffer solutions were adjusted by adding the necessary amount of 2.0 mol l^{-1} NaOH solution in order to obtain the appropriate pH. Graphite powder, mineral oil DMSO and βCD were supplied from Aldrich, Sigma, and Fluka.

2.3. Preparation of the working electrode

The modified carbon paste was prepared by thoroughly hand mixing graphite powder and liquid paraffin oil with βCD in a mortar with a pestle. The components were mixed in the ratio 65:35% (graphite: paraffin oil) for bare CPE and 25:60:15% (graphite: βCD: paraffin oil) for the modified electrode (βCDCPE). The carbon paste was packed into the hole of the electrode body and the electrode surface was smoothed with paper until it had a shiny appearance.

2.4. Assignment of the optimum conditions

To obtain the optimum pH, an appropriate amount of $1.0x10^{-3}$ mol l^{-1} GF or NF solution was placed in the electrolytic cell, which contain 25 ml of BR buffer solution and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values and the optimum pH was obtained.

To study the effect of scan rate (ν) on the peak current (I_p) of GF and NF, the working electrode was immersed in the optimum buffer solution containing appropriate amount of $1.0x10^{-3}$ mol l^{-1} GF or NF solution and the cyclic voltammogram was recorded at different scan rates over the scan rate range of 10-250 mVs^{-1}. Plot log I_p vs. log ν was constructed to know the nature of the process.

To study the accumulation time ($t_{acc.}$) and potential ($pot_{acc.}$), the working electrode was immersed in the optimum buffer solution containing appropriate amount of $1.0x10^{-3}$ mol l^{-1} GF or NF solution for selected times and potentials at 1200 rpm. After accumulation, the cyclic voltammogram was recorded followed by the plot of peak current (I_p) vs. time or potential to obtain optimum accumulation time (t_{acc}) and potential (pot_{acc}).

Voltammetric analyses were performed in 25 ml of BR buffer. The solution was continuously stirred at 1200 rpm when accumulation was applied for a certain time and potential to the working electrode. At the end of accumulation, the stirring was stopped and a 5 sec rest period was allowed for the solution to become quiescent. The used drug was determined by using differential pulse voltammetry DPV method. Aliquots of the drug solution were introduced into the electrolytic cell and the procedures were repeated. The voltammograms were recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All data were obtained were carried out at room temperature.

2.5. Determination of investigated drugs in Pharmaceutical preparations

Ten tablets were weighed and the average mass of per tablet was determined. A portion of the finely grounded material needed to prepare $1.0x10^{-3}$ mol l^{-1} GF solution was transferred into the 100 ml calibrated flask containing 70 ml of deionized water. The content of the flask was sonicated for about 15 min and then made up to the volume with deionized water. The solution was filtered to separate the insoluble excipients. Aliquots of the drug solution were introduced into the electrolytic cell and the general procedure was carried out.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry

In the present work, we examine the electrochemical response of NF and GF at βCDCPE. Fig. 2 illustrated the cyclic voltammograms of $1.0x10^{-5}$ mol l^{-1} of GF and NF in BR buffer of pH 4, following 120 sec accumulation at 0.3 V potential. It can be seen that, in the case of CPE, the voltammograms of GF and NF exhibit just a smaller anodic peaks comparing with the resulted peaks by using βCDCPE. βCD can act as a promoter to enhance the electrochemical reaction and lower the over potential causing the peak potential to shift to more negative value [38]. The increase in peak currents at βCDCPE may be attributed to the ability of βCD to efficiently form an inclusion complex (1:1) with quinone prior to electrochemical methods [39]. The βCD are slightly soluble in water (1.85 g/100 ml), however the use of paraffin oil as on organic binder makes the solubility of βCD the least, thus reducing risk of instability of the electrode surface during the analysis of the drug. The βCD contents of 15, 30 and 60% w/w were tested, respectively in BR buffer solution. It was found that increasing βCD content in the carbon paste would increase the peak current of GF and NF. The quinone group which captured on the surface of βCDCPE would increase the peak current of the drugs as the content of βCD is increased up to 60%, however when the content of βCD was above 60% the βCDCPE was not useful due to low sensitivity by high resistant of the βCDCPE. Thus for analysis of GF and NF the composition of βCD was chosen as 60% in the carbon paste [40].

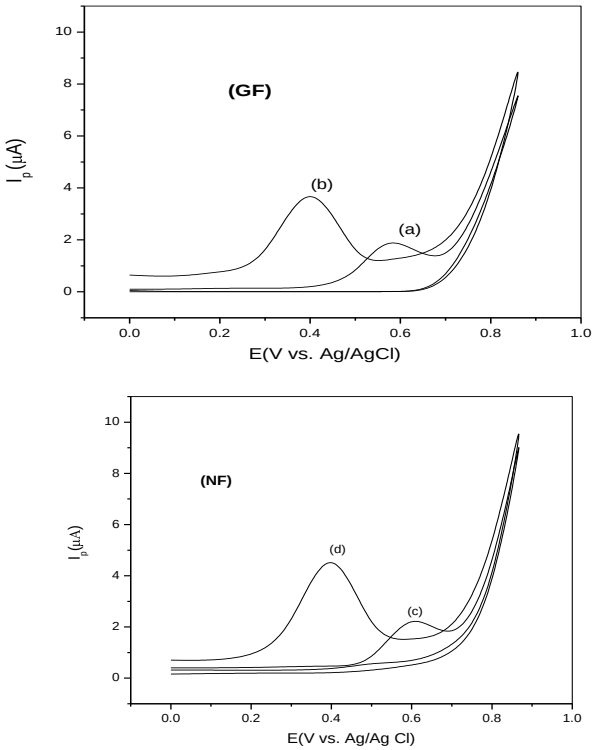

Fig. 2: Cyclic voltammograms of (a) $1.0x10^{-5}$ mol l^{-1} GF solution at CPE (b) GF at βCDCPE, (c) $1.0x10^{-5}$ mol l^{-1} NF solution at CPE (d) NF at βCDCPE in BR buffers of pH 4.0 at a scan rate of 100 mV s^{-1}.

3.2. Effect of solution pH

The effect of the buffer solution has a significant influence on the quinolone oxidation at βCDCNT/E by alternating both peak current and peak potential. Fig. 3 illustrates that the anodic peak currents reach their maximum values at pH 4.0. Therefore, pH 4 was chosen as the optimum pH value.

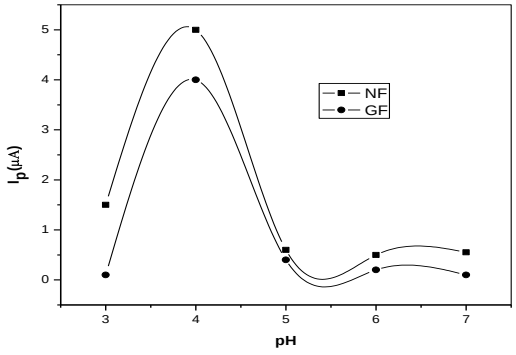

Fig. 3. Effect of pH on peak current of $1.0x10^{-5}$ mol l^{-1} GF and NF solutions in BR buffer at β CDCPE at a scan rate of 100 mV s^{-1}

3.3. The effect of scan rate

The influence of the scan rate on the peak current (Ip) of GF and NF was studied within the range 10-250 mV s^{-1}. The linear increase in the anodic peak current with the scan rate shows the adsorption control process. The plot of log Ip against log ν (fig. 4) displayed linear correlation with slope values of 0.70 and 0.73 for GF and NF, respectively [41].

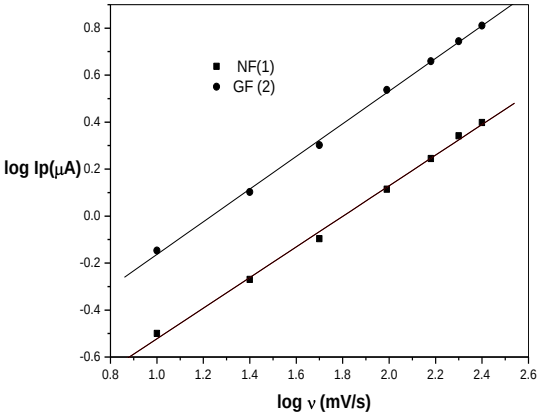

Fig. 4: Anodic peak current response of 1.0x10^{-5} mol l^{-1} GF and NF solution as a function of log scan rate (ν) in BR buffer of pH 4.0 at βCDCPE.

3.4. Effect of accumulation characters

The effect of accumulation time (t_{acc}) on the I_p was studied. Sharp increasing in I_p value was obtained up to 120 sec, the I_p practically level off above this time. This is because the active sites of electrode surface were fully saturated by the analyte, so the preconcentration time of 120 sec at βCDCPE is chosen.
Also, the dependence of peak current on accumulation potential (pot_{acc}) was evaluated over the range 0.0 to 1.0 V for 1.0x10^{-5} mol l^{-1} GF and NF solution at pH 4.0. The results obtained show that the I_p values are maxima for $pot_{acc.}$ 0.3 V, as shown in Fig. 5.

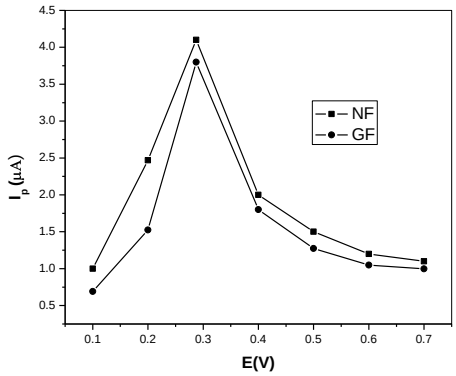

Fig. 5: Effect of accumulation potential on the peak current of 1.0x10^{-5} mol l^{-1} GF and NF solution in BR buffer of pH 4.0 at βCDCPE at accumulation time of 120 sec.

3.5. Analytical application for determination of GF and NF

The determination of GF and NF at the βCDCPE was performed by DPV and the results are shown in Fig. 6. The I_p increased with increasing GF and NF concentration at βCDCPE and there is a linear relation between the peak current and drug concentration in the range of 5.0x10^{-8} to 2.0x10^{-7} mol l^{-1} for both GF and NF. The calibration curves were represented by the following equations:

(GF) Ip (μA) = 1.014C (μmol l^{-1}) + (-0.242) r^2 (Correlation coefficient) = 0.996

(NF) Ip (μA) = 0.941C (μmol l^{-1}) + (-0.261) r^2 (Correlation coefficient) = 0.997

The LOD values were 1.2x10^{-8} mol l^{-1} and 1.0x10^{-8} mol l^{-1} for GF and NF, respectively. The LOQ were 4.3x10^{-8} mol l^{-1} and 3.3x10^{-8} mol l^{-1} for GF and NF, respectively. The percentage recoveries were found in the following ranges: 99.57-100.36% and 99.85-100.64% for normal and differential pulse voltammetry, respectively. The relative standard deviations (RSD) were found in the following ranges: 0.426-0.828% and 0.316-0.937% in case of GF and NF, respectively. The results were given in Table 1.

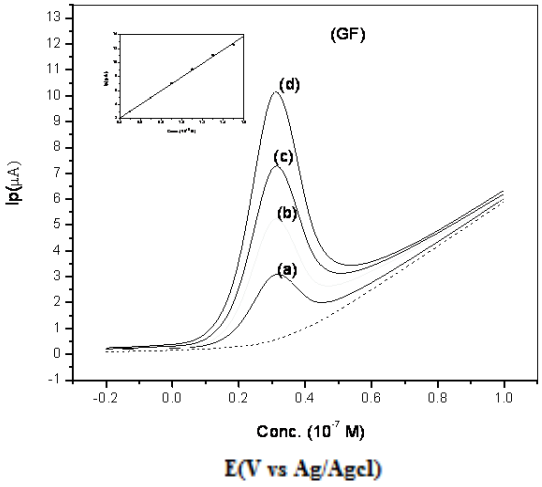

Fig. 6. Calibration curve of GF at βCDCPE by using DPV method.
(a) 0.5, (b) 0.7, (c) 1 and (d) 1.2) x10^{-7} mol l^{-1} of GF

Table 1. Analytical parameters for voltammetric determination of GF and NF using DPV.

Drug	Linearity range (mol l^{-1})	r^2	Recovery (%)	RSD*(%)	LOD (mol l^{-1})	LOQ (mol l^{-1})
GF	$5.0x10^{-8}$ to $2.0x10^{-7}$	0.996	99.57-100.36	0.426-828	$1.2x10^{-8}$	$4.3x10^{-8}$
NF	$5.0x10^{-8}$ to $2.0x10^{-7}$	0.997	99.85-100.64	0.316-937	$1.0x10^{-8}$	$3.3x10^{-8}$

** Five different concentration of GF and NF; number of replicates (n) = 5.*

3.6. Interference study

To check the interference from excipients present in pharmaceutical formulations, recovery experiments were carried out in the presence of some common excipients (e.g., cellulose, lactose, talc and magnesium stearate) added in the same ratio as in pharmaceutical preparation. The data showed that there was no interference of excipients in the analysis of GF and NF in pharmaceutical formulations by using DPV method. The results were presented in Table 2.

Table 2. Recovery study of GF and NF solutions in the presence of excipients.

Drug	Concentration		Recovery (%)	RSD* (%)
	Added (µg/ml)[E]	Found (µg/ml)		
GF	2	1.96	98	0.75
	4	3.96	99	0.82
NF	2	1.97	98.5	0.91
	4	4.02	100.5	1.10

** Five different concentration of GF and NF; number of replicates (n) = 5.*

[E] Prepared solutions of GF and NF containing excipients.

3.7. Assay of tablets

It must be noted that the NF is a new drug and does not exist in the form of pharmaceutical dosage forms in Egypt; currently in this paper the study of the overlap (interference) is sufficient. The adequacy of the developed method was evaluated by quantifying GF in commercial pharmaceutical dosage form (Gemique tablets). The direct determination of GF in commercial tablets using DPV method was carried out without any sample extraction prior to analysis. The content of GF in tablet was found to be 99.38±0.85% and 100.55±0.92% of the label claim by investigated method, respectively. Statistical analysis (t- and F-tests) [42] showed that there is no significant difference between these values at the 95% confidence level compared to the official method [43] indicating the method has similar accuracy and precision. The results were shown in Table 3.

Table 3. Application of the proposed and reference methods for the analysis of GF in Gemique tablets.

Method	Label claim (mg/tablet)	Amount found	% found ±RSD*	t-test[a]	F-test[a]
DPV	320	318	99.38±0.85	1.9	3.1
Official [43]		321	100.55±0.92	2.1	2.9

[a] The tabulated values of t- and F- at 95% confidence limit are 2.31 and 6.39, respectively.

** Five different concentration of GF; number of replicates (n) = 5.*

4. CONCLUSIONS

A rapid, inexpensive, sensitive and selective DPV method is proposed for the determination of GF and NF in pure form and pharmaceutical formulations using βCDCPE. βCDCPE can greatly improve the sensitivity compared with bare CPE. Such an improved sensitivity in voltammetric response would be of advantage for voltammetric determination of GF and NF. The high percentage of recoveries in pharmaceutical formulations without any preliminary treatment confirms the suitability of the proposed method. Further, due to stability, accuracy and low cost, the method offers promise as a substitute for the previous approaches used in routine analysis.

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