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Determination of Norfloxacin using glassy carbon electrode for pharmaceutical formulations

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Abstract
Norfloxacin (NOR) is a fluoroquinolone antibiotic commonly used to treat bacterial infections in humans. Detection of Norfloxacin (NOR)in this study was carried out Differential pulse voltammetry an electrochemical method at Glassy carbon electrode (GCE) . The voltammetric behavior of NOR was investigated in a suitable supporting electrolyte, and the effects of various experimental parameters such as scan rate, pulse amplitude, pulse frequency, pH were optimized. This electrochemical sensor shows excellent sensitivity and high selectivity for an electrochemical detection of NOR. The response is linear over the NOR concentration range of 1.5×10^{-8}M to 1.0×10^{-3} M, the LOD of 1.27×10^{-9}M and LOQ of 3.84×10^{-9}M. The developed DPV technique was successfully applied for quantitative detection of NOR in pharmaceutical formulations.

Keywords: Norfloxacin, Differential pulse voltammetry, Glassy carbon electrode.

1. INTRODUCTION:

Fluoroquinolones belong to antibiotic drug class mainly used for the treatment of bacterial infections. Norfloxacin(NOR) is a synthetic broad-spectrum fluoroquinolone antibiotic utilized practically to treat bacterial infections so has an improved potency and stability as an antibacterial agent. It works on the infections by entering the bacterial cells, causing interference with the machinery of replication, and leading to the death of the bacterial cell. Despite its therapeutic benefits, the extensive use of Norfloxacin has underlined concerns about the development of antibiotic resistance and its persistence in the environment [1].In this regard, Norfloxacin level monitoring in a clinical setting becomes very key in establishing its therapeutic efficiency while at the same time reducing side effects. Further, the presence of Norfloxacin in wastewater and natural water bodies resulting from pharmaceutical wastes imposes environmental risks [2.3].Hence, there is a need to have reliable, rapid and sensitive method for detection and quantification of Norfloxacin. Also, to affirm that the drug should not be misused and does not cause danger for human beings, there is a need to evaluate and monitor the level of NOR in human. Techniques like capillary electrophoresis[4], extractive spectrophotometry [5], HPLC [6], and photoinduced spectro-fluorimetry [7] have been used for its simultaneous determination in combined formulations, both in pharmaceutical and biological samples.

The electrochemical technique is cheaper, with shorter analysis time and greater sensitivity. It is rather preferred and fast in situ pretreatment technique being easy to handle. [8-10]. Glassy carbon electrode

represents the most used carbon-based electrode in analytical laboratories. Sometimes, it is referred to as vitreous carbon as it is a form of glass-like carbon that combines some of the properties of glass with those of normal industrial carbons. Because of its excellent electrical and mechanical properties, its wide working potential window, high chemical inertness, and relatively reproducible performance, it has enjoyed immense popularity. In the present work, a very simple and inexpensive pretreatment bare GCE was applied to the determination of NOR, the work is exploited in extending the applicability of GCE for real sample analysis.

Differential Pulse Voltammetry (DPV) is a highly sensitive electrochemical technique widely used for the analysis of various analytes, including pharmaceuticals, biomolecules, and environmental contaminants. Its application in the determination of NOR provides a valuable tool for precise and reliable quantification of this essential compound [11-13]. Differential Pulse Voltammetry (DPV) stands out due to its high sensitivity and selectivity, making it well-suited for detecting low concentrations of nicotinic acid. In DPV, a series of voltage pulses are superimposed on a linear scan of the potential, and the resulting current responses are recorded. This technique enhances the resolution of redox processes and reduces background noise, allowing for the precise determination of analytes even at trace levels. This study shows that GCE provides a powerful approach for the precise determination of NOR in pharmaceutical samples.

2. MATERIAL AND METHODS

2.1 Chemicals:

All chemicals were of Analytical grade and were used as received without further purification,Norfloxacin Boric Acid, o-Phosphoric Acid, Glacial Acetic Acid, NaOH,
Conc. HCl, Sodium Citrate, Citric Acid anhydrous, Disodium Hydrogen Phosphate,
Potassium Dihydrogen Phosphate, Sodium acetate, Acetone. Double distilled water was used for the preparation of aqueous solutions having a specific conductivity 0.4 -0.9 µS

2.2 Methods:

i. **Preparation of Standard Norfloxacin Solution (1×10^{-4} M) :**
250ml Standard solution was prepared by dissolving 0.0079g of NOR in distilled water and made the volume upto 100ml with the same solvent.

ii. **Preparation of Buffer Solution:**

1. **BR Buffer (0.04M) :** Prepare buffer solution by adding 4.948g boric acid, 4.56ml glacial acetic acid and 5.84ml of o-phosphoric acid in distilled water
2. **Citric acid-sodium citrate buffer:** Prepare 0.1M buffer solution in 1litre by adding 24.2g sodium citrate and 3.358g of citric acid anhydrous solution in distilled water
3. **Phosphate Buffer:** Prepare buffer solution in 500ml by adding 14.1g disodium hydrogen phosphate and 11.45g of potassium dihydrogen phosphate.
4. **Acetate Buffer:** Prepare 1litre by adding 7.72g sodium acetate and 0.35ml acetic acid.

iii. Preparation of different pH solution of BR buffer from BR buffer of pH 2:
Take 100ml buffer solution and then adjust at different pH from 2 to 9 with NaOH or HCl. Dilute 10ml standard solution with adjusted pH solution.

iv. Preparation of Different concentration solution:
Perform serial dilutions of the stock solution to prepare lower concentration solutions from 1×10^{-4}M with citrate buffer solution for concentration study and for the determination of NOR in real samples.

v. Preparation of solution for the determination NOR in Tablets:
Three samples containing NOR each were accurately weighed and finely powdered with a mortar and pestle to the corresponding weight of the real sample. 50ml of distilled water was added. The mixture was shaken for 10minutes and filter through a filter paper, made into 100ml volumetric flask. Appropriate volume of the sample was diluted to 50ml with citrate acid buffer solution(pH 6)and then transferred to an electrolytic cell for the determination of NOR.

vi. Preparation of solution for the determination NOR in Eye drops:
A known volume of the Eye drop was taken and made up in a 50ml Standard flask with citrate acid buffer solution (pH6) and then transferred to an electrolytic cell for the determination of NOR

vii. Preparation of modified Glassy carbon electrode
Polish the GCE using alumina slurry on a polishing cloth to remove any surface impurities and create a smooth surface. Allow it to dry at room temperature. Perform electrochemical tests (e.g., CV and DPV) to evaluate the performance of the doped electrode and ensure that the nanoparticles are effectively enhancing the electrode's properties.

2.3. Instrumentation:

All voltammetric measurements study has been performed on PhadkeSTAT 20 potentiostat. A three electrode system employing an Ag/AgCl (3M KCl) as reference electrode, platinum electrode as counter electrode and glassy carbon as working electrode was used. The pH measurements were performed using an ELICO LI 120 pH meter.

2.4. Determination of Norfloxacin

Differential pulse voltammetric (DPV) studies were carried out with appropriate quantity of the analyte (NOR) in 50mL standard volumetric flask and then making up to the mark with pH 6.0 Citrate buffer (Cit). The solution was then transferred into an electrochemical cell and the measurements were carried out at 25 ± 0.2°C. N_2 gas purging was not required as oxygen did not interfere in the measurements. DPVs were recorded within the potential range 0.0 to − 1.6 V with a scan rate of 50 mVs^{-1} and modulation amplitude of 50 mV.

3. RESULTS AND DISCUSSION:

3.1. Effect of pH:

The effect of change in pH on peak potential for NOR was investigated by different pulse voltammetry

from pH 2 to 8 in BR Buffer of 0.04M. Standard solution of NOR (1×10^{-5}) was used to find the optimum pH of the supporting electrolyte at GCE. Fig. (1) represents the Voltammogram of Ip Vs Ep in pH 2 to 8 in BR buffer . Increase in pH shifted the potential to the less positive values suggesting the involvement of protons for the electro-reduction of NOR. With the increase in pH the peak currents were found to increase showing maximum at pH 6 and decrease thereafter. The reason could be to the fact that the repulsion electrostatic interaction of the molecule with the surface of the electrode made the reduction of NOR at the electrode surface become kinetically less favorable. Therefore, pH 6 which shows maximum peak current for molecule was selected as the optimum pH for further studies.

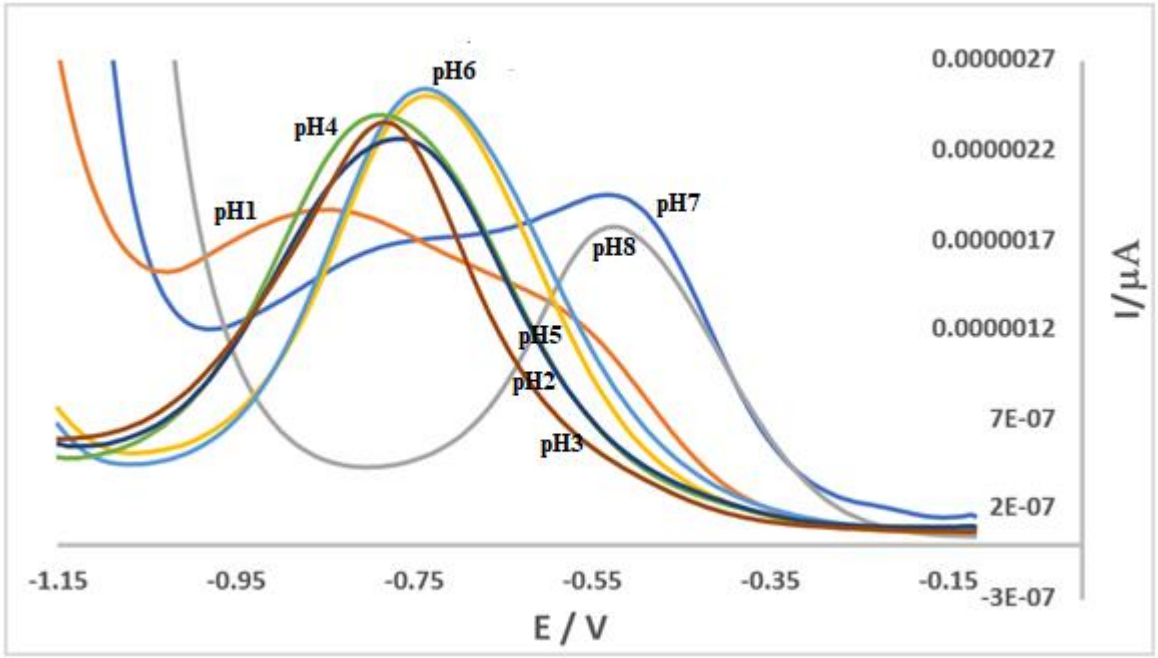

Fig. 1: pH study by Differential pulse voltammetry for reduction of 1×10^{-5}M NOR at; glassy carbon vs. Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25^0C

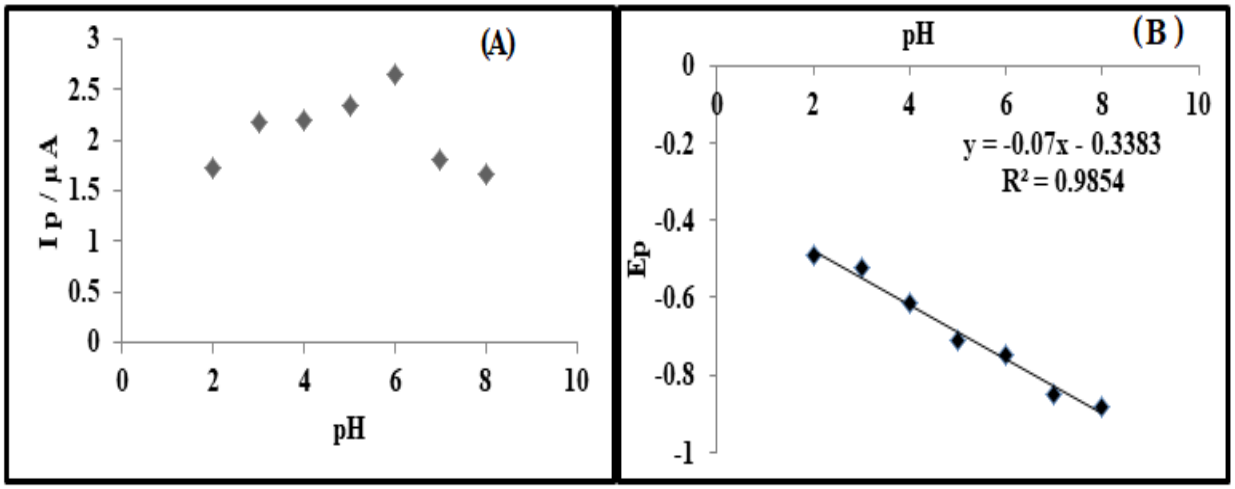

Fig. 2: Plot of Ip vs pH and Ep vs pH by Differential pulse voltammetry for reduction of 1×10^{-5}M NOR at; glassy carbon vs. Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25^0C.

3.2 Effect of supporting electrolyte

The effects of several supporting electrolytes viz. phosphate buffer, acetate buffer, citric acid- sodium citrate buffer, of 0.1M at pH 6 for 1×10^{-5}M NOR on peak current was tested in Fig.3. Of this citric acid- sodium citrate buffer gave the best response in terms of peak height and peak shape. Further optimization of buffer concentrations was carried out by varying citric acid- sodium citrate concentration in the range from 0.05M, 0.1M.0,15M and 0.2M the best peak response was observed for 0.05M for citric acid- sodium citrate (pH 6) and hence was used for the further studies

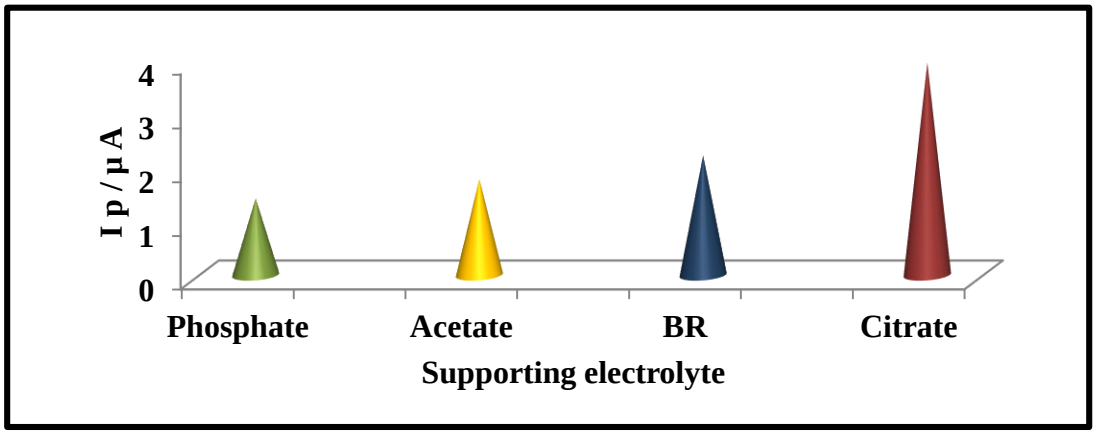

Fig. 3: Plot of Ip vs supporting electrolyte by Differential pulse voltammetry for 1×10^{-5}M NOR at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s at 25^0C.

3.3. Determination of NOR by Differential Pulse Voltammetry (DPV):

3.3.1 Effect of pulse time

The effect of pulse time was studied of 1×10^{-5}M NOR for the purpose of investigating their reaction mechanism which are shown in Fig (4). The influence of pulse time was studied from 0.2 to 0.4 secs for the NOR at GCE in 0.05M citric acid- sodium citrate buffer (pH 6). The peak current varied linearly with the increase in the pulse time for NOR

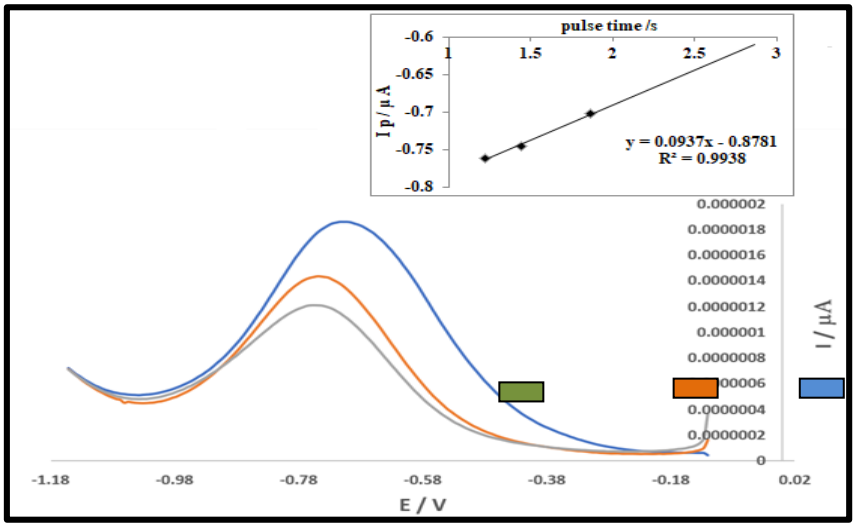

Fig: 4 Voltammogram of NOR at pulse time 0.2sec ( ), 0.3sec ( ) and 0.4sec ( ) at 1×10^{-5}M NOR in 0.05M Citrate buffer ; at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s at 25^0C.

3.3.2 Effect of pulse size

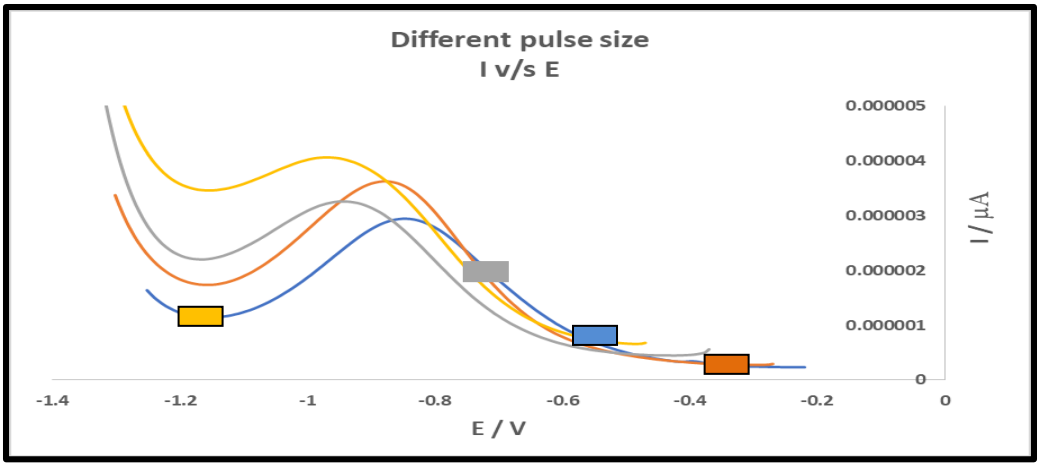

Fig: 5 Voltammogram of 1×10^{-5}M NOR at pulse size 20mV ( ), 30mV( ), 40mV ( )and 50mV( ) at pH=6 in 0.05M Citrate buffer ; at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s at 25^0C

The effects of pulse size were studied for 1×10^{-5}M NOR for the purpose of investigating their reaction mechanism which is shown in Fig (5). The influence of pulse size was studied from 100 to 1000V for the NOR at GCE in citric acid- sodium citrate (pH 6) . The peak current were independent with variations in potential (100V to 1000V) and time (0.2 to 0.4secs) for 1×10^{-5}M NA reconfirming that the process of NOR reaching the GCE surface was purely by diffusion.

3.3.3 Effect of concentration

DPV is a type of pulse voltammetry where the current response is measured while applying a series of pulse potentials to the working electrode. DPV involves discrete potential pulses superimposed on a constant base potential. The DPV technique was used for determination of NOR at GCE. The instrumental variables where pulse size 50mV, pulse time 0.2sec, current range 200µA. the linear working range (LWR), empirical limit of detection (LOD) and correlation coefficient were determined and are presented in Table 1.

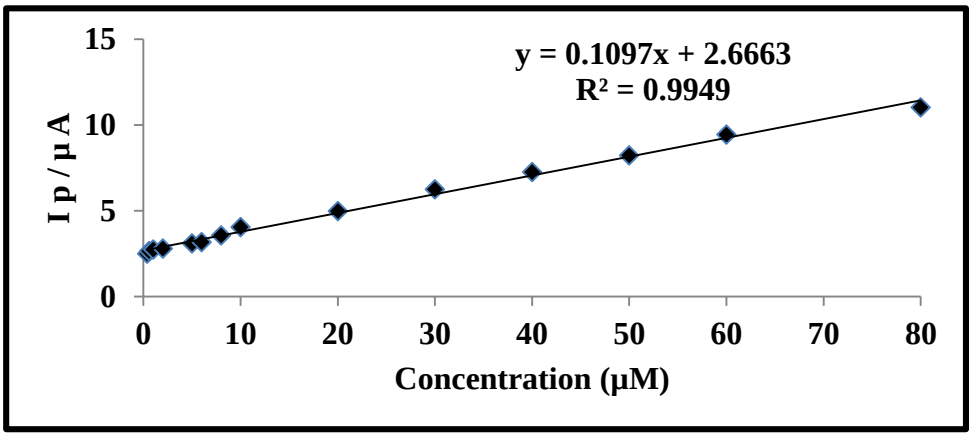

Fig. 6: Plot of Ip vs concentrations (µM) of NOR at glassy carbon electrode in 0.05M citrate buffer

pH=6; at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s, 0.2sec, 50mV at 25^0C.

Molecule	LOD (M)	LOQ (M)	%RSD	LWR	LRE	r
NOR	1.27×10^{-9}	3.84×10^{-9}	1.56	1.5×10^{-8} to 1.0×10^{-3}	I_p (µA) = 0.1097(µM) + 2.663	0.9949

Table 1: Analytical parameters for electrochemical determination of NOR at glassy carbon electrode in 0.05M citrate buffer (pH 6.0) at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s , 0.2sec, 50mV at 25^0C

3.3.4 Validation studies, interference studies and analytical applications:

Various parameters such as repeatability, reproducibility, precision and accuracy of the analysis were obtained by performing five replicate measurements of 1×10^{-5}M NOR for validation of the proposed method over intraday assay (single day, n = 5) and inter-day assay (for a period of 1 week). Mean percentage recoveries (%R) and relative standard deviations (% RSD) were obtained which was satisfactory are presented in Table 2. The recoveries obtained confirmed high precision and accuracy of the proposed method. The validity of the proposed method, verification of the matrix effect on NOR by DPV was studied. The influence on the peak heights of some interferents commonly present, some of them which form the major components of multivitamin pharmaceutical preparations were evaluated.

Molecule	Concentration taken (mol L^{-1})	Mean concentration found (mol L^{-1})	Mean recovery %	Bias %	Precision % RSD
NOR	**Intra day**				
	1×10^{-5}	0.98×10^{-5}M	98.0	- 2.0	2.85
	Inter day				
	1×10^{-5}	1.05×10^{-5}M	105.0	5.0	2.1

Table 2: Precision and Bias of assay for standard NA solution by DPV (n =5)

The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error in terms of ΔI_p less than ± 6.0% at a concentration of 1×10^{-5}M NOR Five replicates of each experimental set were performed. The results showed tolerance limit of 50 fold of ascorbic acid, 100 fold for biotin , 50 fold for thiamine hydrochloride, 50 fold for tartaric acid, 20 fold for riboflavin and 10 fold for cyanocobalamine showing that the present modified electrode was highly selective towards the determination of NOR in the presence of common physiological interferents. The validity of the electrode was verified in the determination of NOR in various pharmaceutical preparations by standard addition method (Table 3)

Pharmaceutical preparation	**Norfloxacin**	
	Amount of drug in the sample (mg)	Amount of drug obtained in the proposed method (mg) ± RSD
Norflox TZ RF	400.0	402.9 ± 2.1
Norflox 400	400.0	400.5 ± 1.2

Norflox eye/ear drop	24.0	22.8 ± 2.8

Table 3: Assay of NOR in pharmaceutical preparations (n =5)

4. CONCLUSION:

A glassy carbon electrode sensor was used for the detection and quantification of Norfloxacin.A linear working range and detection limit were obtained. Differential pulse voltammetric method was applied for the determination of Norfloxacin in tablets and drops with good sensitivity and selectivity in the pharmaceutical dosage forms. The reliability and stability of the electrode offers possibility to be used in quality control laboratories for identification and quantification of real samples

References

1. J. C. Brocklehurst and S. Brocklehurst, Br. J. Urol., (1978), 50, 102–105
2. D. A. Leigh, E. C. Smith and J. Marriner, J. Antimicrob. Chemother., (1984), 13, 79–83
3. K. J. Marians and H. Hiasa, J. Biol. Chem., (1997), 272, 9401–9409
4. T. He, Z. Xu and J. Ren, Microchem. J., (2019), 146, 1295–1300
5. B. Kaur, R. Kumar, S. Chand, K. Singh and A. K. Malik, Spectrochim. Acta, Part A, (2019), 214, 261–268
6. M. Gamal, H. M. Ali, S. M. Fraihat and T. A. Seaf Elnasr, Luminescence, (2019), 34, 644–650
7. M. S. El-Hamshary, M. A. Fouad, R. S. Hanafi, H. S. Al-Easa and S. M. El-
8. Moghazy, Spectrochim. Acta, Part A, (2019), 206, 578–587
9. Z. Liu, M. Jin, J. Cao, J. Wang, X. Wang, G. Zhou, A. van den Berg and L. Shui, Sens. Actuators, B, (2018), **257**, 1065–1075
10. E. Laviron, L. Roullier and C. Degrand, J. Electroanal. Chem. Interfacial Electrochem., (1980), 112, 11–23
11. Guangli Li, et.al. J.Hazard. Mater, (2022) 129107
12. Huang, K. J., Liu, X., Xie, W., & Yuan, H. Colloids and Surfaces B: Biointerfaces, (2008),02 003
13. Muungani, G., & van Zyl, W. VRSC Advances, . (2023), 1702.
14. Canales, C., Ramos, D., Fierro, A., & Antilén, M.. J.Electrochimica Acta (2019), 06 035.