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Research Article

ELECTROCHEMICAL BEHAVIOUR OF FOURTH-GENERATION FLUOROQUINOLONE ANTIBACTERIAL DRUG MOXIFLOXACIN BY DC POLAROGRAPHY AND CYCLIC VOLTAMMETRY

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ABSTRACT

Electrochemical behaviour of fluoroquinolone Antibiotic drug, moxifloxacin hydrochloride has been studied in aqueous medium using DC polarography and cyclic voltammetry. Moxifloxacin exhibits single well-defined cathodic peak in all the buffers like acetate buffer, phosphate buffer and B.R.buffer. The characteristics of the peak have been examined at different concentrations, pH and scan rates. This behaviour may be attributed to the reduction of the C=O double bond of reactant species in the acidic and basic medium. The experimental result shows that the reduction is irreversible and diffusion controlled. A mechanism has been proposed for the reduction of the sample.

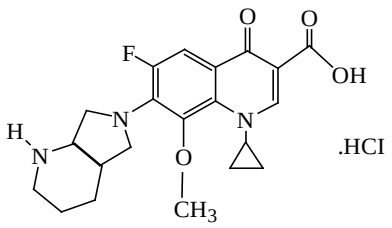
Keywords: Fluoroquinolone antibacterial drug Moxifloxacin, DC Polarography, Cyclic Voltammetry

INTRODUCTION

Moxifloxacin is a fourth-generation fluoroquinolone antibacterial agent active against a broad spectrum of Gram-positive and Gram-negative ocular pathogens, a typical microorganisms and anaerobes[1-2]. It differs from the other quinolones by having a methoxy radical at the 8-position, with an S, S-configured diazabicyclonoyl ring moiety at the 7-position, and by having improved anti-bacterial activity over other similar quinolones[3-5]. Moreover, this compound appears to cover bacterial resistance to second- and third-generation fluoroquinolones [6-7].

Moxifloxacin is bactericidal against a range of Gram-positive and Gram-negative organisms. Such activity arises through the inhibition of DNA gyrase (topoisomerase II) and (topoisomerase IV), which bacteria require for DNA replication, transcription, repair, and recombination. Moxifloxacin contains the C8-methoxy moiety that augments its antibacterial activity and reduces the possibility of Gram-positive mutations. Because the 8-fluoroquinolones use a different mechanism of action than do the aminoglycosides, beta-lactams, macrolides, or tetracyclines, there has been no known cross-resistance between the quinolones and these antimicrobial agents. While cross-resistance does occur between moxifloxacin and other quinolones with Gram-negative bacteria, moxifloxacin continues to have more activity against most Gram-positive bacteria, particularly those now resistant to other fluoroquinolones[8-10]. This agent also shows significant activity against *Mycobacterium leprae*.[11]

Moxifloxacin hydrochloride ($C_{21}H_{24}FN_3O_4$.HCl) has the formula weight 437.9 g/mol and melting point 324-325^{0}C. It is soluble in 0.1 mol/L NaOH; sparingly soluble in water and methanol, and slightly soluble in 0.1 mol/L HCl, N, N-dimethylformamide, and ethanol. The hydrochloric salt of moxifloxacin is off-white to yellow powder. The structure of the compound has been shown in Scheme 1. The molecular structure of the studied compound characterized by the presence of an electroactive reducible carbonyl group adjacent to carboxylic group initiated the present study.



Scheme 1: 1-cyclopropyl-7-[(1*S*,6*S*)-2,8-diazabicyclo[4.3.0]non-8-yl]-6-fluoro-8-methoxy-4-oxo- quinoline-3-carboxylic acid.

Some available methods for the estimation of moxifloxacin in biological fluids and pharmaceutical formulation are spectrofluriometric[12], electrophoretic[13] and chromatographic (HPLC and LC)[14-16]. Although chromatographic[17] and spectrophotometric[18] methods offer high degree of specificity, yet, sample clean up and instrument limitations preclude their use in routine clinical studies. In recent years, the modern voltammetric methods offer another possibility for the determination of compounds of pharmaceutical interest because these methods are faster, easier, cheaper and more sensitive than spectrometric and HPLC methods and the experimental methodology are less tedious. Some voltammetric methods for the reduction behaviour of moxifloxacin hydrochloride at HMDE[19-20] and oxidation behaviour at glassy carbon electrode have been used earlier[21]. The complex formation of moxifloxacin with some transition metal ions have also been studied electroanalytically[22-23].

In continuation of previous work the aim of the present paper is to reconsider the electrochemical behaviour of the drug at DME and glassy carbon electrode in Britton-Robinson (BR) buffer, acetate buffer and phosphate buffer.

MATERIALS AND METHODS

Reagents

A stock solution of 1x10^{-2} M moxifloxacin hydrochloride (moxif 400 mg) (Apollo pharmacy, India) has been prepared in triply distilled water and further diluted with the same solvent to give appropriate concentration for the working range.

Britton-Robinson buffer (0.1 M) solutions (pH, 2.0-12.30), used as supporting electrolyte, were prepared by dissolving a mixture of 6.18 gm of boric acid, 5.7 ml of glacial acetic acid, and 6.7 ml of Ortho-phosphoric acid in 1000 ml of volumetric flask. pH was adjusted with appropriate amount of 0.1 M Sodium hydroxide.

Acetate buffer (pH 3-6, 0.1M) was prepared with acetic acid and sodium acetate. Phosphate buffer (pH 6-11, 0.1 M) was prepared using di-sodium hydrogen phosphate and mono-sodium hydrogen phosphate. All reagents were of analytical-reagent grade (Merck and sigma) and triply distilled water was used throughout.

Instrumentation

DC Polarographic experiments were carried out with Elico D.C. recording polarograph model CL 357. The current voltage measurements were performed with three electrode assembly, a dropping mercury electrode as working electrode (m = 2.768 mg/s, t = 3.0 sec, h = 60 cm.), calomel as reference electrode and platinum electrode as counter electrode. The current responses and applied potentials were recorded at scan rate 100 mV/min.

All the voltammetric measurements were performed with a CH Instruments, USA made model CHI 1230. All experiments were carried out in three-electrode system. Glassy carbon electrode (Part No.CHI 104 of a diameter 3 mm) was used as the working electrode, a platinum wire as counter electrode and Ag/AgCl electrode as reference electrode.

pH was adjusted to suitable range by Elico digital pH meter.

Procedure

Electrochemical measurements in dc polarography as well as in voltammetry were performed in the solution of total 10 ml containing Moxifloxacin, Triton-X-100 (maximum suppresser), supporting electrolyte or buffer of desired pH value. The solutions (10 ml) was placed in the working cell and were purged with nitrogen for at least 15 minutes prior to each experiment and the nitrogen atmosphere was maintained thereafter, then a negatively directed dc scan was initiated between 0.0 and -2.0 V. The polarograms were recorded in the following order: pure supporting electrolyte and after addition of each aliquot of moxifloxacin hydrochloride.

In cyclic voltammetry before each measurement, the Glassy carbon electrode was polished, at the start of the work, with aqueous slurry of 0.5 µm alumina on a damp silk cloth until a mirror-like finish was obtained, then it was rinsed with distilled water and dried with a non-abrasive tissue paper. All voltammetric measurements were carried out at room temperature after purging with pure nitrogen for 10 minutes.

RESULTS AND DISCUSSION

DC polarographic study

Electrochemical behaviour of Moxifloxacin hydrochloride has been studied in different supporting electrolytes like B.R.buffer, Acetate buffer and Phosphate buffer in aqueous medium. In all the mediums moxifloxacin gave one well-defined cathodic peak due to the reduction of carbonyl group. The plot of i_d versus concentration was found to be linear and also the wave height increases with increasing the mercury height (h); a plot of i_d vs. the $\sqrt{h}$ wave height gave a straight line. Temperature dependence of the diffusion current for the reduction wave was found to be linear. The temperature coefficient calculated was 1.63%/C over the range 20-40^{0}C. Since the temperature coefficients of the diffusion currents of most organic molecules are usually of the order 1% to 2%/deg[24]. These characteristics points to a diffusion controlled process.

Linear plots were obtained for log i/i_d-i versus $E_{d.e.}$ with slope 0.0591/αn volts and zero intercept on y-axis gave the value of $E_{1/2}$. The values of slopes indicated that the reduction of Moxifloxacin hydrochloride is irreversible. The kinetic parameters were calculated from Meites-Israel method and also by the further modified Gaur-Bhargava's method[25].

According to them the equation for irreversible wave was found to be

$$E_{d.e.} = \frac{0.05915}{\propto n} log \frac{1.349\, K^0_{fh} t^{1/2}}{D^{1/2}} - \frac{0.0542}{\propto n} log \frac{i}{i_d - i} \quad \ldots\ldots(1)$$

which may be written as-

$$E_{d.e} = E_{1/2} - \frac{0.0542}{\propto n} log \frac{i}{i_d - i} \quad \ldots\ldots(2)$$

$$E_{1/2} = \frac{0.05915}{\propto n} log \frac{1.349 K^u_{fh} t^{1/2}}{D^{1/2}} \quad \ldots\ldots(3)$$

Where,

K^o_{fh} = formal rate constant for forward reaction

D = diffusion constant

α_n = transfer coefficient

And other terms have usual significance

Thus the value of αn was obtained from the slope of the straight line corresponding to $E_{d.e.}$ V/s log i/i_d-i. The intercept of the same plot gives the value of $E_{1/2}$ which was used to calculate K^o_{fh} after getting the value of $D^{1/2}$ from the Ilkovic equation. Meites Israel has extended the Koutecky's graphical method into comparatively more precise mathematical form. Further, Gaur-Bhargava has also extended the Koutecky's treatment for irreversible wave, since according to them the diffusion to the electrode surface (mercury drop) is spherical and not a linear one as assumed by Meites and Israel[26]. Gaur Bhargava's modification:-

$$E_{1/2} = \frac{0.05915}{\propto n} log \frac{K^u_{fh} t^{1/2}}{antilog(c) D^{1/2}} \quad \ldots..(4)$$

Effect of concentration

Polarograms were run of solutions containing moxifloxacin hydrochloride in concentrations ranging from 1×10^{-3}M to 2×10^{-3}M in Acetate buffer (pH=6, 0.1 M)(Fig 1).The results indicate that the reaction is irreversible. The half-wave potential shifts towards less negative value with the increasing concentration. Further, the height of the wave was found to vary directly with the concentration indicating the electrode reaction to be diffusion controlled. The values of K^o_{fh} indicate that the electrode reaction is more irreversible at low concentrations[25]. The results are shown in table 1

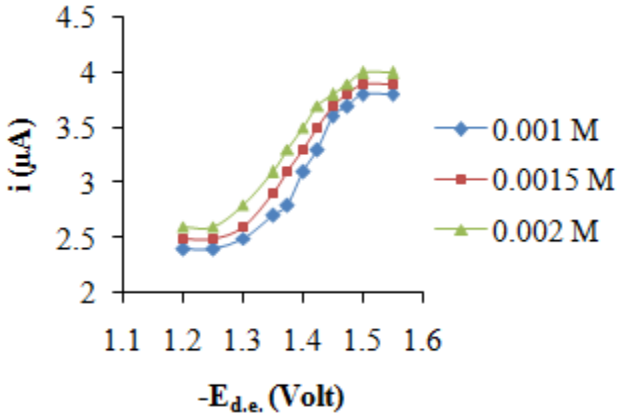


Fig. 1: Polarograms of moxifloxacin at different concentrations in acetate buffer at pH= 6.

Table 1: Effect of concentration on the polarograms of gatifloxacin in 0.1 M acetate buffer (pH=6)

Con^c(M)	-$E_{1/2}$ (Volt)	i_d (µA)	Slope (Volt)	αn	D×10^{-8} (cm^2sec)	K^o_{fh}(cm s^{-1}) Meites Israel method	Gaur bhargava's method
0.0010	1.413	0.8	0.056	1.084	7.77	4.049×10^{-26}	4.959×10^{-26}
0.0015	1.381	1.1	0.070	0.872	6.53	1.715×10^{-21}	1.340×10^{-21}
0.0020	1.353	1.4	0.077	0.792	3.67	1.068×10^{-19}	1.448×10^{-19}

Effect of pH

The pH of the electrolyte medium is one of the variables that commonly and strongly influence the shape of the polarogram, and therefore it was important to investigate the effect of pH on the electrochemical system. The effect of pH on the reduction of moxifloxacin has been studied in phosphate buffer (pH=6-10) (fig 3) and B.R. buffer (2-12.30) (fig 4).

In both buffers moxifloxacin gave one well defined cathodic peak. B.R.buffer has been selected for further studies due to its wide acidic or basic pH range and the composition of the buffer did not affect the

i_d values. With rise in pH, the half wave potential shifted to more negative value in phosphate buffer as well as in B.R.buffer indicating the involvement of protons in electrode reaction[27-28]. A plot of $E_{1/2}$ versus pH (fig 5a) gave two straight lines, with an intersection point at pH=6.8 which corresponds to the first pK_a value of moxifloxacin. This was also proved by spectrophotometry[2] and the pK_1 was found to be 6.25±0.02.

The relation between $E_{1/2}$ and pH of the solution is expressed by the following equation:

$E_{1/2}$ = – 0.056 pH – 1.075 (R=0.999)
Over the pH range 2 – 6.5
$E_{1/2}$ = – 0.099 pH – 0.783 (R=0.990)
Over the pH range 7 – 10.4

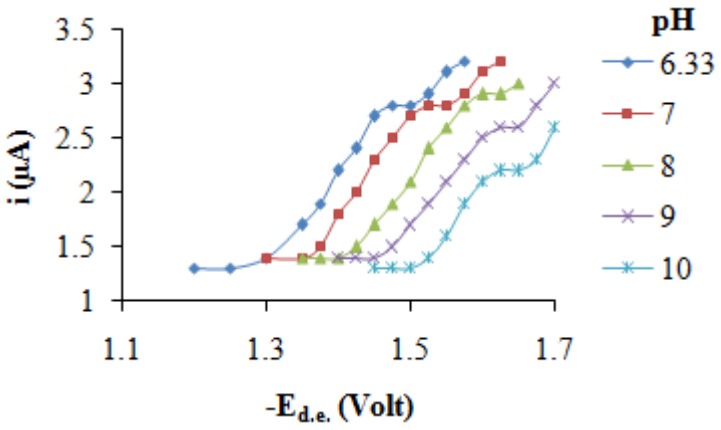


Fig. 2: DC polarograms of 2×10^{-3}M moxifloxacin at different pH values in phosphate buffer using DME.

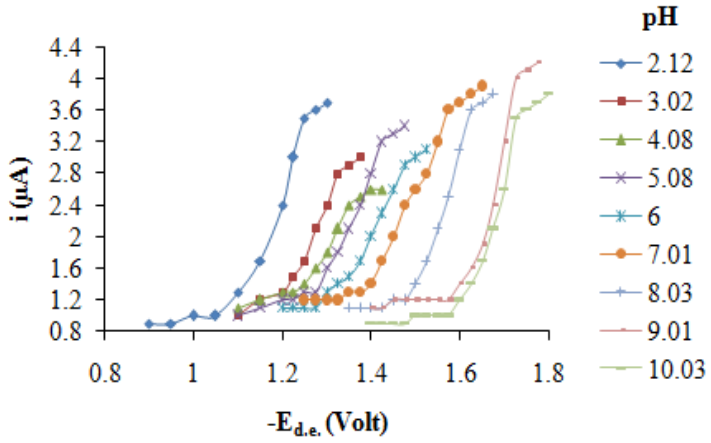


Fig. 3: DC polarograms of 2×10^{-3}M moxifloxacin at different pH values in B.R.buffer using DME.

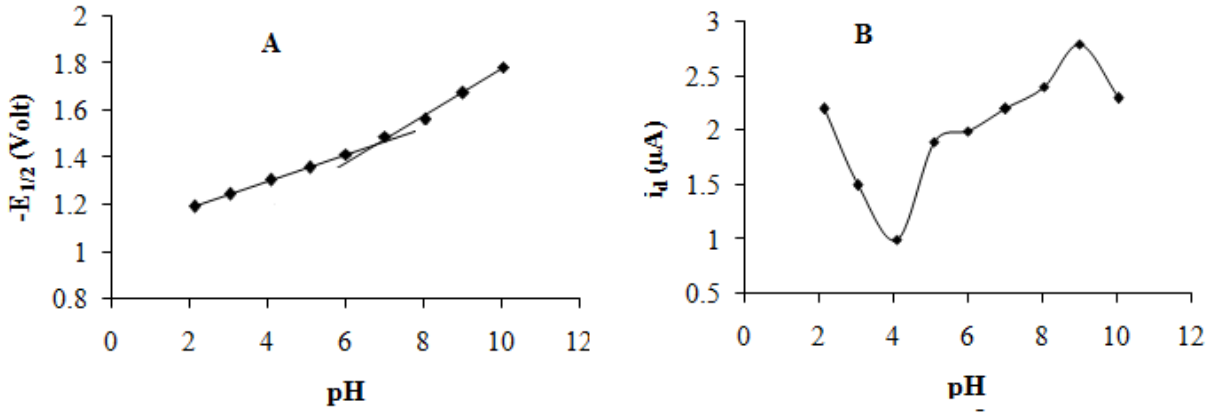


Fig. 4: The dependence of $E_{1/2}$ and i_d on pH, A and B respectively.

The logarithmic analysis of the reduction waves at different pH values resulted in straight lines with the slope values 0.045 to 0.078 V indicating the irreversible nature of the electrode process (Fig 6). From the values of the slope the value of αn (product of transfer coefficient α and number of electrons n transferred in the rate-determining step) were calculated and was found to be 0.78 to 1.33. Assuming that the electroreduction of C=O double bond involves two electrons[29] in the rate determining step the transfer coefficient should be 0.39 to 0.66 which is very close to the totally irreversible (α=0.5) electrode process. The results are listed in table 2 and 3.

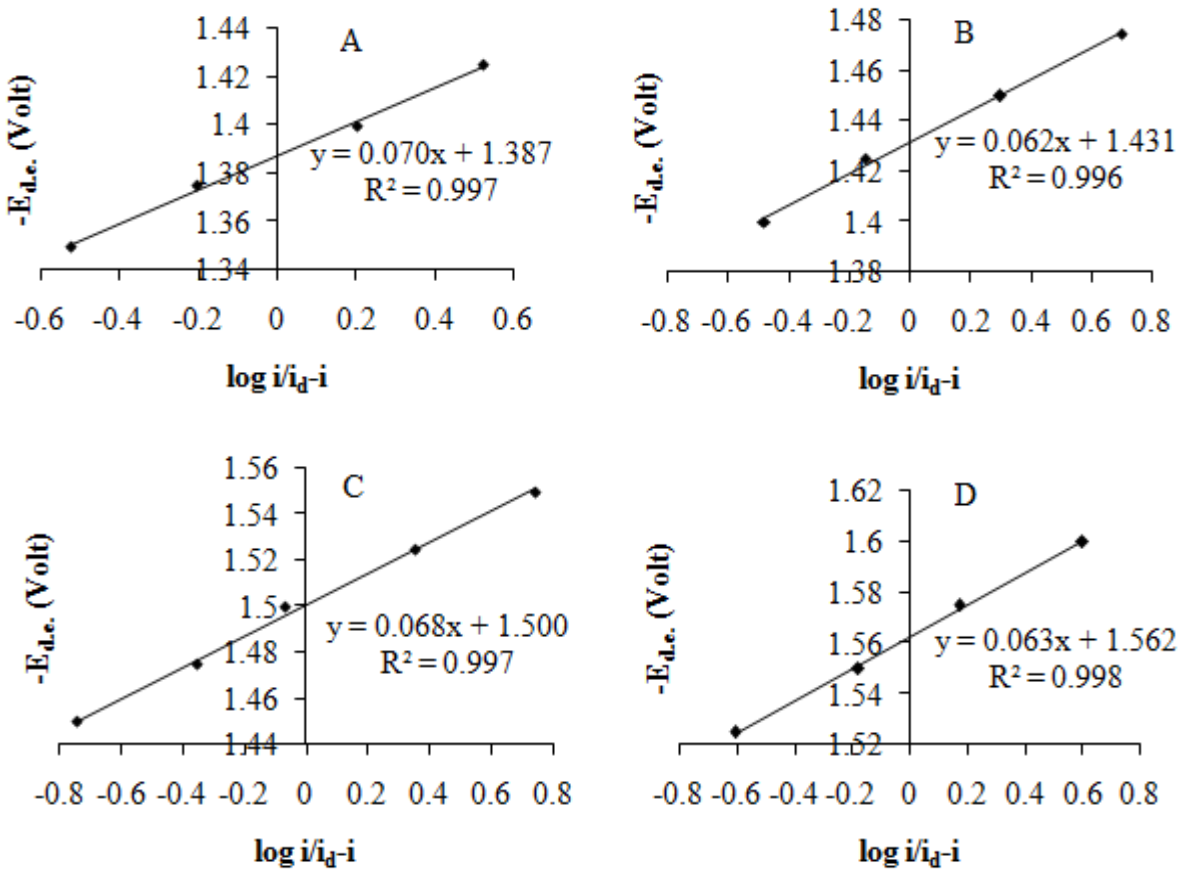


Fig. 5: Plot of $-E_{d.e.}$ vs log i/i_d-i of moxifloxacin in phosphate buffer at pH 6.33 (A), 7.0 (B), 8 (C) and 9 (D).

The number of protons, Z_{H^+}, consumed in the electrode process for each pH value was determined by the following equation[30]:

$$\frac{\Delta E_{1/2}}{\Delta pH} = \frac{0.05915}{\alpha n}(Z_{H^+}) \quad \ldots\ldots (5)$$

where the values of αn (transfer coefficient) were calculated by meites-israel method[24]. The value of Z_{H^+} was found to be 1.14 and 1.05 at pH≈7 in phosphate buffer and B.R.buffer respectively, i.e. two protons probably are consumed in the electrode process.

Further, the effect of pH on diffusion current indicates that different ionic species present in the solution having different diffusion coefficients (fig 5b). Absolute value of i_d where the reduction peak shape is well defined passes through a maximum at 9.01. Also the irreversibility of the system is more in basic medium. From the pk_1 values it is clear that this compound is weaker acid. The intra-molecular hydrogen bond formation between carboxyl and keto group in the quinolone ring contributes to lower the acidic character and also decreased the free availability of carbonyl group for the reduction in acidic medium. Thus the drug is best reduced in basic medium.

Table 2: pH effect on the polarogram of moxifloxacin (2×10^{-3}M) in 0.1 M phosphate buffer

pH	$-E_{1/2}$ (Volt)	i_d (μA)	Slope	αn	A	Z_{H^+}
6.33	-1.388	1.3	0.070	0.869	0.44	0.97
7.0	-1.432	1.2	0.063	0.975	0.49	1.14
8.0	-1.501	1.3	0.068	0.899	0.45	0.55
9.0	-1.538	1.0	0.063	0.970	0.49	0.62
10.0	-1.575	0.7	0.063	0.973	0.49	

Table 3: Effect of pH on the polarogram of moxifloxacin (2×10^{-3}M) in 0.1 M B.R.buffer

pH	$-E_{1/2}$ (Volt)	i_d (μA)	Slope	αn	A	Z_{H^+}
2.12	1.194	2.2	0.064	0.958	0.48	0.97
3.02	1.248	1.5	0.059	1.043	0.52	1.09
4.08	1.308	1.0	0.046	1.33	0.66	1.12
5.08	1.359	1.9	0.078	0.786	0.39	0.84
6	1.417	1.4	0.057	1.082	0.54	1.36
7.01	1.492	2.2	0.070	0.868	0.43	1.05
8.03	1.565	2.4	0.063	0.974	0.49	1.85
9.01	1.678	2.8	0.067	0.915	0.46	1.66
10.03	1.787	2.3	0.067	0.915	0.46	

Cyclic voltammetric behaviour

The reversibility of reduction process of moxifloxacin was further investigated by cyclic voltammetry at glassy carbon electrode. Cyclic voltammograms for moxifloxacin were recorded within the potential range -1.0 to -1.5 V at different pH and scan rate. Moxifloxacin (2×10^{-4}M) exhibited a single 2-electron irreversible cathodic peak in B.R.buffer of pH values 2.12 to 10.50. The peak potential of the reduction wave shifted to more negative value with rise in pH indicating the existence of the protonation reaction coupled with the moxifloxacin reduction process. No peaks were observed in the anodic direction suggesting the irreversible nature of the electrode process.

Effect of scan rate

The influence of the scan rate on the peak current and peak potential of 2×10^{-4}M moxifloxacin has been investigated in the range of 10-50 mV s^{-1} in B.R.buffer at pH value 9 (fig 6). Any change in i_p and E_p of reduction wave of moxifloxacin due to the changing ν values were observed. The shift of peak potential (E_p) to a more negative value with increasing the scan rate indicates the irreversible nature of the electrode process moxifloxacin at glassy carbon electrode[31].

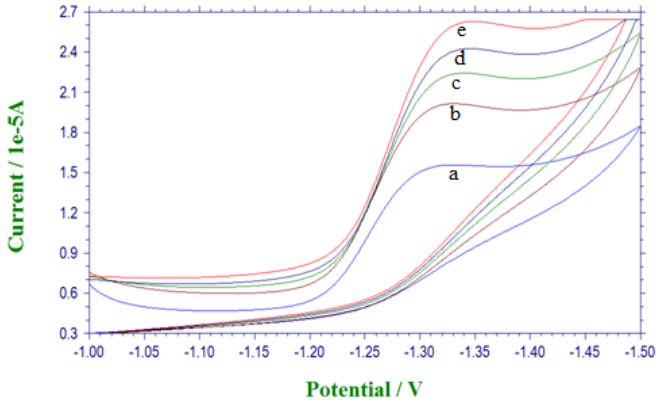


Fig. 6: Cyclic voltammograms of $2x10^{-4}$ M moxifloxacin in B.R. buffer at pH 9 on glassy carbon electrode at different scan rates: (a) 10, (b) 20, (c) 30, (d) 40 and (e) 50 mVs-1

Linear -E_p versus log ν plot[32] (fig 7a) of slope value of 0.026 V was obtained according to the following equation:

E_p = -0.026 logν - 1.302, R^2=0.969

From the slope of the straight line the value of αn was calculated using equation[33-34]; ΔE/Δlog ν =30/ αn and it was found to be 1.15.

Generally, α is assumed to be 0.5 in a totally irreversible electrode process[35]. Thus, two electrons are involved in the electro reduction of moxifloxacin.

The peak current increased steadily with increasing scan rate and the peak current function, $i_p/(AC\nu^{1/2})$, exhibited nearly constancy.

The linear relationship existing between peak current and the square root of the scan rate (fig 7b) showed that the reduction process is predominantly diffusion-controlled in the whole scan rate range studied. The equation is as follows:

i_p = 1.903 $\nu^{1/2}$+5.236, R = 0.985

This finding was confirmed by the linear plots of log i_p verses log υ (fig 7c) with the slope 0.325 mVs^{-1}, which are close to the theoretical value, 0.5[36], expected value for a process controlled by diffusion. The equation obtained was:

log i_p=0.325 logν+0.717, R=0.993.

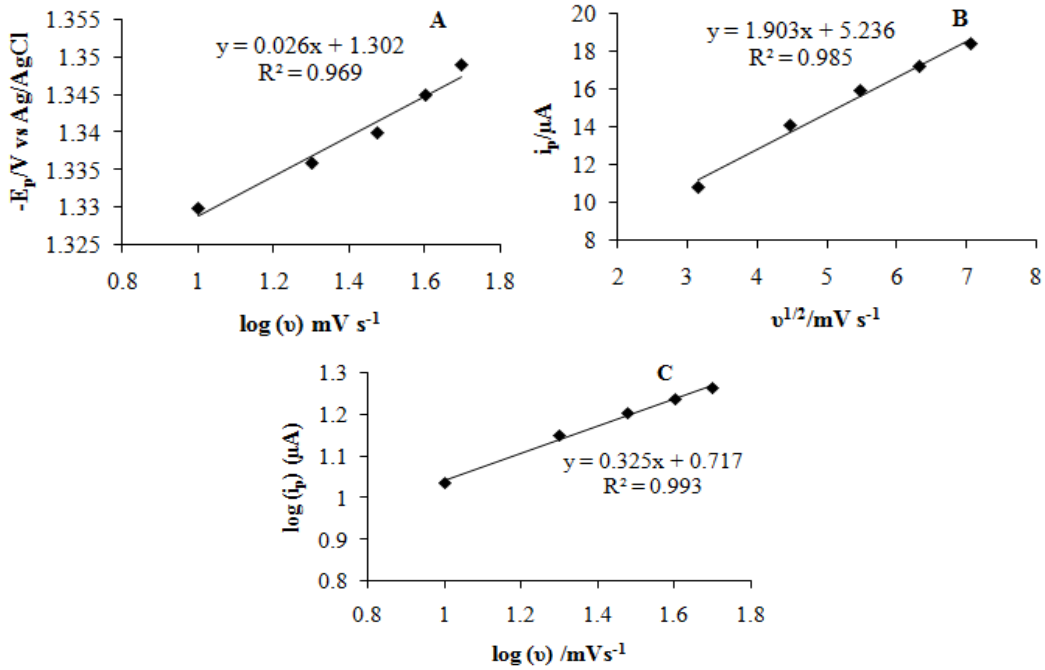


Fig. 7: Plot of -E_p vs. log ν (A), i_p vs. $\nu^{1/2}$ (B) and log i_p vs. log ν (C) from the voltammogram in fig 5.

Determination of electron transfer coefficient

The values of αn (where α is the transfer coefficient and n is the number of electrons involved in the rate determining step) were calculated for the reduction of moxifloxacin at pH value of 9.0 at glassy carbon electrode, according to the following equation[37]:

$$\alpha n = 0.048 / (E_p - E_{p/2})$$

Where, $E_{p/2}$ is the potential at which the current equals one-half of the peak current ($i_p/2$). The values of α obtained are 0.31 and 0.32 at different scan rate showing the total irreversibility of the electron transfer reaction. The results are listed in table 4

Determination of diffusion coefficients

For a redox electrode reaction, assuming that the rate of electron transfer at electrode surface is so rapid that the diffusion process in the bulk of the electrode is the rate determining step, the diffusion coefficient of an irreversible system can be calculated by the following equation [38-39]:

$$i_p = 2.99 \times 10^5 n (\alpha n)^{1/2} A C D^{1/2} \upsilon^{1/2}$$

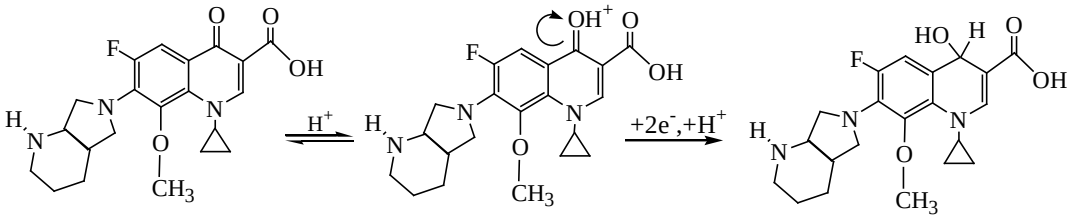
The values of diffusion coefficients decrease as the scan rate of the system increase. The values of diffusion coefficients (D) are listed in Table 4. These small values of diffusion coefficients are due to the bulky nature of the compound.

Table 4: Values of the transfer coefficient, α and diffusion coefficient, D, obtained from cyclic voltammogram of moxifloxacin at different scan rate

Scan Rate (mV s^{-1})	$-E_p$ (Volt)	$-E_{p/2}$ (Volt)	$E_p - E_{p/2}$ (Volt)	α	i_p (μA)	$Dx10^{-6}$ (cm^2s^{-1})
10	1.330	-1.2542	0.0777	0.31	10.87	1.16
20	1.330	-1.2585	0.077	0.31	14.14	0.97
30	1.340	-1.2640	0.076	0.32	15.98	0.82
40	1.345	-1.2680	0.0765	0.31	17.26	0.72
50	1.349	-1.2713	0.0758	0.32	18.39	0.65

Reaction Mechanism

It has been reported that C_5H_4NO or –COOH is the active group of quinolone. But the C_5H_4NO is the more active one, reported by the calculation of wiberg bond orders of similar compound[40] and based on the observation of polarographic and voltammetric technique two electrons and two protons are involved in the electrode reaction. Thus the possible mechanism of the electrode reaction may be as follows:



Scheme 2: possible reduction mechanism of moxifloxacin

CONCLUSION

The reduction behaviour of moxifloxacin has been studied in B.R.buffer (pH=2.12-10.50) by two simple and sensitive electrochemical methods. In both the methods the drug gave one well defined cathodic peak which is diffusion controlled and irreversible in nature. The reduction process was found to be pH dependent, as one would expect for an organic reaction, the shift per pH unit exceeds the theoretical values predicted for reversible reactions and in reaction mechanism two hydrogen ions and two electrons are involved. The reduction process was found to be more irreversible at lower concentration. If proper attention is given to the factors of pH, concentration and scan rate, the wave could be of value for qualitative identification.

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