Indian Journal of Chemistry
Vol. 38B, May 1999, pp. 553-562

Electrochemical studies of 1-cyclopropyl-6-fluoro-1,4-dihydro-7-(*N*-piperazinyl)-4-oxo-3-quinoline carboxylic acid and its synthetic precursors

K Srinivasu[†], M Sunil Kumar, A Ramachandraiah* & A Veera Reddy[†]
Department of Chemistry, Regional Engineering College, Warangal 506004

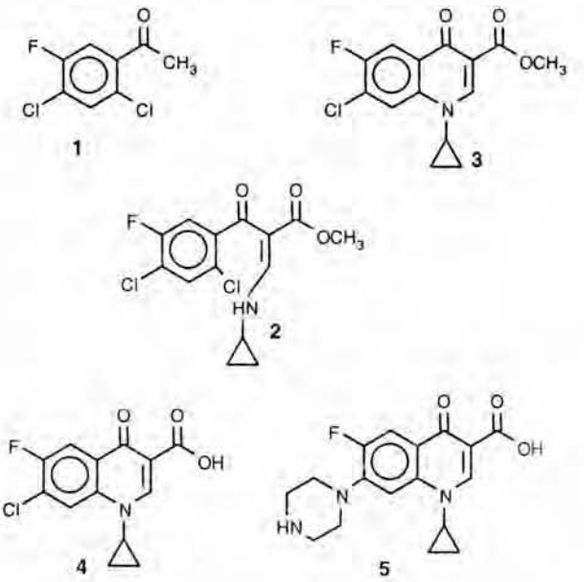
Received 5 May 1998; accepted (revised) 30 December 1998

Electrochemical studies of 1-cyclopropyl-6-fluoro-1,4-dihydro-7-(*N*-piperazinyl)-4-oxo-3-quinoline carboxylic acid **5** and its synthetic precursors, *viz.*, 2, 4-dichloro-5-fluoroacetophenone **1**, 3-cyclopropylamino-2-(2,4-dichloro-5-fluorobenzoyl)acrylic acid methyl ester **2**, 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid methyl ester **3** and 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline-3-carboxylic acid **4** are reported. Plausible electrochemical mechanism for the reduction of the series of compounds is suggested based on cyclic voltammetry, coulometry and spectral studies. The role of resonance isomerism aided by intramolecular hydrogen bonding and aromaticity, in the electrochemistry of compounds **2-5**, is discussed. The acid-base equilibria of the compounds are revisited based on PCMODEL MMX Molecular Energy Minimisation Software and cyclic voltammetry. An excellent electro-analytical method in differential pulse polarography for the quantitative analysis of the drug **5** and its synthetic precursors **1-4** is developed.

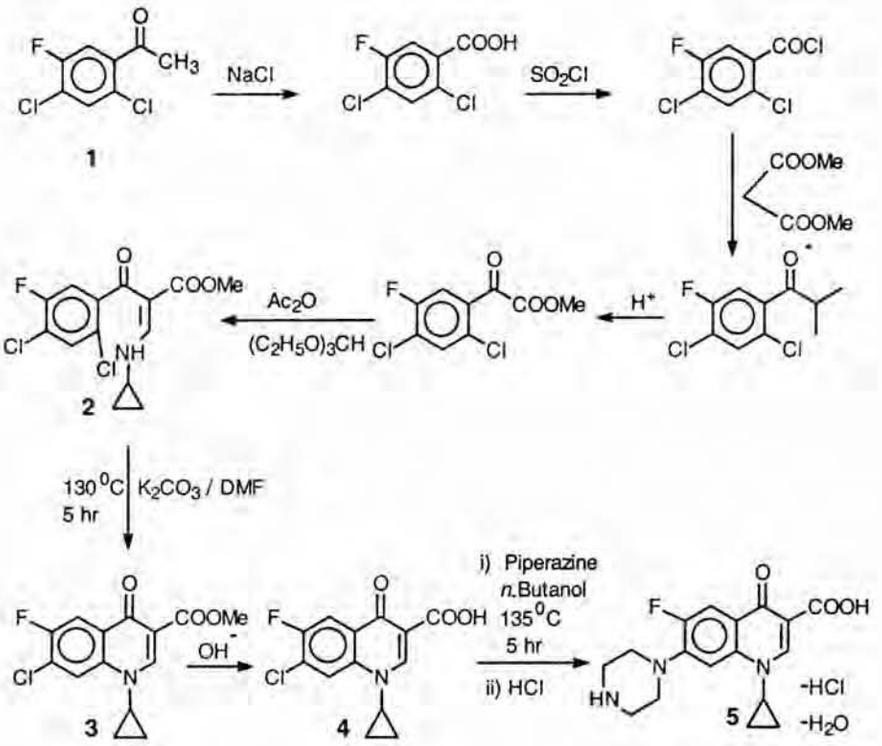
1-Cyclopropyl- 6-fluoro-1,4-dihydro-7-(*N*-piperazinyl)-4-oxo -quinoline-3- carboxylic acid **5** is an important wide spectrum drug that is vastly used in the treatment of conjunctivitis and other ophthalmological diseases, muscular disorders and pains and also in genital diseases like gonhorrea, syphilis etc. Ever since the drugs, Ciprofloxacin **5** and its family of compounds have been discovered to possess antibacterial activity due to their topoisomerase enzyme-DNA-gyrase inhibition activity, the bulk production of these drugs is one of the major trades of pharmaceutical industries all over the world[1-5]. The conventional industrial process of production of this drug employs 2,4-dichloro-5-fluoro-acetophenone **1** as the raw material which is subjected to various steps to get **5** during the synthetic route. The studies at Natco Laboratories have resulted in an alternative synthetic route with improved yields of the drug. This considers **1** as the raw material which is subjected to synthetic process passing through the intermediate stages, **2-4** as shown in **Scheme 1**.

On the basis of theoretical calculations and experimental evidence, Volmer and co-workers[6] proposed that the heterocyclic nitrogen in compounds of the type, **4** and **5**, do not involve in any acid-base equilibrium. The compounds **4** and **5** contain both a carboxylic acid and one or more nitrogen atoms to render them behave as amino acids. In fact, the presence of zwitter ion species in aqueous solution of *p*H ~ 7 is attributed for the lack of basicity of the heterocyclic nitrogen. Experimental procedure for the evaluation of micro species concentration of Ciprofloxacin which is stated to exist in the acid-base equilibria of **Scheme II**, is recently reported[7].

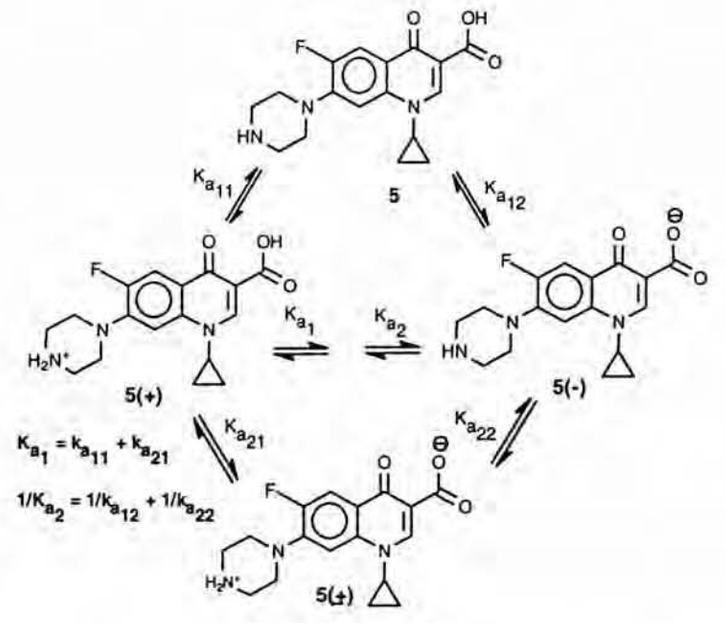
Looking at the pace with which the membership of this quinolone class of drugs is expanding and at the



[†] Natco Pharma Limited, B-13, Industrial Estate, Sanathnagar, Hyderabad 500 018



Scheme I



Scheme II

variety of structure-property relationships these compounds can exhibit, one would suggest a detailed aqueous electrochemical study of these compounds.Barring a few reports, no detailed electrochemical studies are available in the literature on these compounds[8,9] **1-5**. Herein, we present the *p*H-dependent aqueous electrochemistry of compounds **1-5** in a moderate detail.

Materials and Methods

All the compounds **1-5** are of AnalaR grade and were used after repeated recrystallisation from HPLC grade methanol for constant melting point. Comparing their FTIR with their library spectra checked the integrity of the compounds. Buffers used in our studies were prepared according to standard procedures[10]. Their *p*H was measured by an ATI Orion Model 902 Ion Meter under thermosttated conditions (25 ± 0.1° C). The UV-Visible spectra of the solutions were recorded on a Shimadzu Model UV-160A Ratio Recording Spectrophotometer fitted with thermostatted 1-cm-path-length cuvette holder. The electrochemical measurements such as cyclic voltammetry, differential pulse polarography, etc. were recorded on an EG&G PARC Model 264 A3 Polarographic Analyser/Stripping Voltammeter whereas the coulometry and electrosynthesis were performed on a BAS Model CV-27 Voltammograph, accessed with an Omnigraphic 2001 X-Y/t Recorder of Digital Electronics.

Molecular energy minimisation calculations were carried out using PCMODEL Software for Windows (Serena Software Ver. 4.1).

Results and Discussion

2,4-Dichloro-5-fluoroacetophenone, 1. This is the starting compound for the synthesis of Ciprofloxacin. It has only one electroreducible site, i.e. the carbonyl group. Its electronic spectral profiles appear to be very similar and overlap on each other at all the *p*H's. The cyclic voltammograms of this compound at three selected *p*H's are shown in **Figure 1**. The electrochemical reduction of carbonyl group in aqueous buffers is fairly understood and the suggested mechanism[11] is as shown in **Scheme III**. The carbonyl group usually undergoes irreversible reduction by two one-electron one-proton successive steps via a carbinol free radical, which also dimerises with varied speeds.

As expected, the peak potentials shift cathodically with increasing *p*H due to decreasing [H^+]. A plot of E_{pc} vs *p*H gave the number of H^+ participating in electrochemical reduction steps as expected from **Scheme III**.

All the peaks at all the *p*H's are found to be diffusion controlled as indicated by the linearity of i_p vs $v^{1/2}$ graphs, where i_p is the current and v the scan rate. The peak current, i_p, peak potential, E_p, diffusion coefficient, D_o and the heterogeneous electron transfer

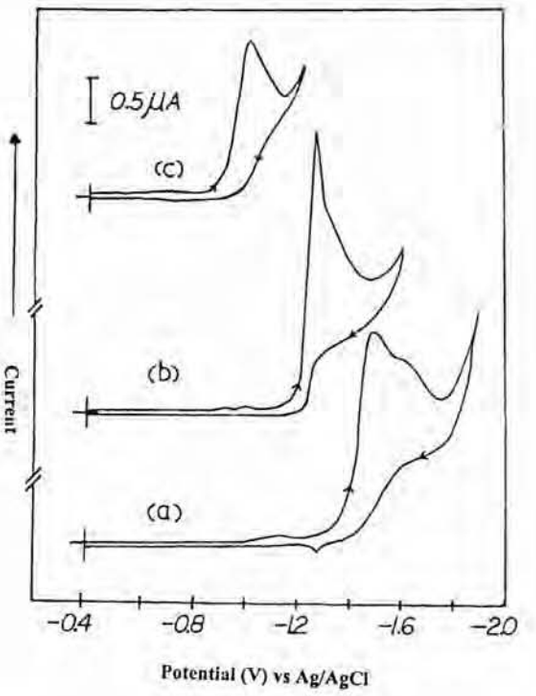
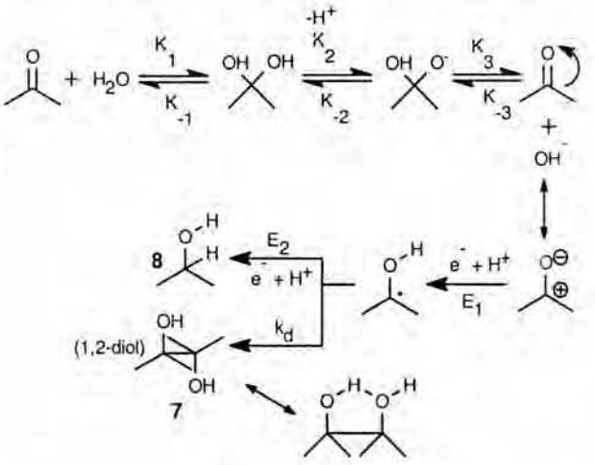


Figure 1—Cyclic voltammograms of **1** (1.03 x 10^{-3} M) at a *p*H of (a) 10.00, (b) 6.00 and (c) 2.00 at 25° C (scan rate, 50 mV/sec)



Scheme III

coefficient, K_h^0 are collected in **Table I**. The values of the corresponding parameters for pure acetophenone under identical experimental conditions are also given in **Table I** for comparison. The heterogeneous electron transfer constant, K_h^0 in the respective *p*H's is lower for acetophenone than that for **1**. This is not unexpected because the presence of strongly electron withdrawing halo substitution on the

Table I—Typical cyclic voltammetric data for the synthetic precursors, **1-4**

Substrate	*p*H	$-E_p$ (V vs Ag/AgCl)	i_p (μA)	$D_0\times10^6$ (cm^2s^{-1})	$K^o_h\times10^3$ ($cm\ s^{-1}$)
	2.00	1.27	0.45	2.37	4.32
Acetophenone	6.00	1.54	1.25	2.94	12.89
	10.00	1.72	0.86	2.52	25.98
	2.00	1.00	1.35	1.98	6.38
1	6.00	1.27	3.03	2.55	15.43
	10.00	1.50	2.18	2.13	30.99
	2.00	1.02	1.00	3.80	42.03
2	6.00	1.26	0.95	3.62	47.83
	10.00	1.62	0.60	4.01	18.05
	2.00	0.86	0.40	3.77	21.70
3	6.00	1.29	0.60	3.82	45.32
	10.00	1.52	0.60	4.01	59.30
	2.00	0.99	0.53	5.22	7.40
4	6.00	1.21	0.36	5.73	31.35
	10.00	1.74	0.45	4.81	44.32

* scan rate at 50 mV/sec

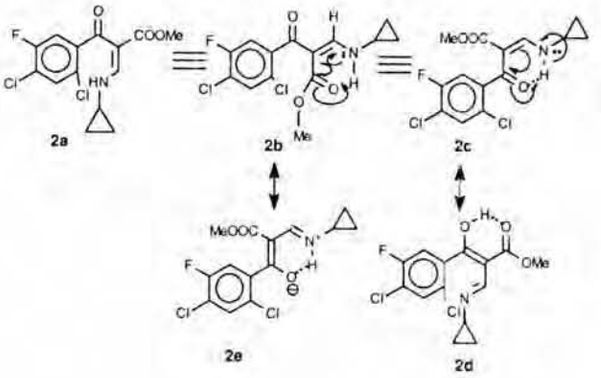
benzene ring of **1** renders the carbonyl group more easily reducible.

The relative populations of the 1,2-diol **7**, generated by the E_1C route and the mono alcohol **8**, obtained through the E_1E_2 route depend on v, k_d (dimerisation rate constant) and K^0_h among others. For moderate k_d values, a lower scan rate results in greater accumulation of **7** causing lower currents at E_2 potential and faster scan rates take the sequence to **8** with increased current heights at E_2 compared to those at E_1.

The effect of *p*H on the cyclic voltammetric profiles of **1** is shown in **Figure 1**. Only it is at *p*H > 8 that the two electrochemical steps at E_1 and E_2 of **Scheme III** are resolved. At lower *p*H's the E_1 and E_2 are very close and are unresolved. Constant potential coulometry and electrolysis at a potential slightly cathodic to the second peak potential gave a value of 2 for the number of electrons and the electrogenerated product was found to be a secondary alcohol, **8**. The FT-IR spectra of this product and an authentic **8** gave good correlation. Constant potential coulometry at a potential between E_1 and E_2 gave the charge as 1 faraday per mole. Hence, we attribute a mechanism similar to that in **Scheme III** for **1**. The peak current varies in a bell shaped curve with increasing *p*H at a given scan rate supporting the involvement of geminal diol in the electrochemical mechanism as reported in the literature[12].

Methyl 3-cyclopropylamino-2-(2,4-dichloro-5-fluorobenzoyl)acrylate 2. Compound **2** is obtained by a series of steps from **1** (**Scheme 1**). The compound in gaseous and liquid states may exist in one or several canonical structures exhibited in **Scheme IV**.

Even though **2c** is the most stable form as found from PCMODEL energy minimisation calculations, **2a** is supposed to be the conformer for the generation of **3** during the synthesis of Ciprofloxacin (**cf. Scheme I**). **Figure 2** shows the electronic spectra of **2** at three distinct *p*H's. All the spectral profiles appear to be very similar to one another suggesting little acid-base chemistry for **2**. There is a finite probability for **2** to exist in the zwitter ion form **2e** and hence the absence of acid-base chemistry to **2**



Scheme IV

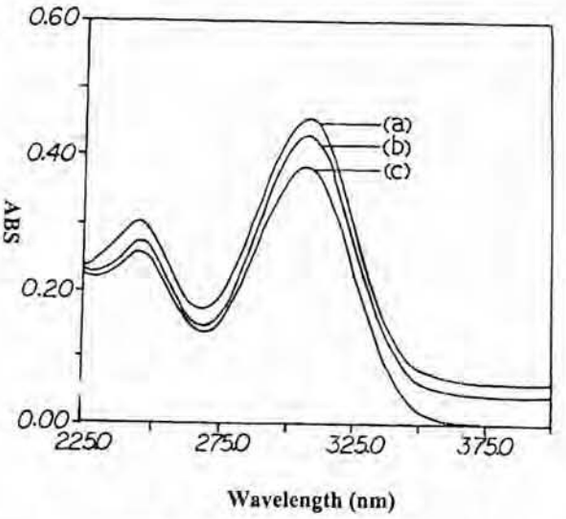


Figure 2—Electronic spectra of **2** (2.01 x 10^{-5} M) at a *p*H of (a) 2.00, (b) 6.00 and (c) 10.00 at 25°C

might be attributed to this zwitter ion as known from the literature[6]. Since each of the canonical/tautomeric forms from **2a** through **2e** has either a carbonyl group or an azomethine group, one expects **2** to exhibit multiple electrochemical reduction peaks. In all the buffers studied, **2** exhibits only one irreversible cyclic voltammetric peak in the cathodic region due to the reduction of carbonyl group. This only suggests that **2** undergoes electrochemical reduction through forms, **2a-2c** whose reduction potentials are too close to be distinguished due to their structural similarities. Since, the occurrence of only one reduction peak rules out forms, **2d** and **2e**, the absence of acid-base chemistry to **2** is to be associated with the stabilisation of geminal diol and its anion equivalents of **2a-2c** by intramolecular hydrogen bonding via amine's nitrogen and ester group's oxygens. Energy minimisation calculations by PCMODEL have revealed that **2** in the geminal diol form is more stable than **2d** and **2e**. A free radical mechanism for the reduction of the carbonyl group may be very unlikely due to the presence of long chain substituents like 2-(*N*-cyclopropyl)ethenyl group and ester group. Further, the presence of a β-double bond destabilises a tertiary free radical carbon. Hence, a single step two-proton-two-electron reduction via the geminal diol, is suggested in all the *p*H's studied.

As expected, the peak potential shifts cathodically with increasing *p*H due to decreasing concentration of H^+. A plot of E_{pc} vs *p*H gave the number of H^+ participating in the electrochemical reduction step as 2 while coulometry gave the number of electrons as 2, at all the *p*H's. The electrochemical reduction was found to be diffusion controlled as indicated by the linearity of i_p vs $v^{1/2}$ graphs. The D_o, K^0_h, E_{pc} and i_p values of **2** are collected in **Table I**.

As form **2a** which is needed for synthesis of **5**, is less stable than forms **2b** and **2c**, it is to be concluded that the high temperature and high alkaline conditions maintained during the conversion of **2** to **3** (**Scheme I**) are to account for this energy barrier.

7-Chloro-1-cylcopropyl-6-fluoro-1,4-dihydro-4-oxo-quinoline carboxylate 3. Compound **3** is a quinolone product derived by cyclisation of **2** (**cf. Scheme I**). Several of its possible canonical structures are provided in **Chart I**. Due to the presence of strongly electron withdrawing carbonyl groups and eventual electronic stabilisation by quinoline-aromaticity, compound 3 prefers the 10 π electron canonical structure **3b** [see **Chart I**]. This, in all aqueous media, would abstract a proton at the alkoxide site that is stabilised further by intramolecular hydrogen bonding. Hence, compound 3 exists essentially as a quaternary cation 3c, in aqueous media at all *p*H's because an alcoholic group does not effectively involve in any acid-base equilibria. Thus there is only one electro-reducible site, i.e., the azomethine site. Hence, one would expect a *p*H-independent electronic spectra and also cyclic voltammetric profile (except for the E_p shift with *p*H). In **Figure 3** are shown the electronic spectra of **3** at a few selected *p*H's. The spectra appear alike with no indication of any isosbesticity suggesting no basicity for the heterocyclic nitrogen.

Only one reduction peak with an adsorptive prewave is observed in the cyclic voltammetric response of **3**. The typical voltammetric data of **3** are

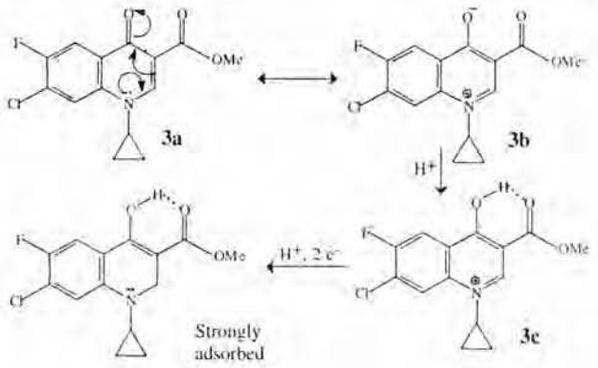


Chart I

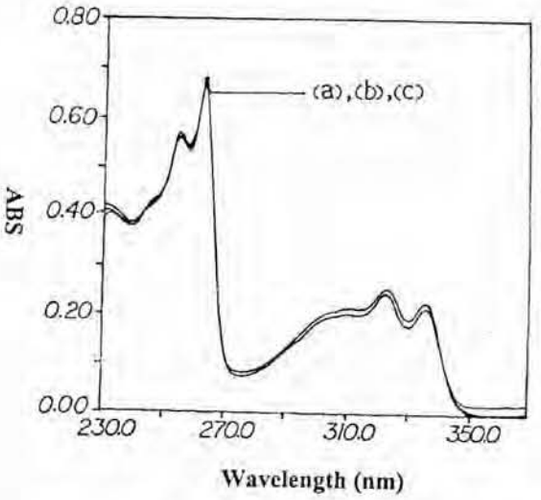


Figure 3 Electronic spectra of **3** (2.14×10^{-5} M) at a *p*H of (a) 2.00, (b) 6.00 and (c) 10.00 at 25°C

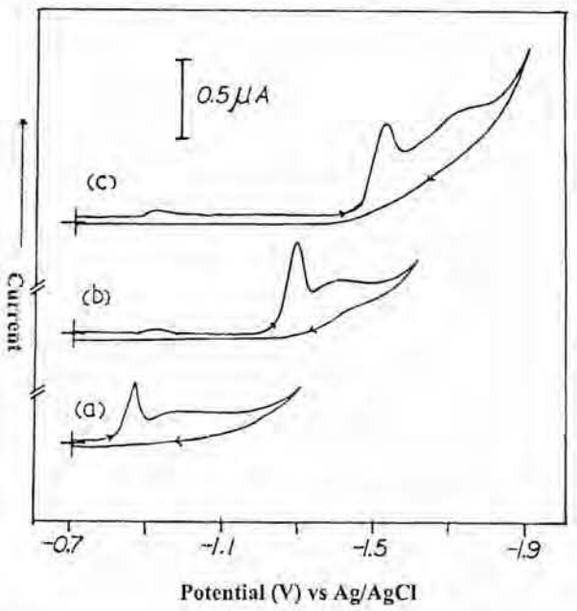


Figure 4—Cyclic voltammograms of **3** (2.13 x 10^{-4} M) at a *p*H of (a) 2.00, (b) 6.00 and (c) 10.00 at 25°C (scan rate, 50 mV/sec)

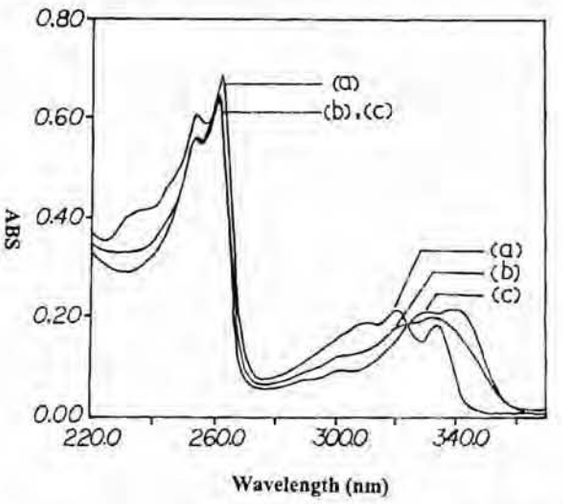
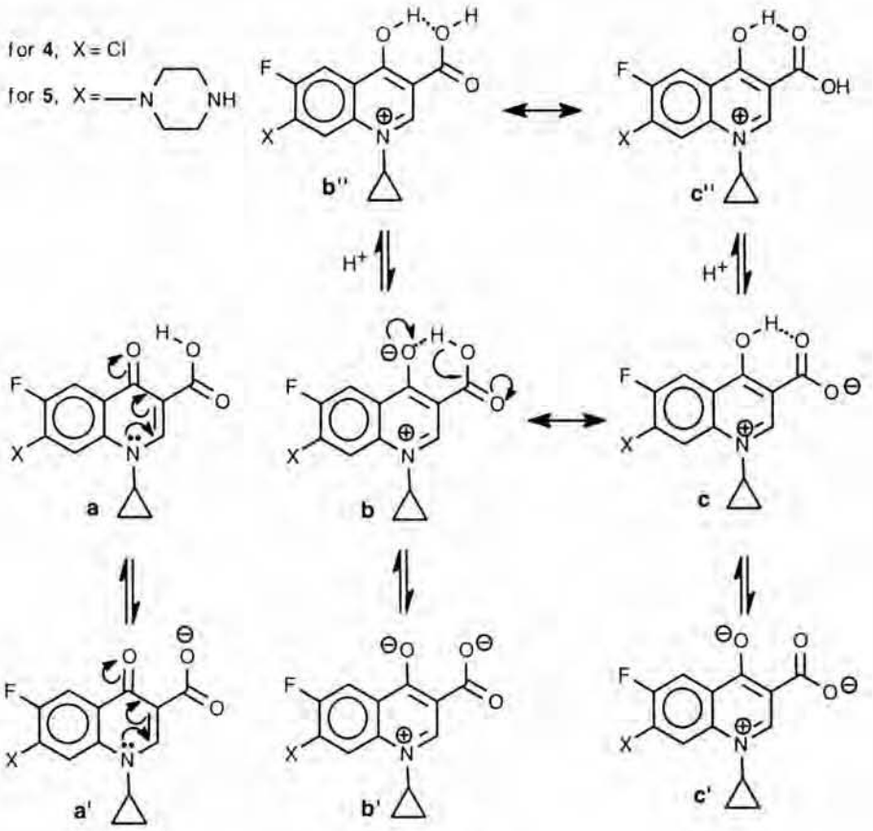


Figure 5—Electronic spectra of **4** (1.93 x 10^{-5} M) at a *p*H of (a) 2.00, (b) 6.00 and (c) 10.00 at 25°C

collected in **Table I**. In **Figure 4** are shown the cyclic voltammograms at three different *p*H's. The voltammograms appear similar in pattern with a cathodic shift of E_p with increasing *p*H. That the first sharp peak is an adsorptive peak is confirmed by the linearity of the plot of i_p vs v and that the second one is a diffusion controlled by that of i_p vs $v^{1/2}$. An E_p vs *p*H plot gave the number of protons involved in the electrochemical reduction as **1** and constant potential coulometry gave the number of electrons as 2. The reduction product was found to be a 4-hydroxy quinolone derivative as proven from the IR spectral correlation of the product with an authentic sample.

The electro-reduction of **3** can then be assigned as shown in **Chart 1**.

7-chloro-1-cyclopropyl-6-flouro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid, 4. This compound **4** is obtained by the hydrolysis of an ester **3**. Since, it is a 1, 3-diketo acid, its pK_a is expected to be closer to that of acetoacetic acid. *p*H-metric titration gave the pK_a value as ~ 6.00. The electronic spectra recorded at different *p*H's are shown in **Figure 5**. An isosbestic point is observed at a wavelength of 324 nm with decreasing and increasing absorbances at 320 nm and 330 nm respectively. The plots of absorbances at these wavelengths vs *p*H also gave the pK_a value as ~ 6.00. This value is slightly greater than those of acetic acid and acetoacetic acid implying that **4** is a weaker acid than acetic acid and acetoacetic acid. This is likely to be from two contributions; (i) the acid form is greatly stabilised and (ii) the deprotonated form (the conjugate base of **4**) is destabilised when compared to those of acetic acid and acetoacetic acid. The acid-base equilibria pertaining to **4** are presented in **Scheme V**, wherein **4** and its deprotonated and protonated forms are shown in several plausible canonical structures. It is suggestive from this figure that the acid form of **4** is more stabilised than acetic acid by intramolecular hydrogen bonding as in **4a-c** and that **4** is more stable than acetoacetic acid because the 4-alkoxide forms of **4b** and **4c** offer aromaticity to the quinolone ring. To the extent of **4c**, which is an alcoholic convert of acid form **4b**, compound **4** can not serve as a proton donor. Further, the alkoxide form destabilises the deprotonated carboxylic group by coulombic repulsion as in **4b′** and **4c′**. Hence, **4** is a weaker acid than both acetic acid and acetoacetic acid. On the basis of same above reasons, the heterocyclic nitrogen is a poor base because of the non-availability of its lone pair of electrons in forms, **4b**, **4b′**, **4c** and **4c′**. At higher *p*H's, however, the carboxylic group is deprotonated and renders the alkoxide untenable due to coulombic repulsions in **4b′** and **4c′** forcing the deprotonated **4** into the ketonic form **4a′**. This means that **4** would have an electronic structure in its pyridine ring varied with *p*H. From Figure 5 it is evident that the spectral profiles vary with *p*H that feature an isosbestic point. On these reasons one expects a *p*H dependent cyclic voltammetric profiles also.



Scheme V

The D_o, K_h^0, E_p and i_p values of **4** are collected in **Table 1**. In **Figure 6** are shown the cyclic voltammetric profiles of **4** at three distinct *p*H's. At a *p*H far less than the pK_a, the compound has only an azomethine site as the electro-reducible centre (forms **4b''** and **4c''**). Hence, one would get a CV pattern similar to that of **3**. Comparison of CV profile in **Figure 6** with that in **Figure 4** reveals this. When the *p*H reaches close to pK_a, both azomethine (**4b** and **4c**) and carbonyl (**4a**) forms co-exist. Hence, one would expect two CV responses. Coulometry has revealed that each peak corresponds to a two-electron reduction. Constant potential electrolysis at a potential centred between the two peaks gave an α-enol, whereas that after the second peak gave a mixture of both α and β-enols.

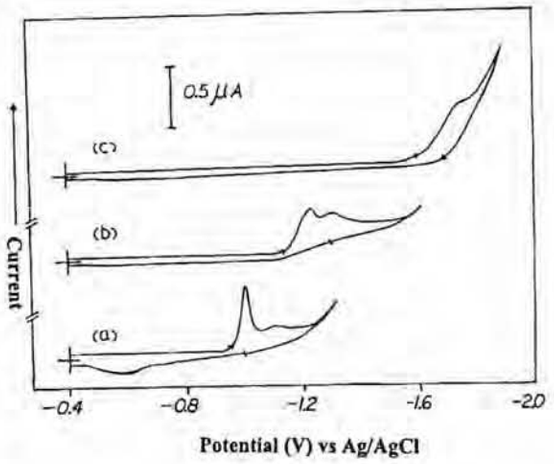


Figure 6—Cyclic voltammograms of **4** (1.93×10^{-4} M) at a *p*H of (a) 2.00, (b) 6.00 and (c) 10.00 at 25° C (scan rate, 50 mV/sec)

1-Cyclopropyl-6-fluoro-1, 4-dihydro-7-(N-piperazinyl)-4-oxo-quinoline-3-carboxylic acid, 5. Compound **5** is generated by adding a piperazinyl group on the benzene ring of **4**. One would expect the pK_{a1} of **5** to be close to the pK_a of **4**, in addition to another, pK_{a2}, due to the piperazinyl nitrogen. The *p*H metric titration gave two values, viz., ~ 6.00 and ~ 8.7. The values of pK_{a1} is to be compared to the pK_a's of acetic acid and acetoacetic acid on the same lines

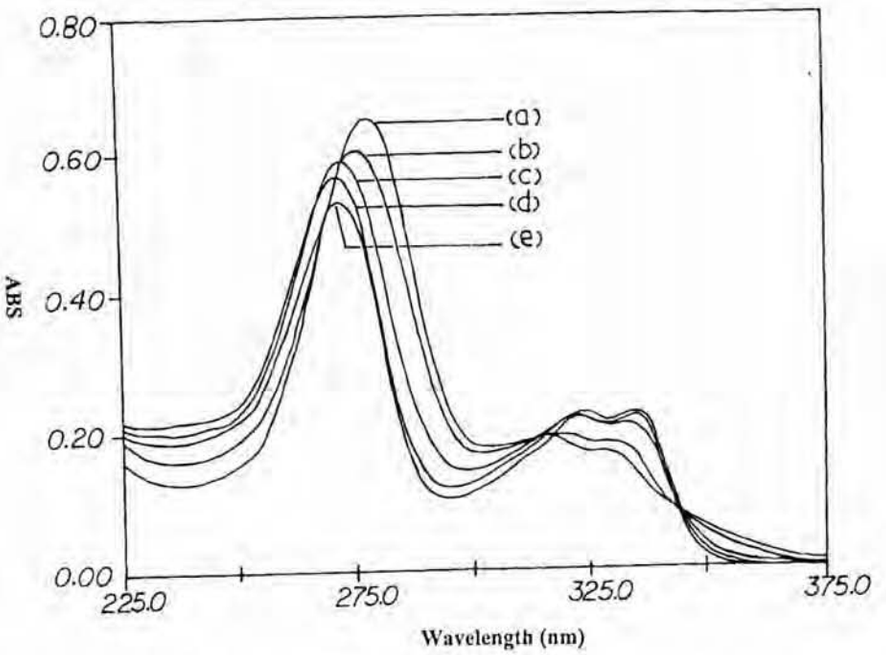


Figure 7—Electronic spectra of **5** (1.69×10^{-5} M) at a *p*H of (a) 2.65, (b) 5.44, (c) 6.63 (d) 7.53 and (e) 10.47 at 25°C

discussed for **4** through **Scheme V**. The electronic spectral profiles of **5** are shown in **Figure 7** on the lines shown in **Figure 5** for **4**.

If form **5**(+) and **5**(±) alone are engaged in acid-base equilibrium as shown in **Scheme II**, a set of spectra with perfect isosbestic point would have been observed. However, the spectra exhibit a slight aberration from isosbesticity, indicating the presence of more than two canonical forms, the relative population of each of which, is *p*H- dependent (**Scheme V**). Spectra with slight deviation from perfect isosbesticity was also reported recently by Hernandez-Borell *et al.*[7] but they overlooked the slight deviation and claimed usual isosbesticity along **Scheme II**.

Compound 5 can exist in three resonance structures, as shown in **Scheme V**, of which two forms **5b** and **5c** are zwitter ions and hence **5** is also a poor base with respect to the quinolone's nitrogen. However, these zwitter ions at low *p*H would result in **5b″** and **5c″** respectively (**Scheme V**) which, in turn, are resonance isomers in them. In acidic media, the resonance forms of **5b″** and **5c″** undergo electron flow reduction at lower potentials whereas forms of **5b** and **5c** at higher cathodic potentials due to the presence of negative charge. Species **5a** is expected to undergo electrochemical reduction at the carbonyl site in the usual mechanism. At very low *p*H's only, reduction corresponding to **5b″** and **5c″** is expected. At elevated *p*H's, these species are absent and only the electrochemistry of **5a** through **5c** is observed. Carbonyl reduction of **5a** is masked by the large hydrogen evolution peak at lower *p*H's (< 3.00) and hence no usual peak for the carbonyl reduction is observed. But at $4.00 < pH < 7.00$, the usual carbonyl reduction occurs. The *p*H dependent cyclic voltammograms in the range of *p*H are shown in **Figure 8**. Typical Voltammetric data of **5** are collected in **Table II**.

Table II—Typical cyclic voltammetric data of **5***

*p*H	$-E_p$	i_p	$D_0 \times 10^6$	$K_h^0 \times 10^4$
	(V vs Ag/AgCl)	(μA)	($cm^2 s^{-1}$)	($cm\ s^{-1}$)
1.13	0.36	0.02	1.11	4.01
	0.94	0.02	1.10	3.23
2.65	0.38	0.02	1.12	5.12
	0.96	0.02	1.11	3.15
3.66	0.39	0.02	1.34	3.98
	1.02	0.03	1.38	4.28
4.62	1.26	0.53	1.81	52.45
5.44	1.27	0.40	2.03	63.84
6.51	1.29	0.41	3.37	66.47

—Contd

Table II—Typical cyclic voltammetric data of **5***— *Contd*

*p*H	$-E_p$	i_p	$D_0\times10^6$	$K^0_h\times10^4$
7.50	1.37	0.38	3.31	84.96
8.50	1.53	1.00	3.41	74.12
9.50	1.53	0.83	3.27	71.66
10.47	1.57	0.25	4.28	70.33
	1.67	0.43	1.43	51.60

* scan rate at 50 mV/sec

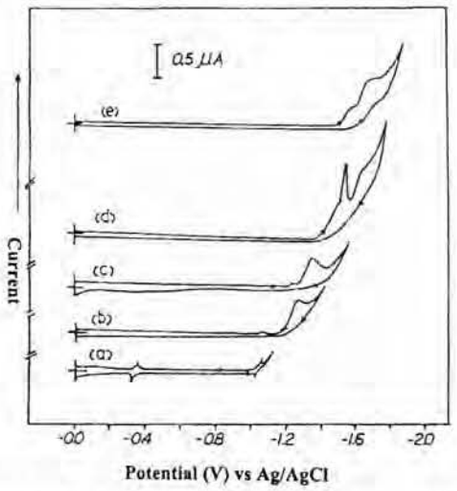


Figure 8—Cyclic voltammograms of **5** (1.67×10^{-4} M) at a *p*H of (a) 1.13, (b) 4.65, (c) 6.56, (d) 8.54 and (e) 10.47 at 25° C (scan rate, 50 mV/sec)

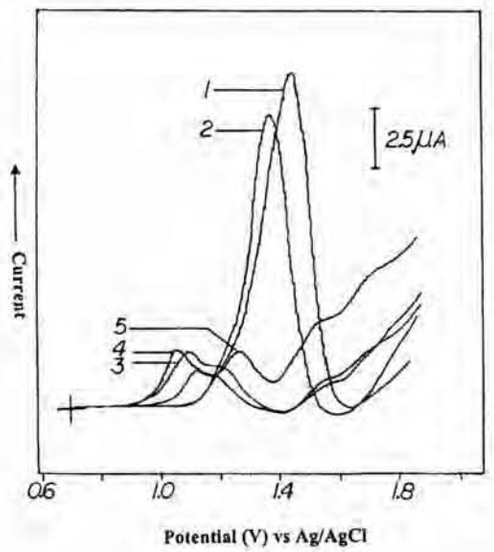


Figure 9—Differential pulse polarograms **1-5** of **1-5** respectively at *p*H 6.00 (pulse height at 25 mV, scan rate at 5 mV/sec and drop time at 1 sec

The trends appear similar to those observed for **4** up to a *p*H < pK_{a2} and the same electrochemistry as that of **4** can be assigned to **5** on the basis of **Scheme V**. However, at *p*H > pK_{a2}, the protonated piperazine ring influences the electrodics of **5**.

Assaying

Based on the electrochemical response of compounds **1-5**, an excellent electroanalytical assaying method of these compounds in differential pulse polarography is developed. The assaying data along with the optimum conditions and statistical information are shown in **Table III**. The DPP curves of **1-5** at *p*H 6.0 are shown in **Figure 9**.

Table-III—Assaying data of **1-5** by differential pulse polarography@ in aqueous buffers

Substrate	*p*H *	Amount labelled(mg)	Amount recovered as analysed # (mg)	Recovery (%)
1	6.00	100	99.32	99.32
2	2.00	100	99.64	99.64
3	6.00	100	98.35	98.35
4	2.00	100	99.46	99.46
5	8.50	100	99.78	99.78

@ pulse height at 25 mV, scan rate at 5 mV/sec and drop time at 1 sec

* *p*H values selected where the compounds exhibit maximum analytical sensitivity

mean value of 5 measurements

Acknowledgements

The authors thank the authorities of Indian Institute of Chemical Technology, Hyderabad for providing ccess to their PCMODEL Serena Software.

References

1 Wise R, Andrew J & Edward E, *Antimicrob Agents Chemother*, 23, **1983**, 559.
2 Koga H, Itoh A, Murayama S, Suzue S & Irikura T, *J Med Chem*, 23, **1980**, 1358.
3 Cornett J B & Wentland M P, *Annu Rep Med Chem*, 21, **1986**, 139.
4 Ferneondes P B & Chu D T W, *Annu Rep Med Chem*, 23, **1988**, 133.
5 Chu D T W, Nordeen C W, Dwight J H, Robert N S, William J G, Andre G P & Jacob J P, *J Med Chem*, 34, **1991**, 168 and references there in.
6 Dietrich A Volmer, Bashir Mansoori & Steven J Locke, *Anal Chem*, 69, **1997**, 4143.
7 Hernandez-Borrell Jordi & Montoro M Teresa, *J Chem Edu*, 74, **1997**, 1311.
8 Raoof S, Wollschalager C & Klan F, *J Antimicrob Chemother*, 18, **1986**, 139; Warowna, Grzeskiewiz, Malgorzata, Chod Kouski, Jerry, Rjalek & Zbegniew (Dept of Inorg / Anal Chem Sch Med, 02097, Warsan, Pol, *Acta Pol*

Pharm, 52, **1995**, 187 (Eng) *Chem Abstra*, Vol. 123, 266325m.

9 *Preparation of most common buffers used in Polarographic Determination*-an educational document supplied by EG&G Princeton Applied Research Corporation, New York as part of their Application briefs and Application notes.

10 O'Dea Philip, Costa Carcia Agustin, Miranda ordieres, Artumo J, Tunon Blanco Paulino & Smyth Malcom R, *Electro Analysis* (N Y), 2, **1990**, 637 (Eng); *Chem Abstra*, Vol. 114, 129234f.

11 Siva Kumar A, Jayarama Reddy S & Krishnan V R, *J electrochem Soc India*, 32 **1983**, 129; Siva Kumar A & Jayarama Reddy S, *Indian J Chem*, 23A, **1984**, 732; Pasternak, *Helv Chim Acta*, 31, **1948**, 753.

12 Zuman P, *J electroanal Chem*, 75, **1977**, 523