Polarography of Flavanone and Isoflavone

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Electro-reduction behaviour of flavanone and 5,7- dihydroxy isoflavone are reported over the entire pH range using DC, AC & Kalousek polarographic methods, CV, millicoulometry and controlled potential coulometry. Heterogeneous rate constant for the reduction is computed and reduction schemes are proposed.

Geissman *et.al.* studied polarography of flavones, flavanones and flavonols under limited set of conditions [1,2]. Later studies [3-6] are also not exhaustive. Niether the study has covered a wide pH range nor extensive electrometric study has been carried out. Isoflavones have not received any attention in these studies. Therefore the present investigation was undertaken to study the polarographic behaviour of flavanone and 5,7- dihydroxy isoflavone over the entire pH range using DC, AC and Kalousek polarographic methods, CV, millicoulometry, controlled potential coulometry and using spectroscopy for identifying the products after controlled potential electrolysis.

EXPERIMENTAL

Aqueous acetate-phosphate - borate universal buffer (each 0.026 $mol.dm^{-3}$) was prepared. Test solution was prepared by mixing 12.5 cm^3 of the buffer, 10 cm^3 of ethanol, calculated amount of tetramethyl ammonium bromide to give 0.1 $mol.dm^{-3}$ ethanolic solution of depolariser (flavanone or 5,7-dihydroxy isoflavone as the case may be) and made up to 25 cm^3. The final polarographic solution would be 0.4 $mmol.dm^{-3}$ in depolariser, 0.04 $mol.dm^{-3}$ in buffer, 0.1 $mol.dm^{-3}$ in ionic strength and 50% v/v in ethanol.

The studies were carried out using Bruker universal Modular Polarograph (Model E310) along with Hewlett-Packard (model 7050A) X-Y recorder. The pH values of solution was checked with digital pH meter with accuracy ±0.01 pH (model LI 120 of M/s Elico Pvt. Ltd., Hyderabad, India).

Capillary characteristics m=1.293 $mg.sec^{-1}$; t = 4.1 sec at h=70 cms and droptime controlled at 2.0 sec.

Constant potential studies were carried out using a locally made 5V, 3A potentiostat-galvanostat to maintain constant potential.

Coulometry was carried out using a digital coulometer (model 640, the Electrosynthesis co., East Amherst, N.Y). Extent of reduction was measured and followed by recording polarograms and UV-VIS spectra of the solution before and after electrolysis (using Cecil CE 599 scanning double beam UV-VIS spectrometer).

RESULTS AND DISCUSSION

Flavanone

The DC polarograms of flavanone are given in Fig.1. At pH 3.20 only one wave is seen, but at pH 4.3, two waves of equal height are found. However, only one wave is seen in the pH range 5.2 to 8.1. At further higher pH values, two more waves appear but at much more positive potentials.

Fig. 2 shows the limiting currents of different steps as a function of pH. From pH 9.70, the current of the main wave decreases but a new wave is seen at positive potentials and this increases with pH. The total limiting current is seen to remain almost constant throughout the pH range. This leads to the conclusion that flavanone is in equilibrium with a second species (2-hydroxy chalcone). The concentration of chalcone increases with pH. Both forms are electro-reducible.

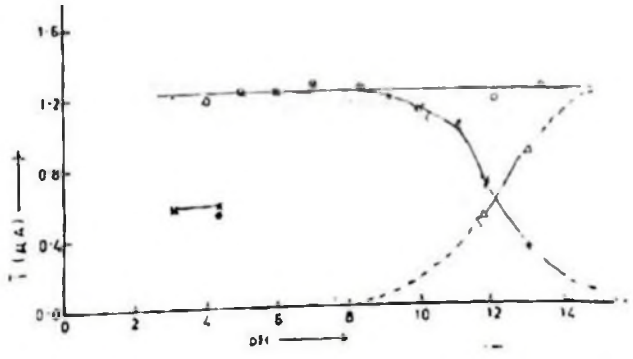


Fig 2. Limiting Currents of Flavanone at Different pH Values
Flavanone Conc 0.4 mol.m^{-3}
D for $i_1 + i_2$; x for i_1; o for i_2; O for i_{total}

The diffusion current - concentration relation has been studied at pH 7.2. Diffusion current (i_d) is found to be proportional to

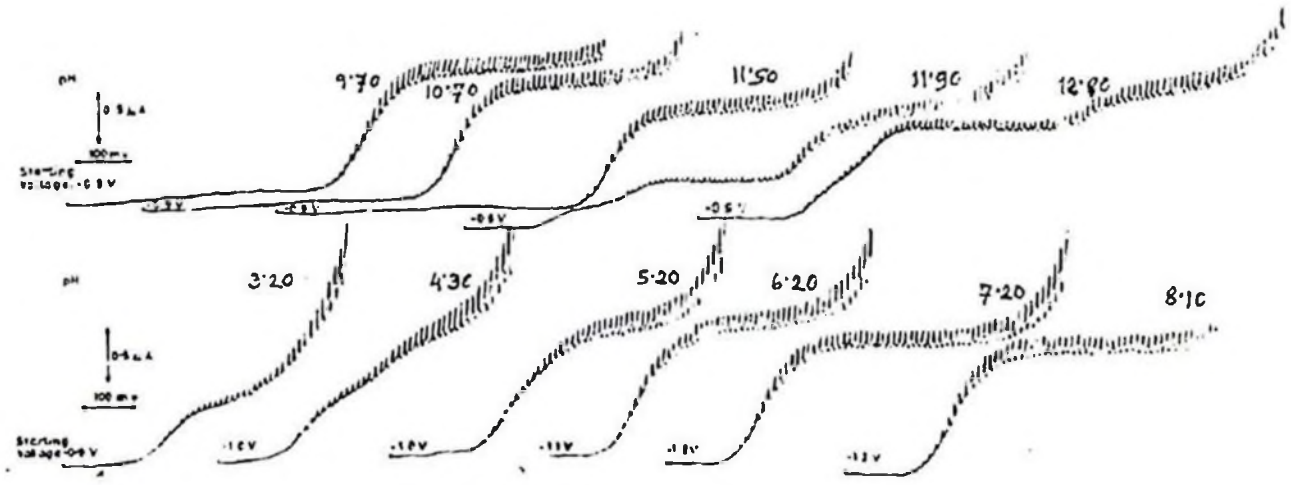


Fig 1. Polarograms of Flavanone at Different Buffers
Britton-Robinson's Buffers - pH & starting Voltage mentioned on the Fig. Flavanone Conc 0.4 mol.m^{-3}

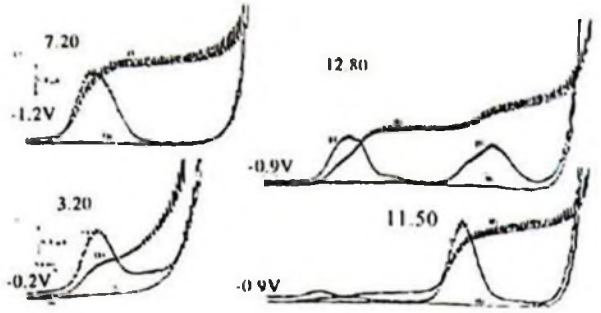


Fig 3. Typical AC & DC Polarograms
Flavanone Conc 0.4 mol.m^{-3}
1 AC; 2 DC; 3 AC of the blank

concentration in the range 0.200 to 1.091 mmol.dm^{-3} and proportional to $h^{2/3}$ (at controlled droptime of 2 sec). This indicates that the main wave is diffusion controlled in nature.

AC polarogram (Fig. 3) very nearly corresponds to DC polarograms. One AC peak is seen upto pH 10.70. However at pH 4.30, a shoulder is seen to the negative side of the peak. At pH 11.50, one more small peak with a shoulder is seen on the positive side. At pH 11.90 and 12.80, the height of the small peak at the positive side is found to increase, while that of the main peak decreases gradually. The base line in the polarogram is above that of the supporting electrolyte, which reveals that adsorption phenonena are absent in the electrode process.

Kalousek polarogram at pH 9.60 [7] did not exhibit any anodic current suggesting irreversible nature of these cathodic steps. Cyclic voltammogram (at HMDE) at different pH values (Fig 4) reveal no anodic peak even at far positive potentials confirming

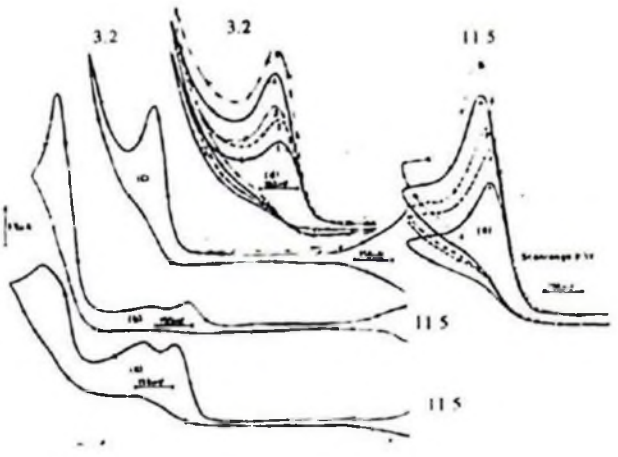


Fig 4. Cyclic Voltammograms
Flavanone Conc 0.4 mol.m^{-3}
ab & c at 0.150V.S^{-1} d & e different scanrates.

irreversible nature of the cathodic wave. i_p *vs* (scanrate)$^{1/2}$ and i_p *vs* C were found to be straight lines passing through the origin indicating uncomplicated diffusion controlled nature of the reduction step [8]. The value of αn_a was computed from the relationship $E_p - E_{p/2} = 0.048/\alpha n_a$ and found to be nearly unity.

5,7- Dihydroxy Isoflavone

DC Polarogram of 5,7-dihydroxy isoflavone (Fig. 5) shows a single step in the pH range 5.20 - 11.50. In solutions of pH < 4.30, the wave nearly merges with the hydrogen discharge curves of the supporting electrolyte. At pH > 11.50, the waves merge with the final current discharge of the supporting electrolyte.

The limiting current remains nearly the same in all pH media. The current - concentration behaviour is studied in medium of pH 7.20 and found to be linear (0.2 to 0.818 mmol.dm^{-3}) indicating diffusion controlled nature of the reduction.

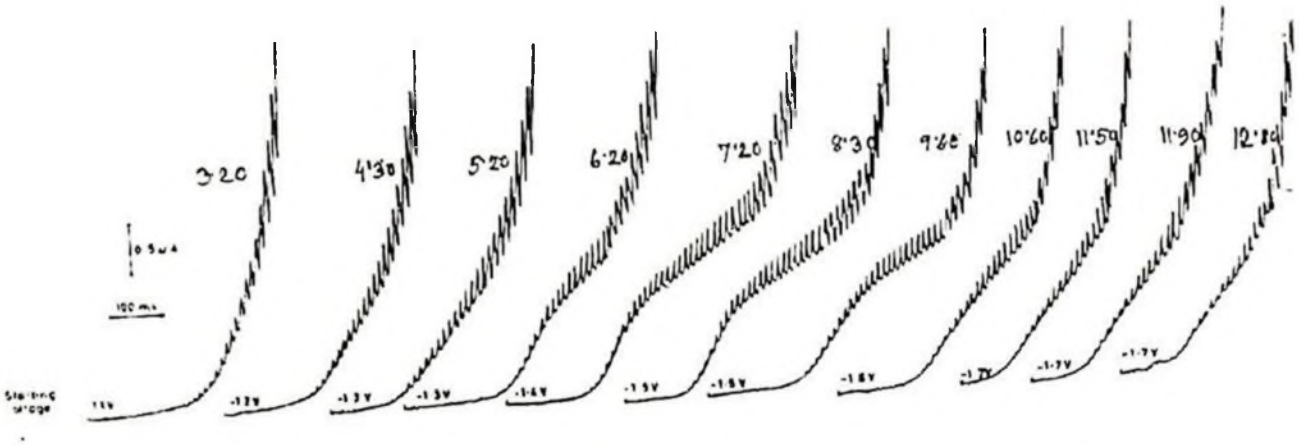


Fig 5. Polarograms of 5,7-isoflavone at Different Buffers
Britton-Robinson's Buffers - pH & starting Voltage mentioned on the Fig 5.7- isoflavone Conc 0.4 mol.m^{-1}

AC Polarogram shows no sharp peak at both low and high pH values (Fig 6). Peaks are obtained for a single step in the pH range 6.7 to 9.6. In the pH range 10.60 to 12.80, the falling part of the AC peak merges with rising part of the supporting electrolyte. In the whole range of pH, the AC polarogram does not go below the base current line indicating absence of any adsorption of the species. The i_p was found to increase linearly with concentration over pH range studied.

Cyclic voltammogram (at HMDE) are recorded at different pH values and at different scan rates (typical curve in Fig. 7). As seen in DC polarograms, CV also shows only one step in the pH range studied. Further no anodic peak is seen indicating irreversibility of the reduction. The i_p *vs* (scan rate)$^{1/2}$ and i_p *vs* C plots are linear - passing through the origin indicating diffusion controlled nature of the step.

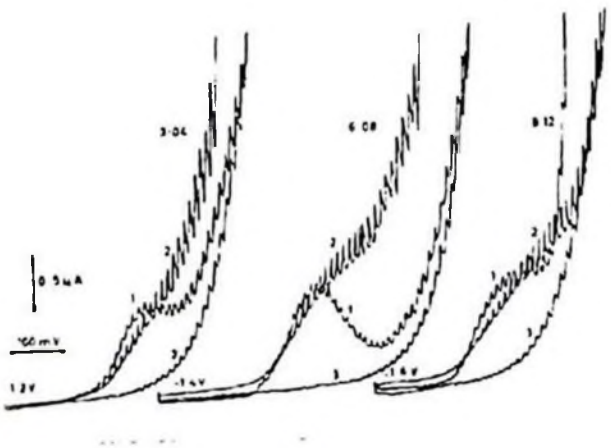


Fig 6. Typical AC & DC Polarograms
5,7-isoflavone Conc 0.4 mol.m^{-1}
1 AC; 2 DC; 3 AC of the blank

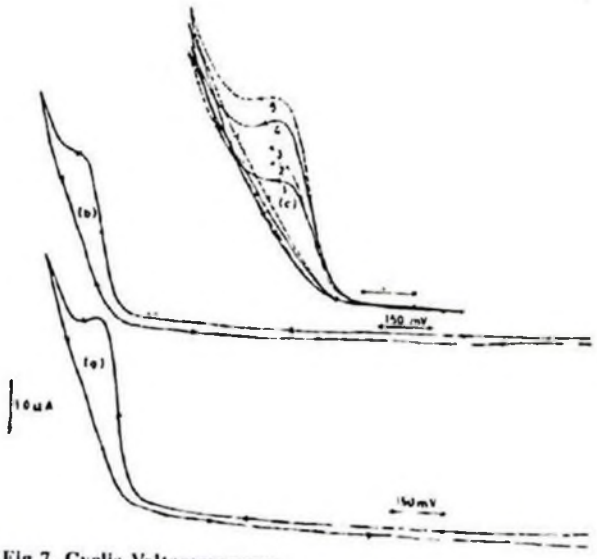


Fig 7. Cyclic Voltammograms
a. pH 7.2 starting voltage - 0.3 V scanrate 0.15 VS^{-1}
b. pH 8.2 starting voltage - 0.3 V V scanrate 0.15 VS^{-1}
c. pH 7.2 starting voltage - 1.3 V at different scanrates
5,7-isoflavone Conc 0.4 mol.m^{-1}

$E_{1/2}$ *vs* pH plots

$E_{1/2}$ *vs* pH plots for the two compounds are shown in Fig 8(a & b). In case of both compounds, the plot consists of 2 linear portions with different slopes. The lines have slopes of 0.075 & 0.037 V/pH meeting at pH 7.10 in case of flavanone and -0.051 & -0.072 V/pH unit meeting at pH 7.4 in case of dihydroxy isoflavone. In addition, flavanone gives two more steps (c & d) at higher alkaline pH. The slopes are -0.059 and 0.0192 V/pH.

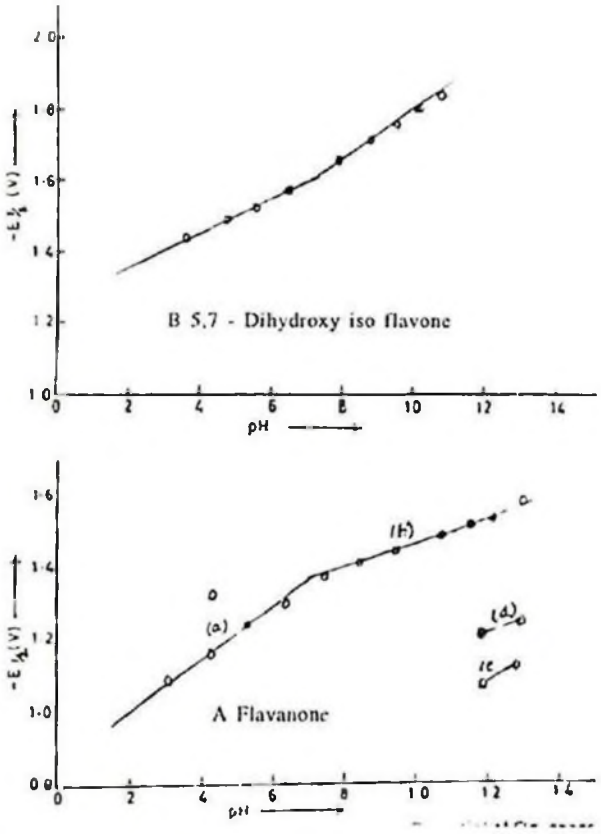


Fig 8. pH Dependance of $E_{1/2}$
A. Flavanone Conc 0.4 mol.m^{-3}
B. 5,7-isoflavone Conc 0.4 mol.m^{-3}

Kinetic Parameters

Heterogeneous rate constants ($k^{o}_{f,h}$) for the two reactions has been calculated [9] at different pH values (Table 1). The results show that the reduction is irreversible in all cases.

Table 1

Heterogeneous Rate Constant $k^{o}_{f,h}$ in cm^{-1}

pH	Flavanone (I Wave)	Dihydroxy isoflavone
3.20	6.31×10^{-23}	-
6.2	-	2.512×10^{-20}
9.6	-	6.310×10^{-24}
10.7	1.0×10^{-23}	-
12.80	1.0×10^{-12}	

n Value

The total number of electrons involved in the reduction is found by comparing the diffusion current constant [I] at pH 6.2 with that of xanthone at the same pH. I in $\mu A.mol^{-1}.m^{3}.mg^{-2/3}.s^{1/2}$ was 2.312 for flavanone, 2.309 for 5,7-dihydroxy isoflavone and 2.443 for xanthone. Xanthone is known to be reduced in a 2 electron step. So, it is concluded that n is 2 for the two steps together in flavanone and for the single step of 5,7-dihydroxy isoflavone.

The n value was confirmed by carrying out electrolysis. Twenty cm^{3} of 1 mmol.dm^{-3}

solution of flavanone in 1 M KCl at pH 7.30 was electrolysed at a constant potential till reduction was complete (2hrs). UV-VIS spectra of the solution taken before and after reduction (Fig 9) shows a band at 280 nm but no band in visible region corresponding to flavylium chloride. The UV-VIS spectra of flavanone over wide range of pH values (Fig 10) shows a band II in the region 256-268 nm and band I of lower intensity in 332-335 nm. Intensity of this band increased in the pH range 11.5 to 13.0 and shifts from 335 to 325 nm. One more band in the 425 nm region in these alkaline media leads one to the conclusion that flavanone is converted into isomeric 2-hydroxy chalcone by ring opening [10].

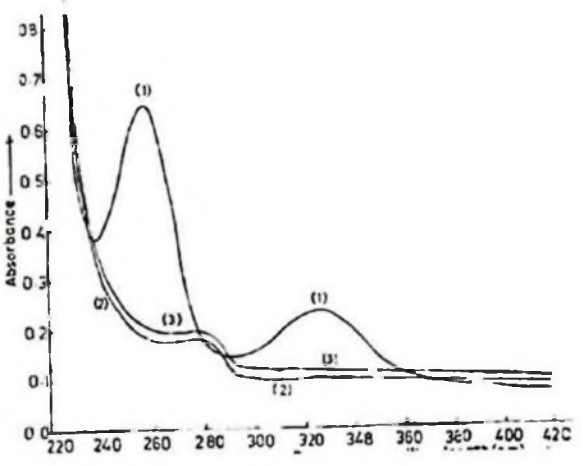


Fig 9. Electronic Spectra of Flavanone and its Reduction Product

1. Flavanone 0.02 mol.m^{-3} at pH 7.30
2. Reduction Product at pH 7.30
3. Reduction Product in 1.0 M HCl

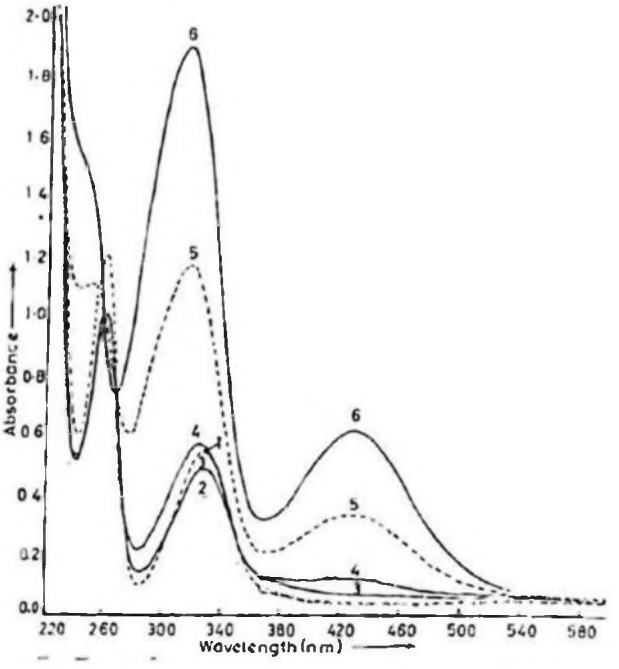


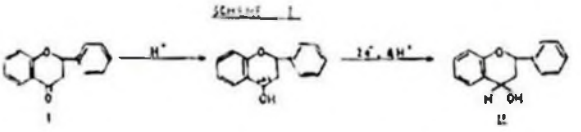
Fig 10. Electronic Spectra of Flavanone at Different pH

1 pH = 3.60; 2 pH = 6.2; 3 pH = 8.2; 4 pH = 11.50; 5 pH = 11.90 and 6 pH = 13.0

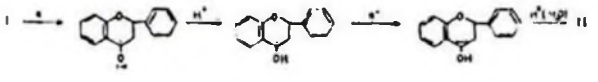
Mechanism of Reduction of Flavanone

Mechanism for the electrode process can be proposed from the following considerations:

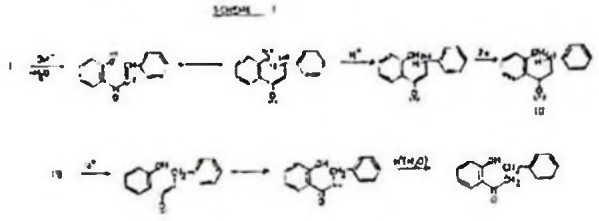
(1) In acidic solution (upto pH 7.20), the slope of $E_{1/2}$ *vs* pH plot for the wave is 0.075 V/pH, which indicates that the reduction step is preceeded by a protonation step. The reduction follows CEEC sequence (Scheme 1)



(2) Above pH 7.10, the slope of $E_{1/2}$ *vs* pH is -0.037 V/pH. The reduction follows scheme2.

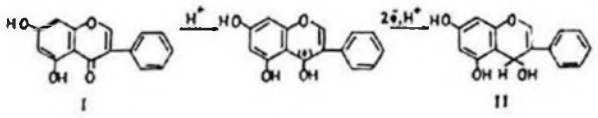


(3) In alkaline solution, flavanone gives rise to isomeric 2 - hydroxy chalkone. The $E_{1/2}$ *vs* pH plot of the two new waves at pH 11.90 and 12.80 indicates that first of these steps (reduction of 2-hydroxy chalcone) is proton dependent; one proton is involved per electron transfer and the second reduction step of the chalkone is nearly independent of pH. One could therefore, conclude that prior protonation occurs at the phenolated anion. Thus the reactivity of the resulting species increase which then takes up two successive electrons.

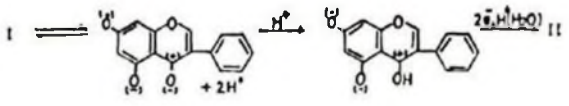


Mechanism of Reduction of 5,7- dihydroxy isoflavone

(1) In acidic solution, pH dependance of $E_{1/2}$ is similar to that of flavanone. The CEEC mechanism is proposed in this region.



(2) In the higher pH range, the shift of pH *vs* $E_{1/2}$ plot indicates involvment of 1 proton only. The reduction scheme is presented below.



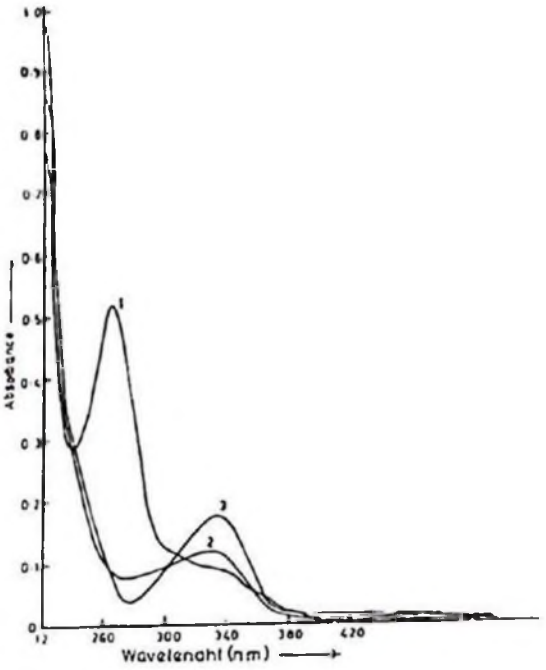
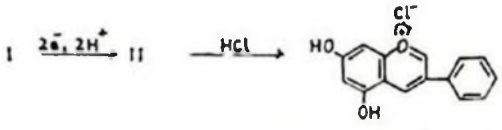


Fig 11. Electronic Spectra of 5,7-Isoflavone and its Reduction Product
1. 5,7-isoflavone 0.016 mol.m^{-1} at pH 7.30
2. Reduction Product at pH 7.30
3. Reduction Product in 1.0 M HCl

(3) The UV band at 330 nm of the two electron reduction product of 5,7- dihydroxy isoflavone (Fig. 11 - spectra No. 3) is found to increase in 1.0 M HCl due to the formation of isoflavylium chloride, the side phenyl group of which does not participate in isomeric transformations.



5,7-Dihydroxy Isoflavylium Chloride

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