



## ELECTROCHEMICAL BEHAVIOR OF ANTICANCER CHALCONE DERIVATIVES ON GLASSY CARBON ELECTRODE

Dr. Shivaraj Yellappa\* and Mallappa M.

Department of Chemistry, Government Science College, Bangalore-560 001, Karnataka, India.

\*Correspondence for Author: Dr. Shivaraj Yellappa

Department of Chemistry, Government Science College, Bangalore-560 001, Karnataka, India.

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### ABSTRACT

The electrochemical behavior of anticancer chalcone and its derivatives was studied at a glassy carbon electrode using cyclic and differential-pulse voltammetric techniques. At the bare glassy carbon electrode (GCE), the anticancer chalcone and its derivatives were shown to be single well defined irreversible cathodic peak at -1.48 V in methanol containing 0.1 M TBAP with scan rate 50 mV/s and the electrode reaction process is found to be diffusion controlled. The various parameters such as effects of anodic peak potential ( $E_p$ ), anodic peak current ( $I_{pa}$ ), scan rate, effect of substituent, heterogeneous rate constant ( $k^0$ ), etc have been discussed. The shifts in peak potential were observed with the various in substituents.

**KEYWORDS:** Chalcone derivatives, voltammetry, glassy carbon electrode,

### INTRODUCTION

Chalcones are the immediate precursors in the biosynthesis of flavonoids, and their structure differs considerably from the others members of the flavonoid family, since chalcones are open-chain analogs in contrast to the other family's members. Chalcones are abundantly present in nature starting from ferns to higher plants.<sup>[1]</sup> Chalcones have been identified as interesting compounds that are associated with several biological activities. Chemically they are 1,3-diphenyl-2-propen-1-ones and are often cytotoxic in vitro<sup>[2]</sup> and some of their derivatives are reported to be antimutagenic<sup>[3]</sup>, tumor reducing agent.<sup>[4]</sup> In an effort to develop a potent anti-inflammatory and cancer chemopreventive agents a series of chalcones were synthesized.<sup>[5]</sup> Chalcones possess conjugated double bonds and a completely delocalized  $\pi$ -electron system on both benzene rings. Molecules possessing such system have relatively low redox potentials and have a greater probability of undergoing electron transfer reactions.<sup>[6]</sup>

It is proposed that the reduction of chalcones involved that the first electron attack takes place on the carbonyl group and the free radical formed undergoes isomerization into another free radical which is further reduced or can be converted into a dimer. The interpretation of the reduction process of o-hydroxy chalcone and its heterocyclic analogues by Meunier *et al.*,<sup>[8]</sup> proved that saturated ketone results from the acceptance of the first two electrons. Zuman *et al.*<sup>[9]</sup> informed the polarographic reduction of chalcone in aqueous medium finding a radical anion which is protonated with an approximate pK value of 10.2.

Furthermore cyclic voltammetric studies<sup>[10]</sup> carried out in DMSO were consistent with a mechanism involving the reduction of the  $\alpha$ ,  $\beta$ -unsaturated ketone to its radical anion followed by irreversible dimerization. Recently<sup>[11,12]</sup> it was found that electron affinities of a series of monosubstituted chalcones computed at the density functional level, were highly linearly correlated with voltammetric potentials measured in aprotic medium. All the previous electrochemical studies about the reduction of chalcones unanimously revealed that the anion radical generated as the consequence of the one electron acceptance is the obligate first step in its reduction mechanism. In the present study, we have used voltammetric techniques such as cyclic voltammetry and differential pulse voltammetry to study the reduction behavior of different substituted chalcone derivatives.

### EXPERIMENTAL

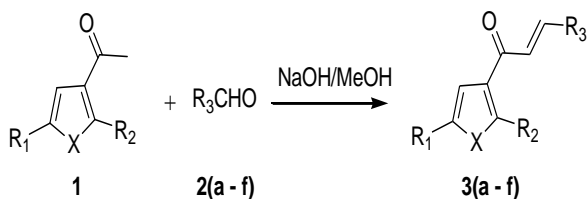
#### Materials and reagents

Chalcones was synthesized as per literature procedure Darshan Raj *et al.*<sup>[13]</sup> Methanol with more than 99.5% purity of spectrochem grade was used as solvent Tetrabutylammonium perchlorate (TBAP, Fluka, 99% purity) was used as a supporting electrolyte. The Stock solutions ( $1.0 \times 10^{-4}$  M) of chalcones were prepared in methanol. All other reagents used were of analytical grade. All solutions were prepared in millipore water.

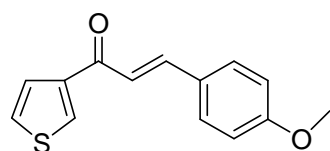
#### General Procedure for the preparation of Chalcones 3(a-f)

To a mixture of ketones (1) (1 mmol) in methanol (15 mL) was added NaOH (0.1 g, 2-3 drops of water) and stirred for 5 min. Then, added substituted aldehydes 2(a-

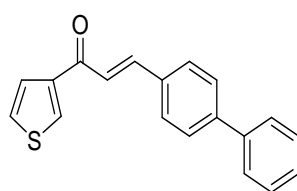
f) and stirred the reaction mixture at room temperature. After completion of reaction (TLC), reaction mixture was poured over crushed ice and acidified with acetic acid. The precipitated solid was filtered, washed with water and oven dried.<sup>[13]</sup>



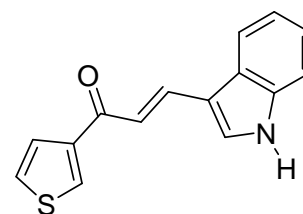
$R_1 = R_2 = \text{H}$ ,  $R_1 = R_2 = \text{Me}$ ,  $X = \text{S}$ ,  $OR_3 = \text{p-OMe (C}_6\text{H}_4\text{)}$ ;  
biphenyl, indolyl, 2-ethyl furyl, pyrrolyl



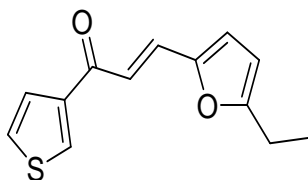
Compound A



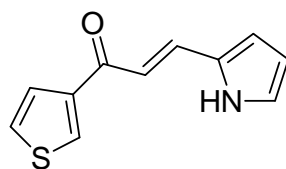
Compound B



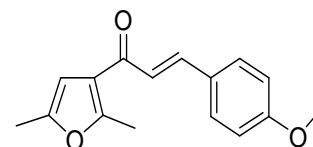
Compound C



Compound D



Compound E



Compound F

Scheme-1. Synthesis of chalcone derivatives.

### Apparatus

Electrochemical measurements were performed on a CH Instruments (USA). Measurements were carried out in a conventional three electrode cell which consisted of saturated calomel electrode (SCE, Fisher Scientific Company cat no.13-639-51) as a reference electrode, a thin Pt wire of thickness 0.5 mm and a glassy carbon electrode (GCE) with a geometric area of 3 mm as the working electrode. All experiments were carried out at an ambient temperature of  $25 \pm 0.1$  °C.

### Procedure

In order to obtain reproducible results, the GCE was cleaned with soft emery paper (600 A), polished with 0.05  $\mu\text{m}$  alumina, then sonicated (ultrasound bath) for 3 min and rinsed with deionized water before making a measurement.

## RESULTS AND DISCUSSION

### Voltammetric behavior of chalcone derivatives

Figure 1 shows the cyclic and differential pulse voltammetric behavior of 0.2 mM chalcone derivative, (2E)-3-(4-methoxyphenyl)-1-(thiophen-3-yl) prop-2-en-1-one (A) at glassy carbon electrode. Cyclic voltammogram of the molecule 'A' exhibited a single well defined cathodic peak at -1.48 V in methanol

The new chalcones (3a-3f) scheme-1 synthesized was found to be useful intermediates in the synthesis of certain medicinally important compounds. (13). New compounds can be synthesized according to the general strategy published in<sup>[10]</sup> using 2, 5 substituted ketones (1) and aldehydes (2a-2f) as the starting compound for the synthesis of novel chalcones (3a-3f) (scheme-1). The present idea relates to study of voltammetric behavior of novel chalcone derivatives and analogs which are useful as angiogenesis inhibitors. The novel chalcones, which are inexpensive to synthesize, exhibit unexpectedly good activity as angiogenesis inhibitors.<sup>[14]</sup>

containing 0.1 M TBAP with scan rate  $50 \text{ mV s}^{-1}$ , whereas No anodic peak was found in the reverse scan. Molecule (A) containing  $R_1$  and  $R_2$  substitution is H,  $R_3$  substitution is PMP and X is S.

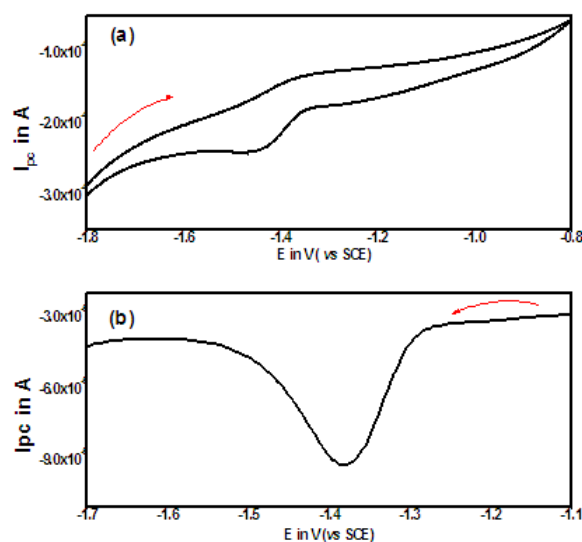


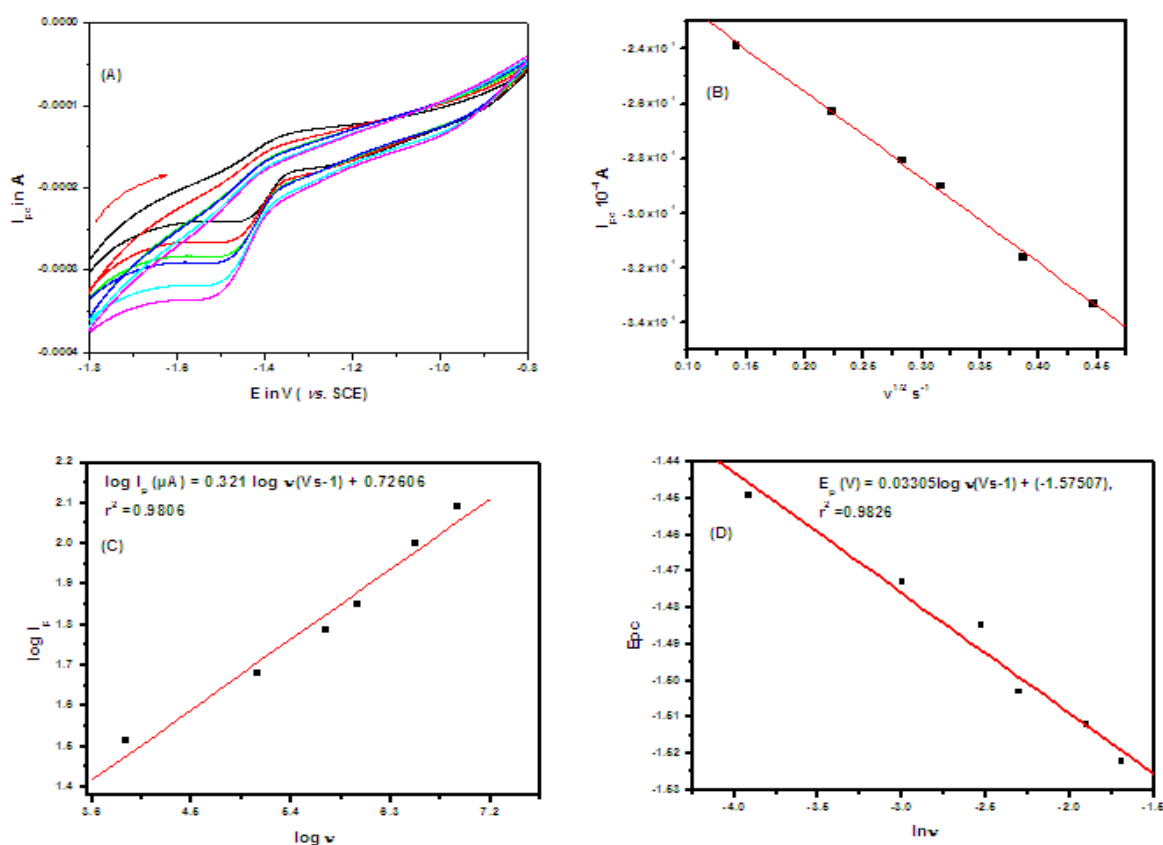
Figure 1. (a) Cyclic and (b) differential pulse voltammograms of 0.2 mM compound (A) on glassy carbon electrode containing 0.1 M TBAP as the supporting electrolyte with scan rate  $50 \text{ mV s}^{-1}$ .

The  $E^\circ$  obtained from DPV for (2*E*)-3-(4-methoxyphenyl)-1-(thiophen-3-yl)prop-2-en-1-one (A) is slight different as that found by CV, however that obtained by DPV for (A) was significantly less negative. To explain this behavior we have to consider the different characteristic times ( $T$ ) of CV and DPV experiments. The time scale for a CV experiment (at  $v = 100 \text{ mV/s}$ ) is  $T = RT/vF = 257 \text{ ms}$  [ $F$  is the faraday constant (96,485 C/eq) and  $R$  is the molar gas constant (8.314 J/mol<sup>-1</sup> K<sup>-1</sup>)] while for a DPV experiment under the conditions here  $T = 17 \text{ ms}$ .<sup>[15]</sup>

### Effect of scan rate

The constructive information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Hence, the voltammetric behaviour of chalcones at different scan rates was also studied using cyclic voltammetry, (Fig. 2A). Scan rate studies were carried out to found whether

the process on glassy carbon electrode was under diffusion or adsorption controlled. The influence of the square root of scan rate on the peak current showed a linear relationship in the range of 0.02-0.2  $\text{mV s}^{-1}$  (Fig. 2B) which is of a typical diffusion controlled process<sup>[16]</sup>, and a plot of logarithm of anodic peak current vs. logarithm of scan rate gave a straight line with a slope of 0.321 (Fig. 2C), which is close to the theoretical value of 0.5 for a diffusion controlled process<sup>[17]</sup> and it as further confirms that the process is diffusion controlled where the electroactive species of chalcone diffuses from the bulk solution to a planar electrode surface. The  $E_p$  of the reduction peak was also dependent on scan rate. The peak potential shifted to slightly positive values on increasing the scan rate, which confirms the irreversibility of the reduction process, and a linear relationship between peak potential and logarithm of scan rate (Fig. 2D).

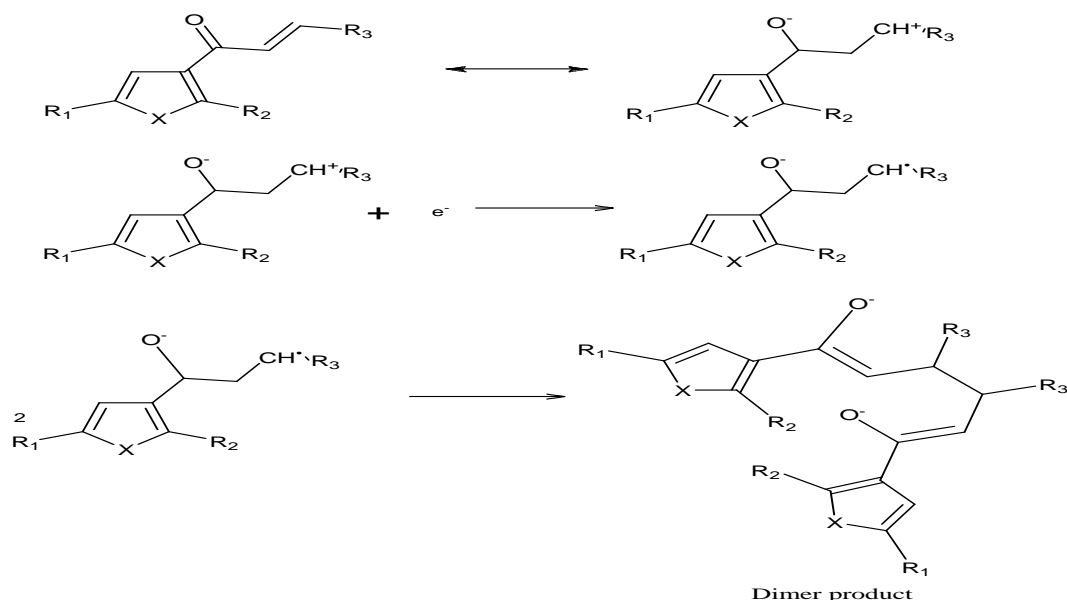


**Figure 2.** (A) Cyclic voltammogram of 0.2 mM chalcone on glassy carbon electrode containing 0.1M TBAP as the supporting electrolyte with scan rates 20, 50, 80, 100, 150, 200  $\text{mV s}^{-1}$  and (B) Plot of cathodic peak current vs. square root of scan rate, (C) Plot of  $\log I_p$  vs.  $\log v$  and (D) plot of  $E_p$  vs.  $\ln v$ .

### Mechanism

All the synthesized substituted chalcones were electrochemically reduced on glassy carbon electrode in methanol and tetrabutylammonium perchlorate (TBAP) as the supporting electrolyte. The reduction behaviour of

the synthesized chalcones was clearly identified from the voltammogram (Fig. 1). The observed reduction peak strongly supports that the peak is due to a one electron reduction to the radical formation and the combination of two radicals to form dimer adduct.<sup>[18, 19]</sup>



**Scheme 2. Probable mechanism for the electrochemical reduction of chalcones.**

The number of electron transfer 'n' is calculated from the difference between peak potential ( $E_p$ ) and half wave potential ( $E_{p/2}$ ) according to equation given below.<sup>[20]</sup>

$$\Delta E_p = E_p - E_{p/2} = (47.7/\alpha n) \text{ mV (irreversible reaction; at 298 K)}$$

Where  $\alpha$  was the electron transfer coefficient and its value taken as 0.5. The value of n is calculated to be 0.89 ~ 1.0. For an irreversible reduction reaction, we may use the following equation to calculate standard rate constant ( $k_0$ ).<sup>[21]</sup>

$$E_p = E^0 + (RT/\alpha n) [\ln(RT k_0 / \alpha n F) - \ln v]$$

Where  $E_0$  is the formal potential, R was the universal gas constant ( $8.314 \text{ J K}^{-1} \text{ mol}^{-1}$ ), T (K) was the Kelvin temperature,  $\alpha$  was the transfer coefficient, n is number electrons transferred,  $k_0$  ( $\text{s}^{-1}$ ) was the electrochemical rate constant and F was the Faraday constant ( $96,487 \text{ C mol}^{-1}$ ). The value of  $E^0$  was obtained from the intercept of the  $E_p$  vs.  $v$  plot by the extrapolation to the vertical axis at  $v = 0$ . The value of  $k_0$  were evaluated from the plot of  $E_p$  vs.  $\ln v$  and found to be  $2.18 \times 10^3 \text{ s}^{-1}$ .

#### Area of the electrode

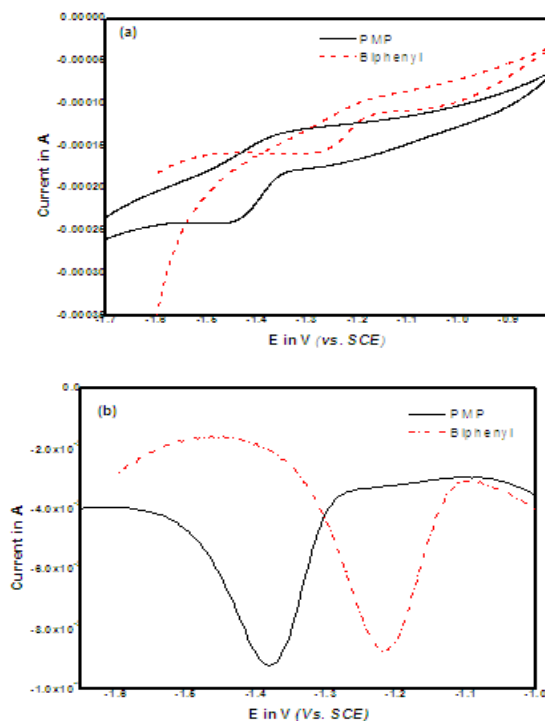
For an irreversible system from Randles–Sevcik equation<sup>[20]</sup>, we have,

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

Where  $I_p$  refers to the peak current, A is the electroactive area ( $\text{cm}^2$ ), C is the concentration of the electroactive species ( $\text{mol cm}^{-3}$ ), n is the number of electrons transferred, D is the diffusion coefficient ( $\text{cm}^2 \text{ s}^{-1}$ ), and v is the scan rate ( $\text{Vs}^{-1}$ ). For 0.2 mM chalcone in 0.1 M TBAP electrolyte, a diffusion coefficient is obtained from the literature (a plot of  $I_p$  vs.  $v^{1/2}$ )<sup>[22,23]</sup> and its value found to  $-3.11487 \times 10^{-4} \text{ cm}^2/\text{s}$ . The surface area of the electrode was calculated by using equation and the value is to be  $0.031 \text{ cm}^2$ .

#### Effects of substituents

Recently the substituent effects on the reduction potentials of a large numbers of chalcone derivatives have been studied, none of the reduction potentials of the current synthesized substituted chalcones can be anticipated from that study as shown in fig.3.



**Figure 3. (a) Cyclic voltammograms and (b) Differential pulse voltammograms of 0.2 mM chalcone (A, solid line) and (B, dashed line) on glassy carbon electrode containing 0.1 M TBAP as the supporting electrolyte.**

By comparing the reduction potentials for the first electron transfer (Table 1) we can describe that the p-

methoxy substituted derivative required more energy to the one-electron transference than the Biphenyl, indole, furyl and pyrrole substituted derivatives. This effect can be ascribed to the electron donor effect of the p-methoxy phenyl (PMP) group on the electron density of the  $\beta$ -carbon of  $\alpha, \beta$  unsaturated ketone moiety. When 4-methoxy group is replaced by phenyl ring the energy required was comparatively less with that of indole, furyl and pyrrole.

In fact, donor groups in ortho and/or para positions of the  $R_3$  will produce an enhanced electron density on the  $\beta$ -

carbon thus hindering the reduction. Only in the case of the PMP derivative the effect of donor groups in ortho and para can be gathered, i.e. in the case of PMP the formation of the anion radical was more difficult than at the other substitution reported in the table. According to the above we have found a direct relation between the  $R_3$ -ring substitution and the radical anion formation. Consequently, it is possible to modulate the radical formation with different substituents on the  $R_3$ -ring. On investigating the substituent effects on the radical anion formation and stability, we have focused our attention only on the first reversible reduction couple.<sup>[24, 25]</sup>

**Table 1: Reduction potentials for substituted chalcones by CV and DPV**

Compound	Ring A substitution $R_1$ & $R_2$ [ $R_1=R_2$ ]	Ring B substitution $R_3$	X	Reduction potential, V	
				CV	DPV
A	H	PMP	S	-1.47	-1.39
B	H	Biphenyl	S	-1.29	-1.22
C	H	Indole	S	-1.46	-1.42
D	H	Furyl	S	-1.31	-1.25
E	H	Pyrrole	S	-1.49	-1.44
F	Me	PMP	O	-1.45	-1.38

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