Global Science Research Journals

ISSN: 2449-0628 Vol. 6 (2), pp. 461-470, March, 2019

http://www.globalscienceresearchjournals.org/

Global Journal of Plant Breeding and Genetics

Full Length Research Paper

Antioxidants / antioxidative agents and superoxide: An electrochemical monitoring device

M. Mohammad[1]*, A. Dar[1], M. Tahir Soomro[2], M. Tariq[1] and M. Latif[1]

[1]Reactive Intermediates – Free Radical Chemistry Group. International Center for Chemical and Biological Sciences, HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan. [2]Institute for Physical and Theoretical Chemistry; Technical University of Graz, Graz-8010, Austria.

Accepted 2 September, 2018

A simple but elegant electrochemical (cyclic voltammetric) device was developed to detect antioxidative property of various substances (pure compounds, extracts of spices etc.). It was employed to investigate the presence of antioxidants / antioxidative agents, in some common herbal and medicinal plant products, for example saffron, clove, herbal tea etc. The concentration of such substances showing antioxidative property was estimated and they were categorized as per the voltammetric responses of some known antioxidants. The effectiveness / relative effectiveness of the antioxidants / antioxidative agents was also investigated.

Keywords: Antioxidants, superoxide, saffron, herbal tea, clove, cyclic voltammetry.

INTRODUCTION

A general definition of an antioxidant may be put as molecules that slow or prevent or inhibit the oxidation of other (substrate) molecules (Graham, 1966; Robert Roskaski, 1996; Pryor et al., 2005; Mohammad et al., 2007). A more precise definition may be: a chemical which reacts with (or scavenges) the reactive reduced molecular oxygen species (superoxide, hydroxyl radical etc) (Graham, 1966; Robert Roskaski, 1996; Pryor et al., 2005; Mohammad et al., 2007). And with the reference to the human body, the product(s) / ultimate product(s) of the reaction between an antioxidant and the reactive reduced molecular oxygen species is (are) not expected to be harmful [Mohammad, 2007]. In the present context / study, antioxidants are taken to be synonym with the antioxidative agents or chemical(s) (or mixture(s)) showing reactions or mopping up property, towards superoxide O_2^-. Thus in the present context those compound, which have higher (more positive) reduction potential than O_2, will, as a rule, exhibit antioxidative property, through electron transfer process. Those compounds which do not have the appropriate reduction reduction potential and still exhibit antioxidative property may be doing so by reacting with or complexing with, superoxide. Thus an antioxidant (antioxidative agent), in the present context, acts through: (a) electron transfer from O_2^- to the antioxidant / antioxidative agent, or (b) protonation of, and /or hydrogen bonding with, or proton atom transfer to, O_2^- and / or (c) making a complex with O_2^- (particularly in the presence of a metal ion to form a ternary complex (Aisha, 2005). The present study thus focuses on the generation of superoxide and its activity vis à vis those agents which have the potentiality of deactivating the superoxide.

It is of general interest to know the antioxidative capacity, amount and constituent of an antioxidant / antioxidant(s) in say, food, we consume or spices and other herbal and related substances. Due to complexity of the composition of food materials, separating each antioxidant compound and studying it individually is costly and time consuming or inefficient. Therefore it is very appealing to search for a reliable and convenient method for the quick monitoring/quantitation of antioxidants and their effectiveness in, say, food ingredients (spices) and/ or herbs.

On the basis of the chemical reaction involved. major antioxidant capacity assay can be roughly divided into two categories: (1) hydrogen atom transfer (HAT) reaction based assays and (2) single electron transfer

*Corresponding author. E-mail: mahboob. Md @ hotmail. com, mahboob_mohammad@iccs.edu. Tel: 92-214824901, 92-214 824930. Fax: 92-21-4819018.

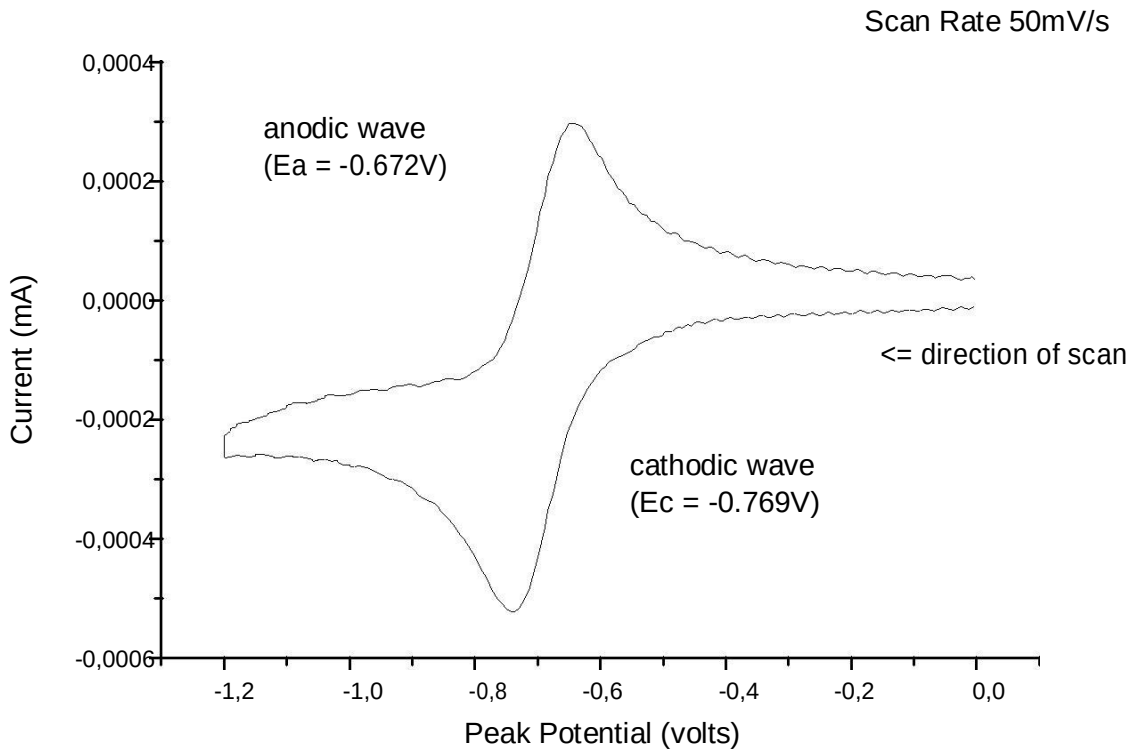

Figure 1. Cyclic voltammogram of O_2/ O_2^- in DMSO (0.1 TBAI); working electrode = Glassy carbon, reference electrode = gold wire QRE, counter electrode = platinum wire.

(ET) reaction based assays (Schaich, 2005) the details of which may be found elsewhere (Schaich, 2005); Jimenez et al., 2004).

Electrochemical methods have been developed in the past for measuring and evaluation of the antioxidant capacity (Kohen et al., 2000; Buratti et al., 2001) . They are based on the reducing property of antioxidants (Kohen et al., 2000) or the use of a flow injection system with an electrochemical detector. The latter method is selective for lipophilic compounds, having antioxidant property (Buratti et al., 2001).

In this work the problem of detecting antioxidative activity of a sample (for example an extract of a herb), the concentration of the antioxidant active ingredient (in the extract, say), the effectiveness of the antioxidant and the type of antioxidative reactivity vis à vis known antioxidants, have been addressed. A simple, elegant and reliable device – cyclic voltammetric device (Graham, 1966; Robert, 1996; Pryor et al., 2005; Mohammad et al., 2007) was used to address the above mentioned problems.

In this paper we report the result of such investigations. The device was tested with some known antioxidants (for example vitamin C, butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), vitamin E) and applied to substance of some common use for example spices, saffron, herbal tea and some other substances. The effectiveness/relative effectiveness of various antioxidants/ antioxidatative agents in scavenging superoxide is also reported.

MATERIALS AND METHODS

Material

DMSO (Riedel) HPLC grade dried over molecular sieve (Linde 3A), tetra n-butyl ammonium iodide (TBAI, Sigma), ethanol (HPLC grade, Fisher), vitamin E (Sigma), P-Benzoquinone (Wako), Ascorbic acid (Wako), butylated hydroxyanisole (BHA, Sigma), butylated hydrooxytoluene (BHT, Sigma), saffron (*Crocus sativus,* purchased from perfume store); clove (*Syzygium aromaticum)*, tisane (herbal green tea) purchased from local market, flower stem of harsinghar (*Nycanthes arbortristis*) (collected from tree in the flowering season, dried under shade and low microware power), methyl gallate (isolated from *Conyza bonariensis*). Care was taken that all extracts were free from moisture.

Instrumentation

A Sycopel AEW2 Analytical Electrochemical was used along with a Ec Prog V3 Software for cyclic voltammetric measurement. A saturated calomel electrode or a gold wire was used as reference or quasi references electrode (QRE) . The latter was found satisfactory Figure 1, as was checked against Methyl Viologen redox system (Mohammad et al., 1981) – used as internal reference. A glassy carbon electrode (BAS, 3 mm in diameter) was used as working electrode. A platinum wire (1 mm in diameter and 15 cm long) was used as counter electrodes. The voltage at the electrode was scanned from 0 mV *vs* Au wire reference electrode.

Usually, a 50 mV/s scan rate was employed.

The device and the procedure

The typical Electrochemical probe (Graham, 1966; Robert Roskaski, 1996; Pryor and Huang, 2005; Mohammad et al., 2007), is a combination electrode which combines the working, reference and counter electrodes into one body. The inner electrode is the working electrode, reference and counter electrodes are the outer electrodes combining both side of the working electrode. The sample is placed in a voltammetric cell which had the electrochemical probe. The potential was applied to the working electrode at a constant scan rate (50 mV/s).

Solution of 0.1M TBAI in dry DMSO was used for recording the CV of the reduction of oxygen to superoxide (Nicholson and Shain, 1964; Mohammad et al., 2001). The following concentration of known samples : 0.1M Vitamin C, 0.1M BHA, 0.1M MG, 0.1M BQ and 0.01M BHT, 0.007M Vitamin E in 0.1M TBAI/DMSO, were and 0.01M BHT, 0.007M Vitamin E in 0.1M TBAI/DMSO, were prepared as stock solutions.

Ethanol 99.99% was used as received from supplier. Extracts of the natural products (the unknown samples) were made in DMSO. Solutions were prepared in DMSO containing 0.1M TBAI as: 2 g/ 50 mL or 0.2 g/ 10 mL for Clove (syzygium aromaticum), 1 g/ 10 mL for Tisane (herbal green tea), 0.05 g / 10 mL for saffron (*Crocus sativus*) and harsinghar (*Nycanthes aboortrisis*).

For testing the antioxidative property of the (suspected antioxidant) samples, typically an aliquot (50, 100, 250, 500 and 1 mL) of the sample solutions were introduced into the electrochemical cell containing 5 – 10 mL 0.1M TBAI/DMSO solution to record the CV of O_2/O_2^- system. The electrodes in a "pin configuration" were introduced into cell and a triangular potential sweep (50 mV/s scan rate) was applied to the working electrode from 0000 mV to – 1200 mV vs Au QRE. The cyclic voltammogram of O_2 was then recorded in the presence of these extract samples, these CV's were compared with the one for O_2 in the absence of these samples. After recording the CV, oxygen was removed by bubbling Nitrogen and CV's of each sample solution was again recorded in the absence O_2/O_2^-. In this way the redox activity of the extract, in the region of interest, was checked. None of the samples studied, except benzoquinone, showed electro-activity in this region (0.000 to -1.200V vs Au QRE).

RESULTS

Detecting antioxidantive property by cyclic voltammetry (C. V)

The cyclic voltammograms (C. V.) of O_2 (dry DMSO, GC working electrode, gold wire QRE) is given in Figure 1. It is a reversible / mild quasi-reversible case but $(ip)_c / (ip)_a = 1$.

The C. V. of some known representative antioxidants are given in Figures 2 - 4. The C. V. data are collected in Table 1. Also C. V. of some representative "unknown" sample extracts (for example saffron, clove etc) are given in Figures 5 - 6. The data obtained from the cyclic voltammograms of these extracts sample are also collected in Table 1.

From figures and data in Table 1 of known antioxidants, it is clear that there are different types of antioxidants – some displaced $(Ep)_c$ of O_2 / O_2^- towards more cathodic while others (displaced) $(Ep)_c$ of O_2 / O_2^- more anodic. In some cases the cathodic peak current $(ip)_c$ height increased while in other cases $(ip)_c$ decreased. In two cases the antioxidant – O_2^- reaction product exhibited another anodic peak (Figure 2), Nevertheless one conclusion is easy to make: these antioxidants react with O_2^-, with different mechanism and that the anodic peak decreased in each case of reaction of O_2^- with a known or an unknown (extracts) sample. The detail mechanism of these reactions will be discussed elsewhere.

However, this categorization of known antioxidants – through the shape of C. V. - helped in categorizing the antioxidant(s) in unknown extract sample.

ESTIMATION OF CONCENTRATION OF ANTIOXIDANT / ANTIOXIDATIVE AGENT IN UNKNOWN EXTRACT SAMPLES

To estimate the concentration of an antioxidant in a given extract sample, the anodic peak height of O_2/O_2^- reversible wave, was used as guidance. For this purpose the $(i_p)_a$ *vs.* concentration for a known antioxidant, here BHT was used as guideline. Figure 7 gives a calibration curve between the concentration of BHT and $(ip)_a$ height (see Table 1). Through this graph between the concentration of BHT and $(i_p)_a$ of O_2^- an estimate of the concentration of an antioxidant/antioxidative agent(s) could be obtained. Results are collected in Tables 1 and 2.

EFFECTIVENESS OF ANTIOXIDANTS

From Table 1, various cyclic voltammograms and Figure 6, the percent of superoxide scavenged by various antioxidants / antioxidative agents are obtained; For example for 2 mM BHA it is 43%, for BHT (2.5 mM ; 46%), Ethanol (1.74 M ; 26%), Vitamin E (5 mM ; 58%), Clove (250 μL extract / 5 mL solution ; 57%) etc. The effectiveness of an antioxidant / antioxidative agent, is defined here as the amount of the O_2^- (estimated as given below) used up divided by amount of antioxidant / antioxidative agent, present in solution, for example, for BHA it is (0.138 mM / 2.5 mM) × 100.

How much fraction (percentage) of an antioxidant/ antioxidative agent, scavenges O_2^- even when it is present, sometime, in large excess, is of interest to know. For this the concentration of superoxide is needed.

Through cyclic voltammetry, the peak height, scan rate, scan width (that is, time *t*) and the approximate thickness of reaction layer $\delta[\delta=(2Dt)^{1/2}$, where D is the diffusion coefficient of O_2], around the electrode, one can estimate the concentration of O_2^- (Mohammad, 1975; Bard and Faulkner, 1980). In the present case with D taken as 1 x 10^{-5} $cm^2 s^{-1}$, t = 12 s (the scan width), δ is obtained. From this δ value along with the geometrical area of the electrode 0.076 cm^2, the volume of the reaction zone is obtained. From peak current and scan width the number

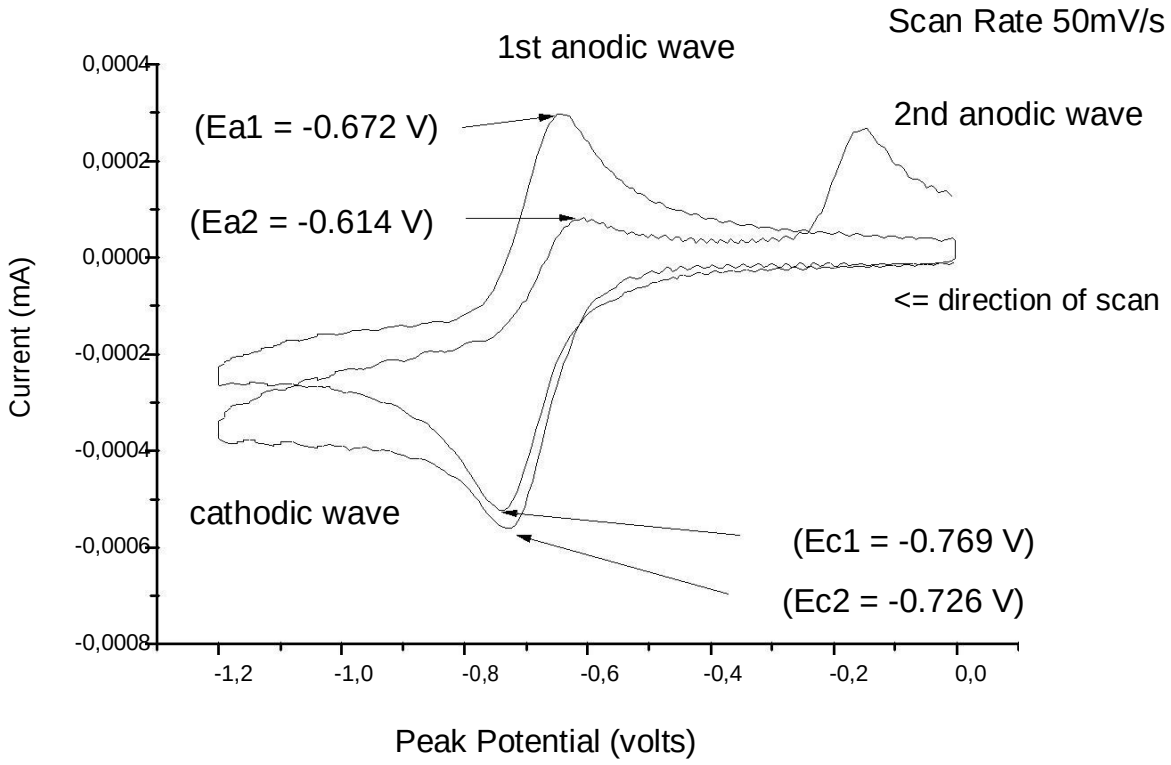

Figure 2. Cyclic voltammogram of O_2/ O_2^- in the presence of BHA; condition same as in Figure 1.

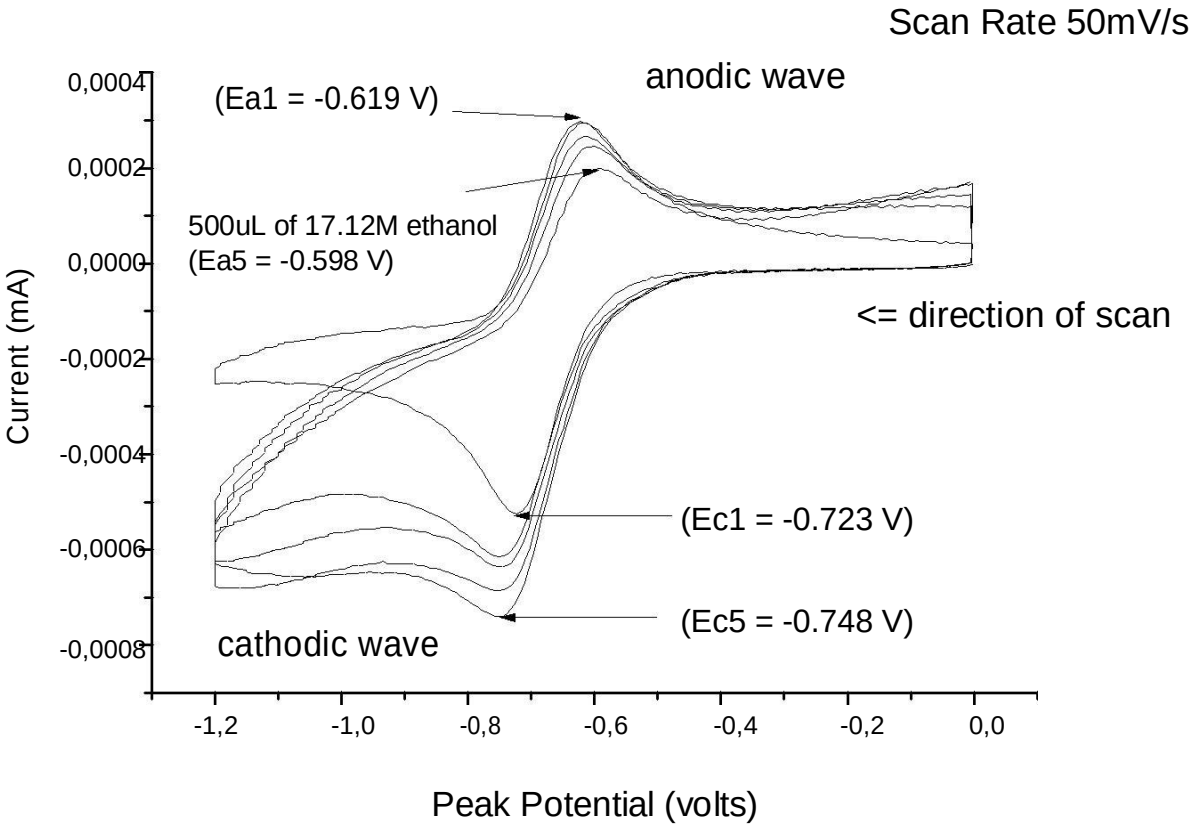

Figure 3. Cyclic voltammogram of O_2/ O_2^- in the presence of ethanol; condition same as in Figure 1.

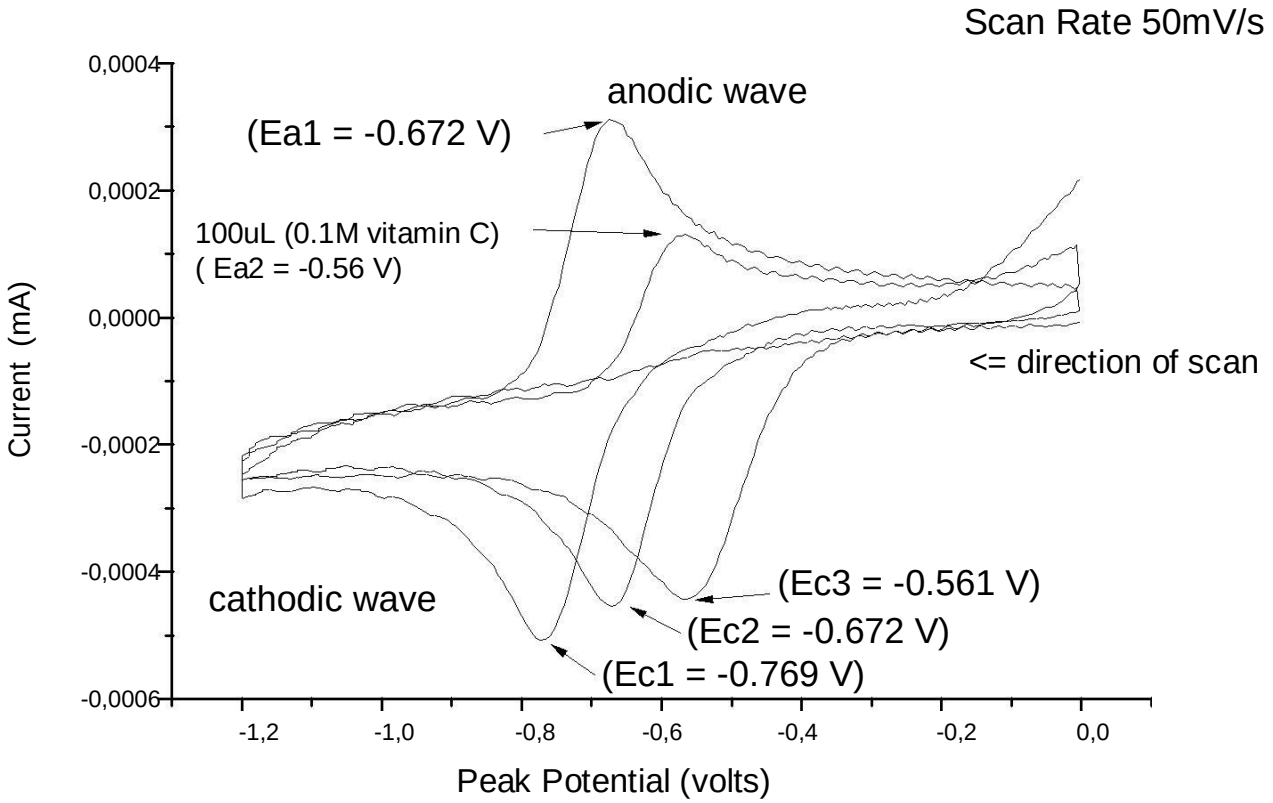

Figure 4. Cyclic voltammogram of O_2/ O_2^- in the presence of vitamin C; condition same as in Figure 1.

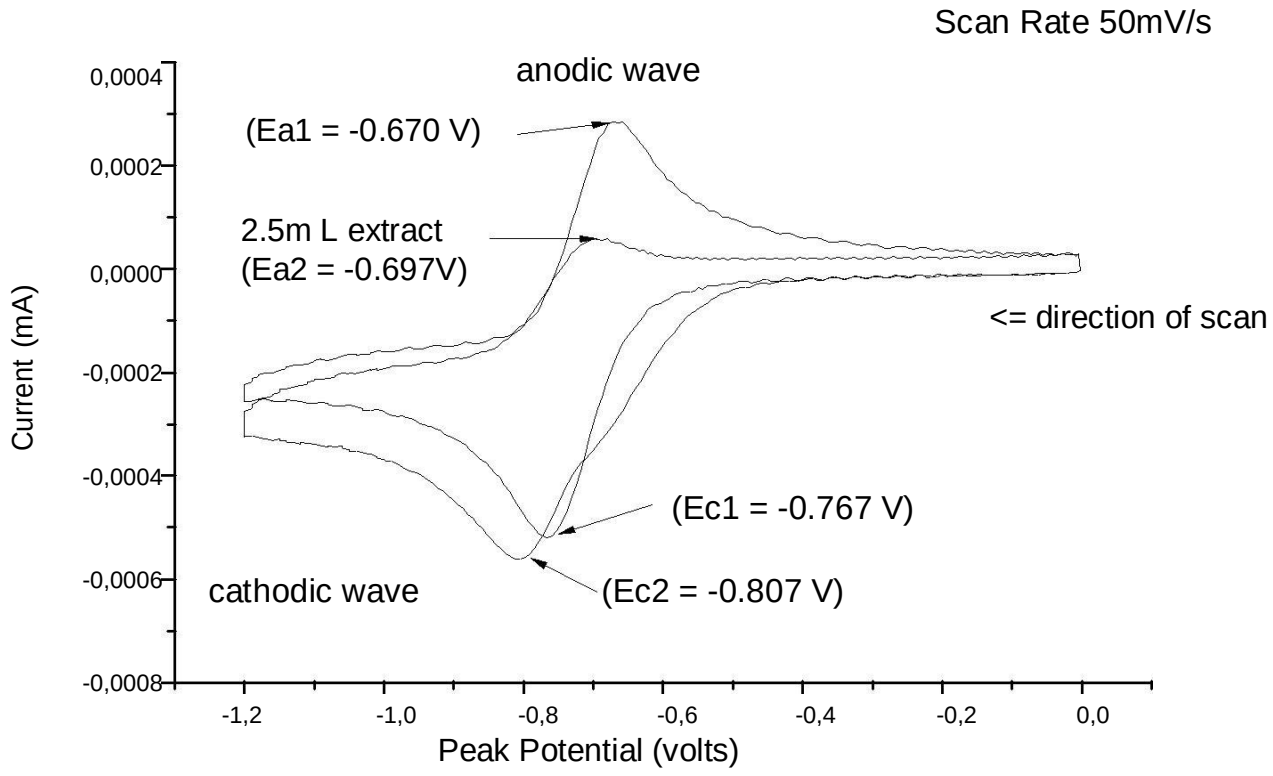

Figure 5. Cyclic voltammogram of O_2/ O_2^- in the presence of saffron extract; condition same as in Figure 1.

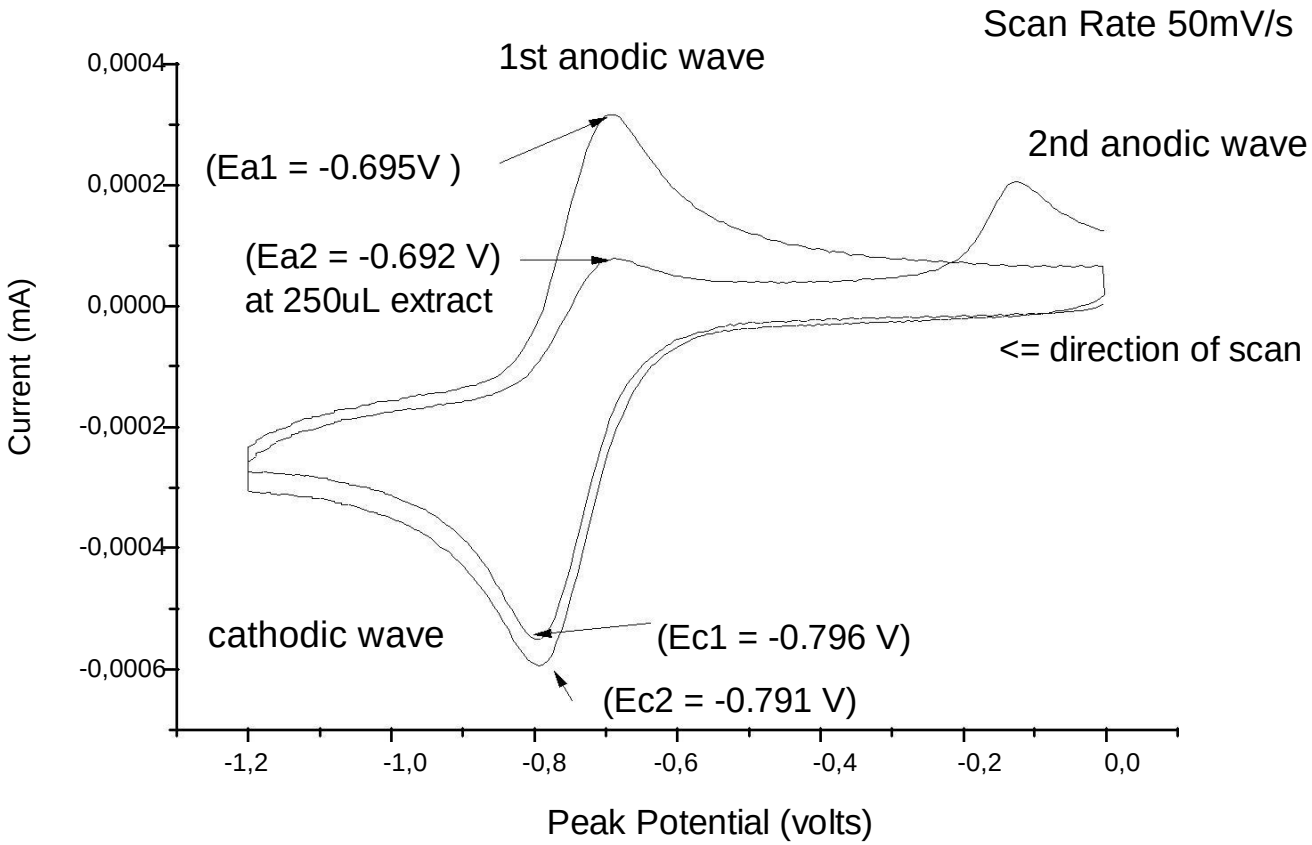

Figure 6. Cyclic voltammogram of O_2/ O_2^- in the presence of clove extract; condition same as in Figure 1.

Table 1. C.V. data, percentage and the concentration of O_2^- used up.

Substances	Concentration	$(i_p)_{cath}$	$(i_p)_{anod}$	$(i_p)_{anod}/(i_p)_{anod,0}$ [b]	% O_2^-, [c] Used up	$[O_2^-]$ [d] used up
		$\times 10^6$ A				$\times 10^4$ M
BHT	0 mM	0.541	0.541	1.00	0.0	---
	0.2 mM	0.590	0.537	0.99	1.0	0.03
	0.5 mM	0.629	0.487	0.90	10	0.3
	0.7 mM	0.669	0.475	0.88	12	0.4
	2 mM	0.613	0.307	0.57	43	1.20
BHA	0 mM	0.508	0.511	1.0	0.0	---
	2.5 mM	0.538	0.298	0.54	46	1.38
Ethanol	0 mM	0.523	0.529	1.0	0.0	---
	1.74 mM	0.785	0.395	0.74	26	0.78
Vitamin E	0 mM	0.488	0.481	0.98	0.0	---
	5 mM	0.560	0.205	0.42	58	1.74
MG	0 mM	0.501	0.581	1.1	0.0	---
	5 mM	0.516	0.239	0.41	59	1.77
Vitamin C	0 mM	0.508	0.511	1.0	0.0	---
	2 mM	0.455	0.333	0.65	35	1.05

Table 1. Contd.

Substances	Concentration	$(i_p)_{cath}$	$(i_p)_{anod}$	$(i_p)_{anod}/(i_p)_{anod,0}$ [b]	% O_2^{-}, [c] used up	$[O_2^{-}]$ [d] used up
		$\times 10^6$ A				$\times 10^4$ M
BQ	0 mM	0.541	0.541	1.0	0.0	---
	1 mM	0.824	0.485	0.88	12	0.36
Saffron	0	0.521	0.586	1.1	0.0	---
(*Crocus sativus*)	2.5/5mL	0.564	0.259	0.44	56	1.68
Tisane herbal tea	0	0.574	0.520	0.96	0.0	---
	150 µL/5 mL	0.575	0.379	0.73	27	0.8
	250 µL/5 mL	0.582	0.320	0.61	39	1.17
	500 µL/5 mL	0.608	0.308	0.59	41	1.24
Harsinghar	0	0.470	0.475	1.0	0.0	---
(*Nycanthes arbortrisis*)	250 µL/5 mL	0.530	0.410	0.86	14	0.42
	500 µL/5 mL	0.549	0.339	0.71	29	0.87
	1.25 mL/5 mL	0.561	0.224	0.47	57	1.59
Clove	0	0.57	0.56	1.0	0.0	---
(*Syzygium aromaticum*)	250 µL/5 mL	0.57	0.24	0.43	57	1.71

(a) Concentration of O_2 in DMSO at atmospheric pressure (air) is about 3 mM, (b) $(Ip)_{anod,0}$ is the anodic peak current when no antioxidant added (c) from the $(i_p)_a$ / $(i_p)_c$ ratio, the decline in anodic peak is manifestation of decay or reaction of O_2^{-}. (d) The concentration of O_2^{-} is estimated as described in text: first only 10% of the reduced species O_2^{-} was produced (0.3mM) and then only the % of O_2^{-} (footnote c) was used up. It was assumed the same amount of antioxidant was used up (reacted with O_2^{-}) on 1:1 stoichiometry.

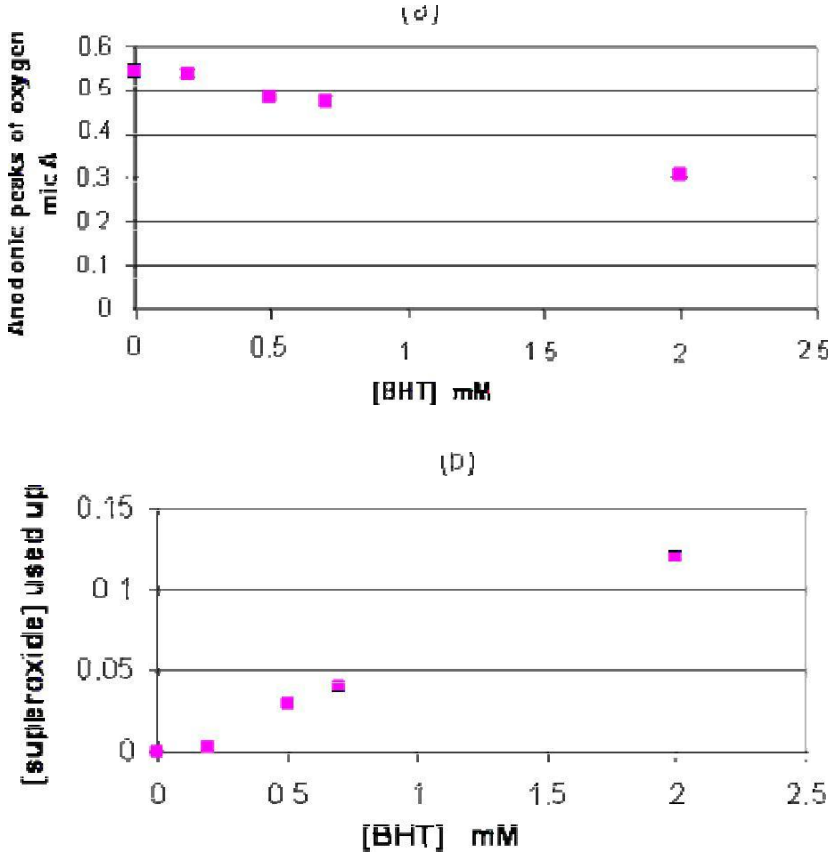

Figure 7. Calibration curves: (a) BHT concentration vs. anodic peaks of O_2; (b) BHT concentration vs. O_2^{-} used up. Concentrations in mM.

Table 2. Effectiveness of the antioxidant[a] .

Antioxidant	Effectiveness [a,e]	Category[d]
BHT	: 6 % = 1[e]	
BHA	: 5.5% = 0.92	
Ethanol1	<0.01% = < 0.002	
Vitamin E	: 4% = 0.66	
MG	: 4% = 0.66	
Vitamin C	: 5% = 0.83	
BQ	4% = 0.66	
Saffron[b] (3.4)	5% = 0.83	Ethanol
Tisane[b] (4)	3% = 0.50	BHT BHA, Ethanol
Harsinghar[b, c] (4)	4% = 0.66	BHA MG
Clove[b] (3)	6% = 1.0	BHT BHA

(b) The effectiveness is defined as the amount of the O_2^- used up divided by amount of antioxidant present in solution (e.g. for BHA it is 0.138 mM/ 2.5mM) × 100, (b) the concentration of the antioxidant / antioxidative agent in the extracts , was estimated from the graph for BHT (fig 7(a)) and the effectiveness is calculated as in (a), (c) the flower is white, the flower stem is highly orange in color. It is the (water) extract of the stem which is used in edibles or coloring clothes (fast color)., Here it is DMSO extract which is used. (d) The antioxidant(s) in the extract sample were categorized according to the shape of their reaction (with O_2^-) CV and comparing this C.V with that of known antioxidant, (e) relative "effectiveness" as superoxide scavenger, with BHT, taken, arbitrarily, nevertheless most potent antioxidant / superoxide scavenger with a numerical value as unity,.

of Coulomb is estimated, hence the concentration of superoxide, in the reaction zone, can be estimated. In the present case it comes out $\leq$ 0.1 mM.

For a reversible reduction of species O at a cathode, to R ;

O + ne € = R ……..(1)

under diffusion controlled condition, it has been proposed (Mohammad, 1975; Allen and Larry,1980) that [R] ; 10% [O].

The theory of stationary electrode polarography (Nicholson and Shain) gives, at time *t*

$$[R]_{electrode} = \frac{C_O^{bulk}}{1+\theta S(t)_{\lambda}} \qquad (2)$$

Where $[R]_{electrode}$ = concentration of R at the electrode, C_O^{bulk} = concentration of O in the bulk, and

$S(t) = e^{-at}$, $a = nFv / RT$, (3)

Where λ is switching time in cyclic sweep of voltage, t is time, v = scan rate (V/s), n is taken as unity, F = Faraday constant, R = gas constant, T = Temperature and

$\theta = \exp[(nF/RT)(E_i - E°)]$ ..... (4)

Where E_i = initial potential and E° is standard electrode potential, may conveniently be taken equal to $E_{1/2} = [(Ep)_c + (Ep)_a] / 2$; $E_{1/2}$ is the half wave potential, $(Ep)_c$ is cathodic peak potential, $(Ep)_a$, corresponding anodic peak potential. In the present case E_i = 0.000 V and $E_{1/2}$ = -0.780V vs. Au QRE.

At 50 mV scan rate and T = 298K, *a* is equal to 1.95. Taking scan time *t* for the total width (in volts) of the wave – scanned from foot of the wave to switching potential - as 12s, then *at* = -23.4 and $S = e^{-23.4}$ whereas $\theta = e^{27.6}$. All these parameters give $[R]_{electrode}$; $< 1 \times 10^{-4}$ M, that is, about 3% of O_2 in DMSO.

However, in the present study we will take (Mohammad, 1975; Bard and Faulkner, 1980) concentration of O_2^- as upper limit as 10% of O_2 in DMSO ~ 3×10^{-4} M (when $(ip)_a = (ip)_c$). The concentration of O_2 in DMSO is taken as 3mM at the ambient temperature.

Thus if we consider a 1:1 stoichiometry in a reaction between an antioxidant / antioxidative agent and O_2^-, and of the concentration of an antioxidant / antioxidative agent, sometime in large excess, less than 10% of O_2 was mopped in reacting with O_2^-. In other words, even when antioxidant was, sometime, in large excess, it failed to mop up all the O_2^- in the time scale of the cyclic voltage scan, this could very well be due to slow reactions between the antioxidant / antioxidatve agent under study and O_2^-.

The effectiveness of antioxidants studied is collected in Table 2. A relative effectiveness is also included in

Table 2. Relative effectiveness is calculated as the ratio of effectiveness of the antioxidant / antioxidative agent and the strongest known antioxidant, here BHA, which is arbitrarily give a value of unity.

Picoline did not show any reactivity towards O_2^- which is not surprising. Both species, picoline and O_2^- are basic in nature.

DISCUSSION

The electrochemical monitoring device, developed and used in the present study, was tested on several known antioxidants. Subsequently various extract samples were subjected to analysis. These results might give impression that everything reacts with O_2^- and that O_2^- is extremely reactive and reacts with every thing (quite fast). It is not so. Inactivity of O_2^- towards pyridine and picoline and slow reaction with various antioxidants antioxidative agents show that O_2^- is superoxide but not a super-reactive species.

Antioxidants may deactivate reactive oxygen species (superoxide, hydroxyl radical; in the present study superoxide) in the following ways (a) electron transfer from O_2^- to an antioxidant (b) H-bonding, (c) protonation of or hydrogen atom transfer to O_2^- , and (d) formation of a complex with O_2^-, with subsequent decomposition of the complex. The possibility of O_2^- acting as an electron abstracting oxidizing agent is ruled out because of the nonexistence of the second reduction wave nearby. Anti-oxidants can be further classified by mechanism of action as primary antioxidants and secondary antioxidants. Example of primary antioxidants include BHT, BHA propyl gallate etc. The shape of the cyclic voltammogram of the reaction of BHT (and BHA) (Figure 2) indicate protonation-type, that is (EC), reaction (Nicholson and Shain, 1964).

Reaction of superoxide with ethanol has been reported in literature before (Mohammad et al., 2001). We have confirmed here that superoxide reacts with ethanol that is, ethanol scavenges or deactivates superoxide (Figure 3, Table 2). In acetonitrile ethanol reacts with O_2^- quite fast, the bimolecular rate constant about 120 $M^{-1}s^{-1}$ (Mohammad et al., 2001). In DMSO the reaction appears to be quite slow. Besides, the reaction product of ethanol-superoxide could be another free radical more lethal than superoxide itself. However, the antioxidative (O_2^- - scavenging/deactivating) behavior of ethanol is of interest since the reported antioxidative effect of the red wine. It is possible that the “red” ingredient of the red wine mops up the product, the [ethanol – O_2^-] complex itself or the product of the complex.

Primary natural antioxidants are also commonly used such as tocopherol (Vitamin E, α -TOH). It has been suggested that α - TOH traps superoxide by hydrogen atom transfer from its phenolic OH group to form the corresponding phenoxyl radical species (Nakanishi et al., 2002). The C. V. depicting reactions of tocopherol with superoxide is similar in shape that for BHT (reacting with superoxide) (Figure 2 and Table 1) there is an anodic wave at – 0.4V (vs. Au QRE). Another compound methyl gallate (isolated from *Conyza banariensis*) showed the reaction with superoxide probably through proton transfer or complexing with O_2^- (and subsequent decomposition).

Another class of antioxidants is designated as second-dary antioxidant. They do not convert reactive reduce oxygen species (here O_2^-) into more stable products. These superoxide scavenger perhaps transfer hydrogen atom, they are like ascorbic acid. The shape of the C. V. (of the reaction) indicates a somewhat more complex reaction than simple EC mechanism.

Antioxidants can also deactivate O_2^- through electron transfer from superoxide. To this class belongs quinones. A quinone unit (ortho- or para- quinone) occurs is many natural products (flavones, say) and are quite effective superoxide scavengers (Aisha, 2005; Ishige et al., 2001). This is demonstrated here by benzoquinone which has $E_{1/2}$ = -0.314 (vs. Au-QRE) whereas $E_{1/2}$ of O_2/O_2^- = -0.720 (vs Au-QRE).

Recently saffron (*Crocus salivas*) has received some scientific recognition as a potential source of new medicinal source (Moss and Abdullayev, 2006). Much re-search has been carried out related to its anticancer and antitumor properties. It is particularly rich in carotenoids, which are antioxidants that protect the body from radical damage (Fridovich, 1982; Mohammad, 1970; Mohammad et al., 2008). In the present study the DMSO extract of saffron gave positive indication of the presence of antioxidant(s)/antioxidative agent(s), in the extract (Table 1). From Table 1, it can be concluded that saffron has high concentration of antioxidant(s) / antioxidative agent(s) and may act as effective antioxidant.

Beside saffron, extracts of some materials of common usage (oriental medicine): clove (*spice Syzyginns aro-maticum*), Miswak (siwak for dental hygiene), harsinghar flower stem (*Nycanthes arobortrisis*, natural food coloring material), tisane (a herbal tea), methyl gallate (isolated from *Conyza benariensis*) were also tested. DMSO extracts of all these materials gave positive result (Table 1). These results establish the wisdom for their traditional use particularly in Asiatic Countries (South East, Middle East, Far East, China). Extract of clove acts as strong antioxidative agent with a relative effectiveness as unity same as BHT.

From Table 1 and 2, it is clear that though antioxidants are present in excess quantities, generally a fraction of O_2^- is mopped. In other words large excess of an antioxidant will be needed for scavenging O_2^- completely. It supports the wisdom of the recommendation of using large excess of Vitamin C by Pauling. Our study supports the recommendation mode/dose of vitamin C by Pauling, particularly if O_2^- is involved in cancer production (Fridovich, 1982; Mohammad, 1970; Mohammad et al., 2008).

CONCLUSION

From our studies following conclusions can be drawn: (a) the present, electrochemical (C.V.), method is an easy, rapid, cost effective and informative method in monitoring antioxidative property of a substance or unknown sample (b) a large excess of these natural occurring substance containing antioxidants are to be consumed to obtain the desired result since only a fraction of O_2^- is mopped by even a large excess of an antioxidant. This tends to confirm Pauling's recommendation for the consumption of vitamin C in rather huge doses.

REFERENCES

Jimenez A, Selga A, Torres JL, Julia L (2004). "Reducing activity of polyphenols with stable radicals of the TTM series. Electron transfer verse H-abstraction reaction in flavon-3-ols". Org. Lett., 6: 4583 – 4586.

Aisha Siddique (2005). "Chemistry of QuinolineQuinone – Cadmium Complexes", M. Sc. Thesis, Chemistry Department, California State University, Northridge: 74.

Aisha Siddique (2005). M. S. Thesis, "Chemistry of QuinolineQuinone – Cadmium complexes", California State University, Northridge California, USA 2004: 102.

Allen Bard J, Larry Faulkner R (1980). " Electrochemical Methods" 1st Ed. John Wiley:129.

Pryor RL, Huang D, E Ou (2005). The chemistry behind dietary antioxidant capacity assays J. Agric. Chem., 53: 1841-1856.

Graham EC (1966). "The Basic Dictionary of Science", McMillan, New York: 24.

Fridovich I (1982). in "Pathology of Oxygen", AP. Autor. Ed. Academic Press.

Nakanishi I, Fukuhara K, Shimada T, Ohkubo K, Iizuka Y, Inami K, Mochizuki M, Urano S, Itoh S, Miyata N, Fukuzumi S (2002). "Effect of magnesium ion on kinetic stability and spin distribution of phenoxyl radical derived from a vitamin E analogue: mechanism insight into antioxidative hydrogen-transfer reaction of vitamins E"., J. Chem. Soc., Perkin Trans., 2: 1520-1524.

Ishige K, Schubert D, Sagara Y (2001). "Flavonoids protect neuronal cells from oxidant stress by three distinct mechanisms", Free Radical Biol. Med., 30: 433-446.

Schaich K, X-Wu, Pryor RL. (2005) "Standardized methods for the determination of antioxidant capacity and phenolic in food and dietary supplements", J. Agric. Food. Chem., 53: 4290-4302.

Mohammad M (1975). "Cyclic Voltammetric Investigation of a Chemical Reaction preceded by a Reversible Charge Transfer: Reaction of Py-ridinyl Radical with 4-nitrobenzyl chloride. Anal. Chem. 47: 958-960.

Mohammad Suny M, Stony Brook NY, Witz G (1970). (Sloan Kettering Cancer Research Inst, New York, – Discussion about the possibility of involvement of free radicals in cancer cell production.

Mohammad M, Dar A, Sajid Jahangir, Tahiri IA, Subhani MS, Khan KM (2008). "In Quest Of Friendly Free Radicals: Reactions of Iodine atom free Radicals with some biologically Important Compounds", in "Innovations in Chemical Biology", B. Sener (ed), Springer.

Mohammad M, Iqbal M, Khan AY, Bhatti MK, Zahir K, Jahan R (1981). "Protonation of higher reduction products of pyridinium salts", J. Phys. Chem. 85: 2816- 2820.

Mohammad M, Tahir Soomro M, Tariq M (2007). Poster Presentation in Conference on, "Free Radicals in Bio-system", Kuwait J. Mol. Biotechnol., (3): 18-21.

Mohammad AY, Khan MS, Subhani, Bibi N, Ahmad S (2001). "Kinetics and electrochemical studies on superoxide", Res. Chem. Inter. 27: 259-267.

Kohen R, Vellaichamy E, Herbac J, Gati I, Tirosh O (2000). "Quantification of the overall reactive oxygen species scavenging capacity of biological fluids and tissues", Free Radical Biol. Med., 28: 871 – 879.

Robert Roskaski (1996). "Biochemistry" Saunders Text and Review Series. Saunders, Philadelphia: 456.

Nicholson RS, Shain I (1964). "Theory of stationary electrode Polarography, Single Scan and Cyclic Methods Applied to Reversible, Irreversible, and Kinetic System", Anal. Chem., 36: 706 – 723.

Buratti S, Pelligrini N, Brenna OV, Manino S (2001). "Rapid electrochemical methods for the evaluation of the antioxidant" J. Agric. Food. Chem., 49: 5136-5141.