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Antioxidant studies of citric acid and citrus fruits towards paraquat by cyclic voltammetry: An antidote of paraquat poisoning

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Abstract: Voltammetric parameters are studied to confirm the antioxidant activity of citric acid towards reduced form of methyl viologen dication (1, 1'-dimethyl-4, 4'-bipyridinium, MV^{2+}, also known as paraquat). In this study the kinetics of the reaction of citric acid with reduced form of methyl viologen is also reported. Out of two oxidative peaks (i.e. MV^{o} to $MV^{+\cdot}$ and $MV^{+\cdot}$ to MV^{+2}) the first peak is almost removed after interaction with citric acid. Shifting in second cathodic peak potential is also obvious and possibility of citric acid to scavenge or making adduct with paraquat is identified. Some real samples (fruit juices) as a rich source of citric acid are also studied. This study presents a simple, relevant and fast voltammetric method by which quick quantitation and monitoring of antioxidant/ scavenging activity of food, herbs and other spices towards paraquat poisoning is possible. It may constitute a new wave of treatment or therapy for the disease caused by paraquat.

Keywords: Methyl viologen, toxicity of paraquat, citric acid, antioxidant activity, cyclic voltammetry.

INTRODUCTION

Paraquat (Kuhn, 1981) (1,1'-dimethyl-4,4'-bipyridinium) exhibit amazing redox system with three oxidation states. Toxicity of paraquat and its mechanism of action is well reported in the literature (Fukushima *et al*., 2002, Dinis-Oliveira *et al.,* 2008). Paraquat causes multiorgan failure includes kidney, liver, lungs and oesophagus (Pavan, 2013). It causes neurological disorder and results in very harmful neurodegenerative disease named as "Parkinson's disease" (Hatcher et al., , 2008, Bastías-Candia *et al*., 2019). Furthermore, it is also found to cause oxidative stress (Black *et al*., 2008, Pang *et al*., 2016). The main target of paraquat poisoning is lungs that accumulate PQ^{+2} and chronic exposure to paraquat may cause pulmonary fibrosis (Han *et al*., 2015).

Toxicity of paraquat is associated with its cyclic reduction and oxidation that generates superoxide anion, singlet oxygen and another radical that causes oxidative damage and leads towards the depletion of NADPH in living organisms (Bus *et al.,* 1974). Several methods were used to overcome the toxic effects caused by paraquat (Vale *et al.,* 1987; Rahmani *et al*., 2015) but unfortunately most of these have been recognized ineffective. Few treatments that proved to be effective are the adsorption by fuller's earth which adsorbs paraquat (Bismuth *et al*., 1982) and the use of haemodialysis or haemoperfusion in which the blood is filters through charcoal to remove the paraquat from lungs (Hsu *et al*., 2012).

Antioxidants provides the first route of defence against the toxicity caused by the radicals (Wojtunik-Kulesza *et al*., 2016). Natural and synthetic antioxidants like vitamin C (Ascorbic acid), flavonoids, vitamin E, carotenoids, super oxide dismutase and glutathione etc. are capable of stabilizing and deactivating the free radicals (Halliwell, 1994; Jacob, 1995). Antioxidant therapy has proven to reduce the oxidation injury caused by paraquat but only few reports have been published that demonstrates the valuable effects of antioxidant on paraquat poisoning (Hong *et al*., 2002; Hosamani and Muralidhara, 2013). A complete inhibition of toxicity of bipyridinium with the intake of natural antioxidant is not yet studied. Recently, Junthip has reported the removal of paraquat by water-insoluble cyclodextrin polymer cross-linked with citric acid (Junthip, 2019).

Citrus fruits are rich in antioxidants and different titrimetric methods have been used in the literature for the determination of antioxidants in citrus and non-citrus fruits (Tareen *et al*., 2015). Citric acid has been chosen for this study because it is abundantly found in variety of citrus fruits (Jamil *et al*., 2015) nevertheless its antioxidant activity/scavenging activity has not been investigated. It is well known that "Lemon" and "Grape fruit" are the rich sources of citric acid. To report an antidote for toxicity of paraquat, the interaction of citric acid with the reduced forms of methyl viologen dication is studied by cyclic voltammetry which has not been studied previously.

MATERIALS AND METHODS

Reagents and chemicals

METHYL VIOLOGEN DICHLORIDE (MV^{2+}) was purchased from Sigma-Aldrich. SODIUM CHLORIDE was used as supporting electrolyte and it was purchased from Sigma Aldrich. CITRIC ACID was purchased from

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BDH Laboratories, was used as received without any further purification. Deionized water was used throughout the study. None of the reagents was found to show any spurious electrochemical signal in the selected potential range of analyte.

Apparatus and method

Electrochemical measurements were carried out on the Electrochemical Analyzer (model CHI660 C series). All electrochemical measurements were performed with three electrode assembly consists of glassy carbon working electrode (3mm in diameter), Ag/AgCl reference electrode and Platinum wire counter electrode. Glassy carbon electrode was polished with 0.05 µm alumina on a polishing pad and rinsed with deionized water before taking each run. A 1.0mM methyl viologen dichloride solution is prepared by dissolving 25.71mg solid compound in 100mL volumetric flask of class A. 0.5850 gm of NaCl is transferred to the same 100 mL volumetric flask.

All solutions contained 1.0mM methyl viologen dichloride, to which 0.1M NaCl (supporting electrolyte) and different amount of citric acid ranging from 20-50 mM were added. All solutions were prepared in deionized water and purged with 99.999 % nitrogen gas for 15 min to eliminate oxygen. Cyclic voltammogram of the solution of supporting electrolyte was recorded, which did not show the presence of any electro active specie. CV's of methyl viologen solution were recorded at different scan rates (0.03-0.5V/s) in the absence and presence of citric acid. CV's of MV^{+2} were also recorded with the addition of 30- 50 µL of natural sample, like lemon and grape fruit juices, at different scan rates.

RESULTS

Determination of reversibility of methyl viologen dication redox couple

Fig. 1 shows two single electron reductions of methyl viologen dication.

$MV^{2+} + e^- \leftrightarrow MV^{+\cdot} + e^- \leftrightarrow MV^o$

CVs of Methyl viologen di cation (1.0mM) was recorded at 0.05 V/s scan rate. In the absence of citric acid anodic to cathodic peak current ratios (Ipa/Ipc) for the first redox process ($MV^{2+}/MV^{+\cdot}$) were found close to unity. Peak current ratio for second redox process ($MV^{+\cdot}/ MV^o$) was found beyond the unity (fig. 1).

Cyclic voltammetric assessment on the interaction of citric acid standard towards paraquat

To study the interaction of citric acid towards methyl viologen, CV's of methyl viologen dication (MV^{+2}) were recorded in the absence and presence of citric acid at 0.05 V/s scan rate. Cathodic to anodic peak separation (ΔEp) was found close to the reversible process in the absence of citric acid. In the presence of citric acid standard, peak current ratio for 1st redox process ($MV^{2+}/MV^{+\cdot}$) was reduced up to 0.44 and the anodic peak corresponding to the second reduction process ($MV^{+\cdot}/MV^o$) was disappeared (fig. 1). In addition to that second cathodic peak potential was shifted 47 mV anodically in the presence of standard citric acid. Cathodic peaks were also observed to shift negatively by increasing the scan rate in the presence of citric acid (fig. 2).

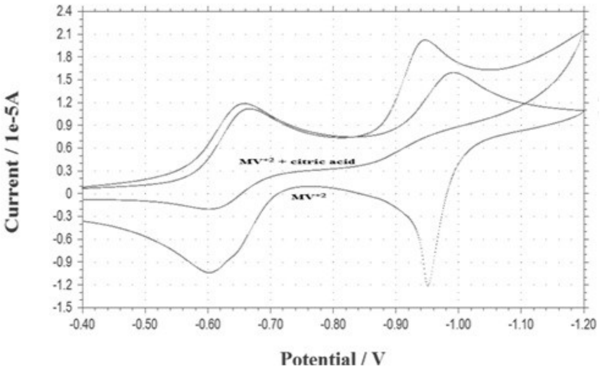


Fig. 1: Overlay of CVs of 1.0mM MV^{+2} without and with 23.215mM citric acid at 0.05 V/s scan rate.

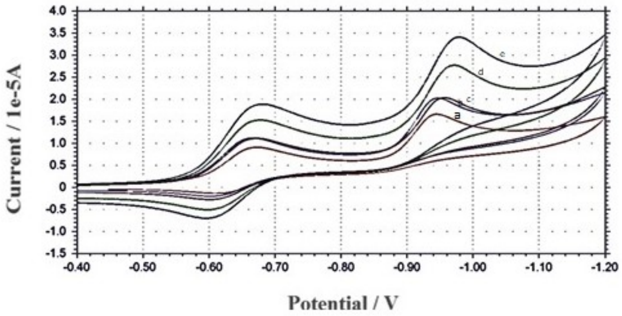


Fig. 2: Overlay of CVs of 1.0mM MV^{+2} with 23.215mM citric acid at different scan rates, where a= 0.03 V/s, b= 0.05 V/s, c= 0.1 V/s, d= 0.3 V/s, e= 0.5 V/s.

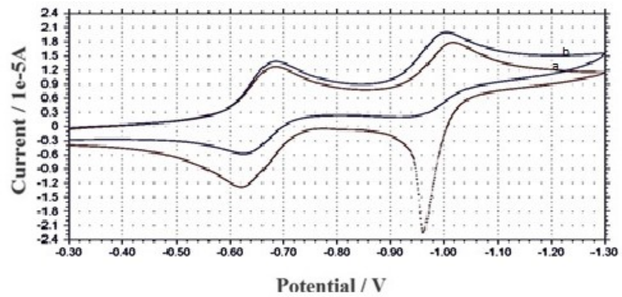


Fig. 3: Cyclic Voltammogram of 1.0mM Methyl viologen dication in the (a) absence and (b) presence of 45 µL grape fruit juice at 0.05 V/s scan rate.

Cyclic voltammetric assessment on the interaction of fruit juices towards paraquat

When CV's of methyl viologen dication (1mM) were recorded in the presence of grape fruit juice, the changes appeared in peak currents and peak potential, were found to be similar as observed for standard citric acid. Anodic peak current for first redox process ($MV^{2+}/MV^{+\cdot}$) was found to reduce and anodic peak corresponding to the second reduction process ($MV^{+\cdot}/MV^{o}$) was disappeared (fig. 3). It was also observed that cathodic peak potentials of viologen were shifted anodically with the addition of grape fruit juice. Cathodic peak potential for second redox process ($MV^{+\cdot}/MV^{o}$) was shifted 13mV anodically in the presence of grape fruit juice (fig. 3). By increasing the scan rate anodic peak corresponding for the second redox process ($MV^{+\cdot}/MV^{o}$) reappeared as observed for standard citric acid.

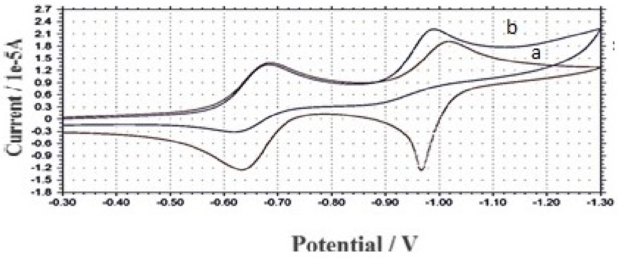


Fig. 4: Cyclic Voltammogram of 1.0mM Methyl viologen dication in the (a) absence and (b) presence of 30 µL lemon juice at 0.05 V/s scan rate.

With the course of investigation of antioxidant/scavenging activity of fruit juice extracts towards MV^{2+}, CVs of methyl viologen dication (1mM) were also recorded in the presence of lemon juice extract and it was noticed that the anodic peak current for 1st redox process ($MV^{2+}/MV^{+\cdot}$) was reduced like standard citric acid while anodic peak corresponding to the second reduction process ($MV^{+\cdot}/ MV^{o}$) was disappeared (fig. 4). The second cathodic peak was shifted 29 mV anodically in the presence of lemon juice (fig. 4). In addition to that, in the presence of lemon juice extract, anodic peak corresponding to the second redox process ($MV^{+\cdot}/ MV^{o}$) reappeared at high scan rate which shows slow kinetics of the reaction of antioxidants present in lemon juice extract.

Determination of percentage of methyl viologen cation radical scavenged by citric acid standard and citrus fruit (lemon and grape fruit) juices

Percentage of methyl viologen cation radical scavenged by citric acid standard was found to be 63.8% (table 1). It was found that 34.3% and 60% of methyl viologen cation radical scavenged by 45 µL grape fruit juice and 30 µL lemon juice respectively (table 2).

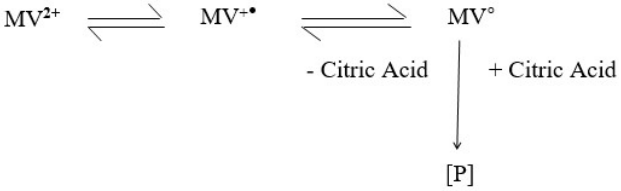
Kinetics of the reaction of citric acid with reduced form of methyl viologen dication

When CVs of methyl viologen di-cation were recorded in higher concentration range of citric acid at various scan rates, it was observed that the anodic peak currents corresponding to the second reduction process ($MV^{+\cdot}/MV^{o}$) increased by increasing the scan rate. It showed that at higher scan rates more MV^{o} left uncomplexed resulting in the appearance of anodic peak (fig. 2), contrary to that at slow scan rate more MV^{o} has complexed by the time the (Ep)a was attained. This indicates that the kinetics of formation of MV^{o} – citric acid adduct, is not quite fast. Bimolecular rate constant, k_2 for the irreversible chemical reaction of MV^{o} with citric acid was calculated as $2.92 \times 10^{3}\ M^{-1}\ s^{-1}$.

DISCUSSION

In this research the interaction of methyl viologen with citric acid as an antioxidant/scavenger has been explored by cyclic voltammetry. Coupling of chemical reactions with electron transfer reactions can be characterized by the change in peak currents and peak positions. In the absence of citric acid standard, peak current ratio for second redox process of methyl viologen ($MV^{+\cdot}/ MV^{o}$) was found beyond the unity because it deposited on the surface of electrode and giving rise a sharp and symmetrical oxidation peak. With the addition of citric acid, oxidation peak currents are reduced verifying the antioxidant activity of citric acid towards methyl viologen. Positive shifting of the peak potential confirms the presence of EC mechanism (Fiaz *et al*., 2019; Perveen and Naqvi 2016; Nicholson and Shain, 1964) between MV^{o} and citric acid. From this observation it is concluded that MV^{0} forms electro inactive product with citric acid according to EC mechanism.

EC mechanism can further be verified with the help of shifting of cathodic peak with the increase in scan rate. For the present case reduction peaks were observed to shift cathodically by increasing the scan rate in the presence of citric acid (fig. 2). This negative shifting is prominent for second redox process ($MV^{+\cdot}/MV^{o}$) as compare to the first redox process ($MV^{2+}/MV^{+\cdot}$). These results confirm the presence of some coupled chemical reaction between reduced form of viologen and citric acid and show antioxidant/scavenging activity of citric acid towards reduced form of methyl viologen dication. Based on shifting in peak position and changes appeared in peak currents, an EEC mechanism (Nicholson and Shain, 1964) is proposed which highlights all possible electron transfer reactions and chemical reactions. This mechanism is given below:



Citric acid scavenges the reduced form of MV^{2+} by making some adduct which may be a protonated adduct, P. How much fraction (percentage) of MV° scavenge with citric acid is the interesting point to know. In this study, the percentage of $MV^{+\bullet}$ scavenged by the citric acid is

Table 1: Scavenging/antioxidant activity of citric acid standard towards reduced form of methyl viologen dication

Concentration/mM	*Ipa₁*/µA	$\lvert Ipa_1/Ipa_1^0 \rvert^c$	*% MV^+ Used up*
$1.0^a + 0.00^b$	-11.36	1.00	0
$1.0^a + 23.215^b$	-4.111	0.361	63.8

a. Concentration of Methyl viologen dication

b. Concentration of citric acid

c. Ipa_1 and Ipa^0 are the anodic peak currents measured in the presence and absence of citric acid for the 1st redox process.

Table 2: Scavenging/antioxidant activity of fruit juice extracts towards reduced form of methyl viologen dication.

Juice extract + 1mM MV^{+2}	*Volume of fruit juice added*/µl	*Ipa₁*/µA	$\lvert Ipa_1/Ipa^0 \rvert^a$	*% $MV^{+\cdot}$ Used up*
Grape fruit	0.0	-12.42	1.00	0
	45	-8.163	0.657	34.3
Lemon	0.0	-13.82	1.00	0
	30	-5.525	0.399	60.0

a. Ipa_1 and Ipa^0 are the anodic peak currents measured in the presence and absence of citric acid for the 1st redox process.

determined and it is found to be 63.8% (table 1). It has been calculated by the anodic peak height of MV^{2+}/ $MV^{+\bullet}$ reversible wave, in the presence and absence of citric acid i.e. $\lvert Ipa_1/Ipa_1^0 \rvert$. This voltammetric parameter can be used as a simple tool to estimate the scavenging /antioxidant activity of food, herbs and other spices towards paraquat poisoning.

In this study fraction of total current of $MV^{+\cdot}$ was used as an analytical tool to determine the antioxidant/scavenging activity of citrus fruits in terms of citric acid. For the present case, percentage of $MV^{+\cdot}$ mopped, by total antioxidants present in the grape fruit and lemon juice is found to be 34.3 and 60 % respectively (table 2). Antioxidant / scavenging activity of these fruit extracts in terms of citric acid can be estimated from the fraction of the total current of $MV^{+\cdot}$ used up by standard citric acid. Thus, total antioxidant activity/scavenging activity of grape fruit and lemon juice in terms of citric acid, has been estimated as 21.8 and 38.3% respectively.

To obtain the rate constant of the [MV^0 - Citric Acid] product (P), an electrochemical approach was used which is superior to peak current ratios method (Akbar *et al.,* 2014). Rate constant for the reaction of MV^o with citric acid could easily be obtained using the Nicholson-Shain theory (Nicholson and Shain, 1964) as adopted by our group for the case in which an irreversible chemical reaction follows a reversible charge transfer (Akbar *et al.,* 2014).

$$E_p = E_{1/2} - (RT/nF) \quad [0.78\text{-}\ln (kf'/a)^{1/2}]$$

Where Ep is the peak potential for MV^o in the presence of citric acid; $E_{1/2}$ is the half wave potential of MV^o in the absence of citric acid, a=nFυ/RT (υ is scan rate, Vs^{-1}). From this pseudo-first order rate constant, second order rate constant k_2 could be obtained. Pseudo-first order condition was maintained using [citric acid] about 20 times of [MV^{2+}].

The bimolecular rate constant of the product is found as $2.92 \times 10^3\ M^{-1}\ s^{-1}$.

In living cells, if enough concentration of $MV^{+\cdot}$ is produced by the oxidation of enzyme NADPH, it may through disproportionation reaction generate enough MV^o to react with oxygen and produce more superoxide anion, (MV^o is highly reactive towards oxygen as compared to $MV^{+\cdot}$). In this context antioxidant/scavenging property of "Citric Acid" may constitute a new wave of treatment or therapy for the disease caused by "PARAQUAT''.

CONCLUSION

This study opens a new avenue for researchers to study the antioxidant/scavenging capacity of medicinal material, herbs and spices towards paraquat by using simple, reliable and fast voltammetric method. These herbal and food materials might be able to attenuate the paraquat mediated toxicity and may be used as antidote.

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