Int. J. Electrochem. Sci., 9 **(2014)** 5751 - 5762

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Determination of Ascorbic Acid Content of Some Capsicum Cultivars by Cyclic Voltammetry performed at G.C.E. by External Standard Series Calibration Method

Sadia Ghafoor[*], *Azizuddin, Rafi Arain, Kousar Yasmeen, Fareed Ahmed, Iftekhar Saeed*

Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi-75300, Pakistan
[*]E-mail: sadiachemist@yahoo.com

Received: 31 July 2013 / *Accepted:* 23 April 2014 / *Published:* 16 July 2014

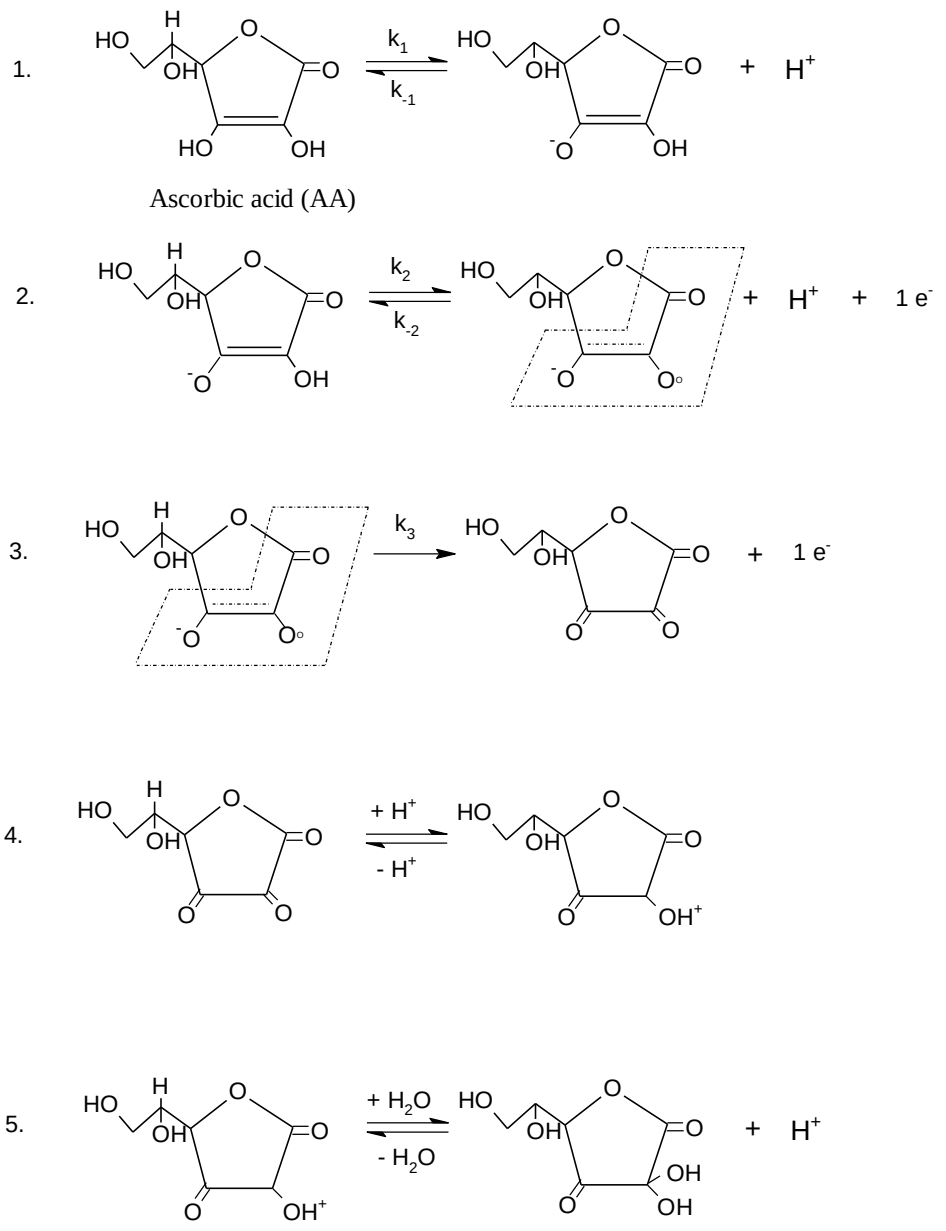
A method has been developed to find out ascorbic acid content in five different chili cultivars included in regular diet viz Green ghotki, Yellow ghotki, Green maliri, Green germane, and Surkh. Samples were analyzed for their ascorbic acid content to evaluate their nutritional importance by cyclic voltammetry by external standard series calibration method. Data evaluated were subjected to investigate daily intake of ascorbic acid in the Pakistani diet. The results showed that almost all cultivars contain appreciable amount of ascorbic acid, may varied according to type of samples. In our studied samples, Green (mature/immature) have high antioxidants in contrast to mature (Yellow) and sun dried over mature (Surkh). AA concentration and daily intake were discovered in an alignment of Green germane [(4.06 mg/100 g) & (0.49 mg/d)] > Green maliri [(3.08 mg/100 g) & (0.37 mg/d)] > Green ghotki [(2.11 mg/100 g) & (0.25 mg/d)] > Yellow ghotki [(2.08 mg/100 g) & (0.25 mg/d)] > Surkh [(0.8 mg/100g) & 0.06 mg/d)], respectively. Intake of fresh green capsicum is recommended for fulfillment of daily antioxidant requirement. This method is found to be efficient and accurate as percentage recovery is found to be 98.34%.

Keywords: Cyclic voltammetry, Ascorbic acid (AA), Capsicum, Daily intake.

1. INTRODUCTION

An electro-active compound vitamin C is often considered equivalent to ascorbic acid, is an essential vitamin required in a healthy diet varies with age and gender. Health Canada recommends daily intake for children, female and male about 15 mg/day, 75 mg/day and 90 mg/day, respectively [1]. Antioxidant properties of foods have to be carefully monitored because variation in content is occurred due to thermal liability during storage and preservation [2-3]. Man, guinea pigs, Indian fruit

bats, bulbul, primates and some fishes are incapable of for biosynthesis of ascorbic acid among most of plants and animals [4-5]. This incapability initiated dependency on external uptake assets for ascorbic acid intake for example, vegetables and fruits as well as pharmaceutical supplements [6]. An excess level does not pose any serious health effect, due to its water solubility it can be easily excreted from body [7]. The deficiency is problematic, because it directs to decrease in infection opposition initiated by free radicals [4]. Ascorbic acid is act as a vital electron donor; by this it takes a major role in the prevention of oxidative damage to protein and DNA [8]. Proposed mechanism for the electro oxidation of ascorbic acid in acidic or neutral solution is described in Scheme 1 [9].



Scheme 1. Proposed mechanism for the electro oxidation of ascorbic acid in acidic or neutral solution.

Vegetable are supposed to be protective agents due to high antioxidant containment, which can prevent oxidative damage caused by reactive oxygen species such as free radicals that are generated by a variety of sources including pesticides, tobacco smoke, exhaust fumes, certain pollutants and organic solvents [10]. Furthermore; ascorbic acid intake prevents sperm agglutination thus making them more motile with resultant improvement in male fertility. It also enhances sperm quality [11-12]. Health effects of ascorbic acid is also added in Table 1 [13]. Total antioxidants share a major role and contribution along with phenolic compounds and sugars in complex environment of peppers [14].

Table 1. Health effect of ascorbic acid (AA)

Disease	Mode of action	Effects
Coronary heart disease	Improved vascular flow	↓
Hypertension/blood pressure	Improved vascular flow	↓
Diabetes/hyperglycemia	Blood flow, impaired endothelium-dependent vasodilatation	(Restores)
Pre-Eclampsia	Plasminogen activator inhibitor (PA-I), a marker	↓ in PA-I
Cancer	Protecting tissues against oxidative damage	↓
Atherosclerosis	Protect LDL from oxidation, increase HDL level and decrease total cholesterol	↓
Cataracts	Descending free radical formation	↓
Men's fertility/oxidative stress	Reduce oxidative damage to sperm-DNA (in smokers)	↓

↓ Means reduction in effects.

Due to high importance no of analytical methods has been proposed for précised determination, but exclusively having drawbacks. Most of these give erroneous results in different matrices due to presence of oxidisable species other than ascorbic acid [15]. These methods include titrimetric, fluorometric, complexometric, liquid chromatography, high-performance liquid chromatography, spectrophotometric, amperometric and enzymatic methods [16-25]. Beside these electrochemical detections are found to be more accurate and reproducible in food ascorbic acid determination due to its electro-activity i.e. ascorbic acid is easily oxidized to dehydroascorbic acid. The electrode surface employed in such determinations can be a powerful tool in such applications [26]. It can determine easily all the way through electrochemical methods such as the determination of vitamin C has been described from differential pulse voltammetry [27-28] and square-wave voltammetry [29].

Cyclic voltammetry is an electrochemical technique in which measure the potential of the electro-active specie on the working electrode against reference electrode is determined [30]. The aim of the present work was to determine the individual ascorbic acid concentration in different chili cultivars include in daily diet namely; Green germane, Green maliri, Green ghotki, Yellow ghotki and Surkh. Data generated were used to estimate daily intake of antioxidants. For this purpose of quantification of ascorbic acid, we used cyclic voltammetry by external standard series calibration method.

2. MATERIAL AND METHODS

2.1 Reagents and solutions

All chemicals and solvents used were of analytical grade. Dimethyl sulfoxide (DMSO), tetrabutyl ammonium perchlorate (TBAPC), ascorbic acid (AA) used were purchased from Merck and Sigma-Aldrich. The distilled water used throughout the research work was doubly distilled in order to minimize the any impurity or interfering specie.

Supporting electrolyte is formed by dissolving 0.314 g TBAPC in 10 mL DMSO (99.9 % pure). The concentration of the resulting solution is 0.1M. Different concentration of ascorbic acid (40, 80, 120, 160 and 200 μM) was prepared by using alcohol (6:4 ratios with distilled water) as a solvent. Preparation of analyte for the analysis of CV: Each analyte was prepared by adding 100 μL of sample and making up the volume to 100 mL with ethanol (6:4 ratios with distilled water).

2.2 Plant material and preparation of extract

The samples were obtained from the model farm at place near the town Kunri, district Umerkot (Namely; Green ghotki, Yellow ghotki, Green maliri, Green germane and Surkh). 2 g of each chili fruit was crushed in a mortar with a pestle. 50 mL of ethanol diluted with distilled water (in 6:4 ratios), was added to the mortar and the mixture transferred to a beaker and sealed with a teflon tape and left for 96 hours. Resulting mixture was then filtered by Whatman (#40) filter paper and washed with another 25 mL of absolute alcohol. The filtrate was then transferred to a glass stopped flask.

2.3 Procedure

After polishing the working electrode. 10 mL of supporting electrolyte was added in the cell of CV and then 20 μL of 40 μM ascorbic acid solution added. The CV of 40 μM ascorbic was taken. This procedure was repetitively done over 40 μM, 80 μM, 120 μM, 160 μM and 200 μM concentrations of standard. The CV of each chili extract was taken by polishing the electrode and 100 μL of each chili extract added in 10 mL supporting electrolyte in the cell of CV. The conformation of ascorbic acid plot was obtained by adding ascorbic acid in the cell of CV after taking the sample CV. It was observed that the CV of sample with added ascorbic acid increased as compare to the CV of the only sample. The concentration of ascorbic acid in the different varieties of chilies determined by external standard series calibration method. The standard series concentration was optimized between 40-200 μM.

2.4 Cyclic voltammetry

The model of cyclic voltammetry was CHI600c USA. The circuit of cyclic voltammetry mainly based on three electrodes namely; working electrode, auxiliary electrode and reference electrode. A potentiostate equipped is required to change the potential of working electrode. Glassy carbon (GCE) was used as a working electrode; the diameter of GC was 2 mm and area was 0.0201 cm^2. The

reference electrode was saturated calomel electrode (SCE) with a diameter of 2 mm. Auxiliary electrode was Pt wire. The volume of voltammetric cell was 10 mL. Potentiostate was built-in in instrument. A window XP Professional was launched on PC (personal computer). For the removal of pre-history of the working electrode alumina solution was used as the polishing material on polishing pad. The potential applied was 0.0-1.6 V as all the done measurements observed in this range. The scan rate was 0.05 V/sec.

2.5 Daily intake of vitamin C

Daily intake of vitamin C was calculated as method described in previously published literature [31]

Total antioxidant intake from vegetables and fruits (mg person^{-1}day^{-1}) = CiVi

Where, Ci is concentration of vitamin C (mg/100 g)

Vi is daily ingestion (mg).

3. RESULTS AND DISCUSSION

3.1 Supporting electrolyte

Supporting electrolyte (SE) in cyclic voltammetry was used to suppress or minimize the effects of migration and to maintain the lamina on the interface of working electrode as well as in solution. This self supporting electrolyte has no effect on electro-active specie as it is electro-inactive; the main purpose of supporting electrolyte as explained above is to maintain ionic strength, to increase the conductivity of solution and to minimize the ohmic drop of electro-analytical or electro-synthetic experiments [32-33].

As shown in the Fig. 1, the voltammogram did not show any electro-active specie. The choice of the supporting electrolyte is depended upon the medium either it's aqueous or non aqueous. TBAPC was used as SE, because of non aqueous medium.

3.2 Ascorbic acid standards

Different concentration of standard ascorbic acid ranged 40, 80, 120, 160, and 200 μM used to plot the standard addition method for the determination of concentrations of samples. The overlay also plotted between the standard to determine the effect of increasing order of concentrations as shown in Fig. 2 (A & B). As the concentration of standard increases the anodic peak current also increases. This shows that there is direct relationship between them. It suggests that there is linear relationship between the rate of oxidation and concentration or anodic peak current and concentration of ascorbic acid with coefficient of determination (R2) of 0.9421.

3.3 Extracts of chilies

The concentrations of ascorbic acid in 5 samples were obtained by plotting the calibration curve for each sample the results are shown in the Fig. 3 (A, B, C, D & E) and Table 2. The total ascorbic acid content varied widely on varieties. The concentration of ascorbic acid ranged from 1.369-4.608 μM as obtained from anodic peak current. It is also found theoretically in cyclic voltammetry the concentration of the substrate has direct relation with the peak current [34].

It was found that Green germane contains maximum amount of ascorbic acid while Surkh (fully sun dried) dried contains the minimum amount. This result shows that Surkh chilies contain the lowest amount of ascorbic acid that's why it have low level of antioxidant potential as compare to the Green chilies. The green chilies of Ghotki, both pigmentations contains different concentration, this suggest that pigmentation can caused in variation, and the Green germane contains highest value of ascorbic acid the above data explain Green germane are more beneficial for health. Green maliri found in midest of all them data shown in (Table 2). AA concentration and daily intake were discovered in an alignment of Green germane 4.06 mg/100 g > Green maliri (3.08 mg/100 g) > Green ghotki (2.11 mg/100 g) > Yellow ghotki (2.08 mg/100 g) > Surkh (0.8 mg/100 g), respectively.

Finely this result shows that each sample contains certain amount of ascorbic acid concentration and it is directly proportional to anodic peak current or current. The limit of detection and quantification of this instrument is 0.1 - 5.0 mM based on the area of working electrode as the area reduced sensitivity increase, such as in the case of ultra-micro working electrode. These are more sensitive because of smaller area of electrode small amount of current passed through it. The limits of quantitation and detection in cyclic voltammetry were approximately three orders of magnitude below the concentrations determined in any of the fruit juices [35].

In conclusion, we modeled and explain that CV is more sensitive as compare to the other techniques. This technique is easily handled, low cost, and sample preparation steps do not requires too much solvent and chemicals. It is fast scanning technique and quite reproducible. The wide range of electrochemical or potential window used in CV for determination of electro-active compounds and nearly all electro-active compounds determined in this range. In which specie interference is normally not occurred and only electro-active compounds detected. To remove the interference species on electrode, polishing done. This is necessary to remove the effect of prolong atmospheric exposure and other functionalities.

3.4 Daily intake of AA as antioxidant

Daily intake of antioxidant is mandatory for a healthy life style and well being [1]. Capsicum is considered as a rich source of antioxidant [31, 36–38]. In our studied samples, Green (mature/immature) have high antioxidants in contrast to mature (Yellow) and sun dried over mature (Surkh). The results showed that almost all cultivars contain appreciable amount of ascorbic acid. Values observed were in alignment of Green germane (4.06 mg/100 g) > Green maliri (3.08 mg/100 g) > Green ghotki (2.11 mg/100 g) > Yellow ghotki (2.08 mg/100 g) > Surkh (0.8 mg/100 g). Notable difference was recorded from values obtained from these five cultivars. In literature, AA concentration

was found in different varieties of chili [37-39]. Furthermore, daily intake were found in an order of Green germane (0.49 mg/d) > Green maliri (0.37 mg/d) > Green ghotki (0.25 mg/d) > Yellow ghotki (0.25 mg/d) > Surkh (0.06 mg/d), respectively. An average is found to be 0.28 mg/d, which is far below than previously reported data [31]. Previously no data were reported in this regards for local consumers. Complete daily intake criteria is accessible in Table 2.

3.5 Accuracy of method

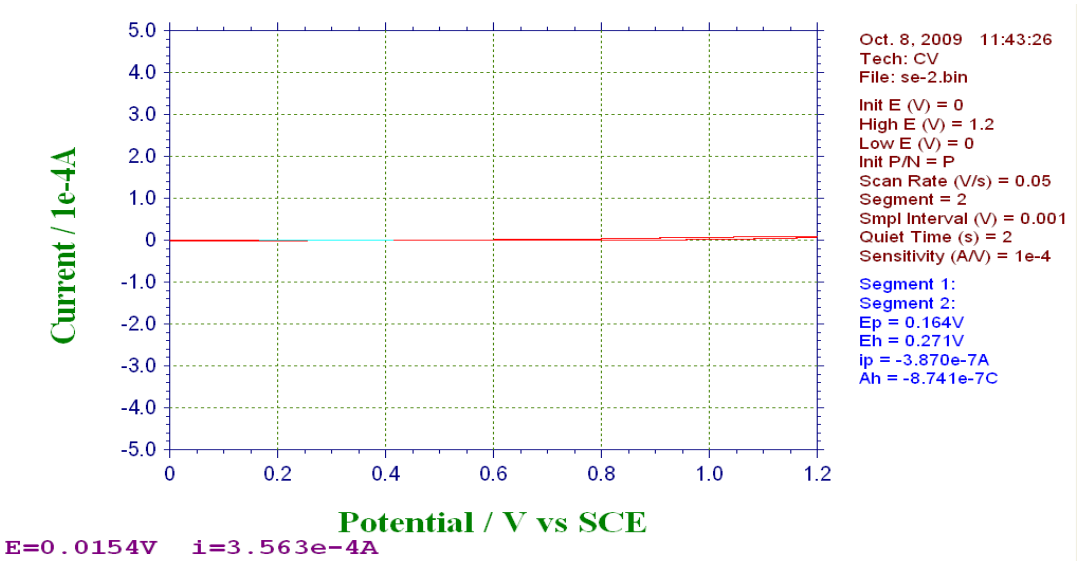
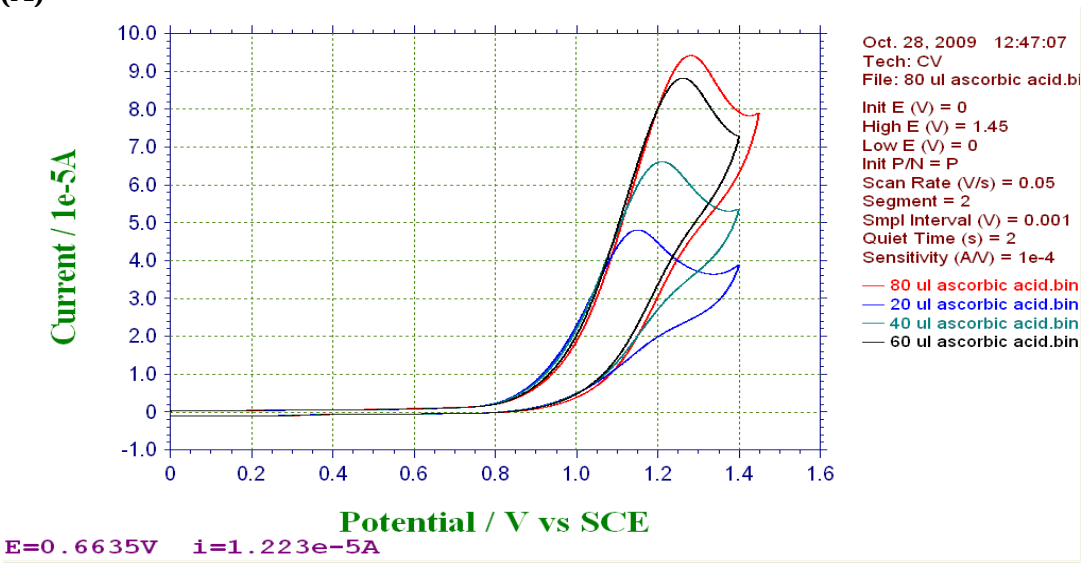


Figure 1. Cyclic voltammogram of supporting electrolyte (SE)

(A)



(B)

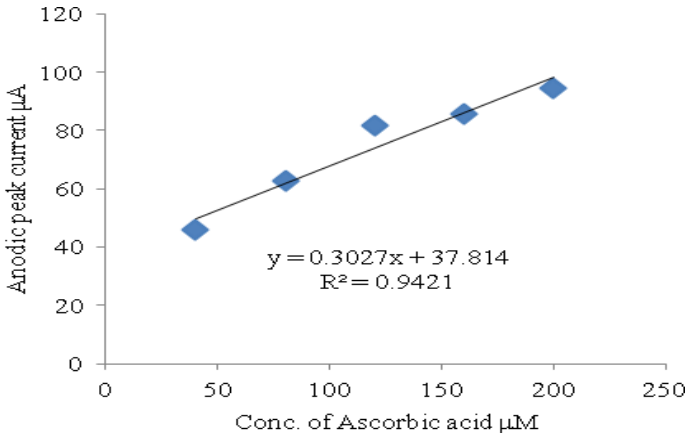
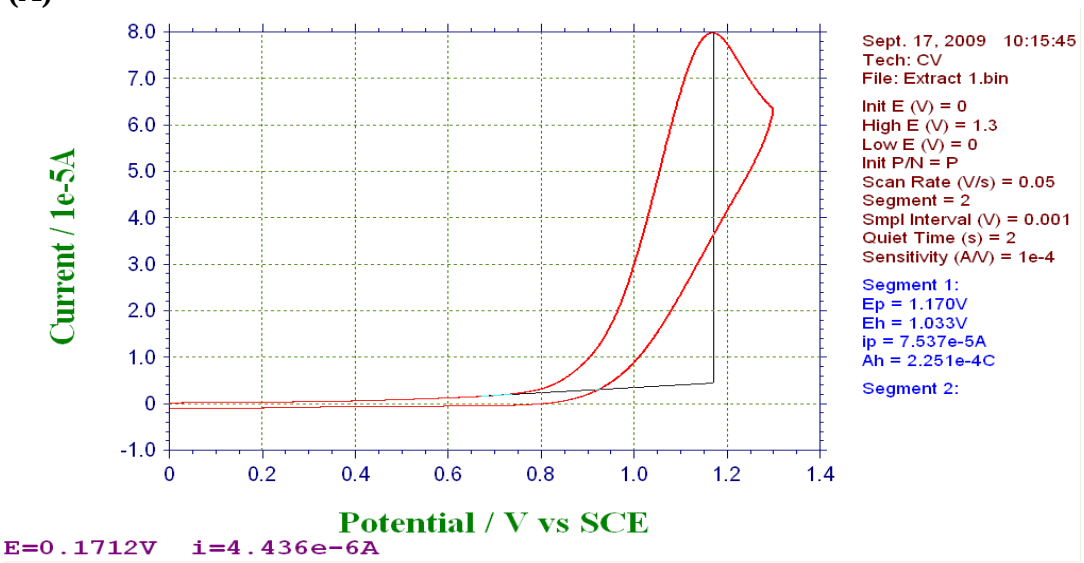
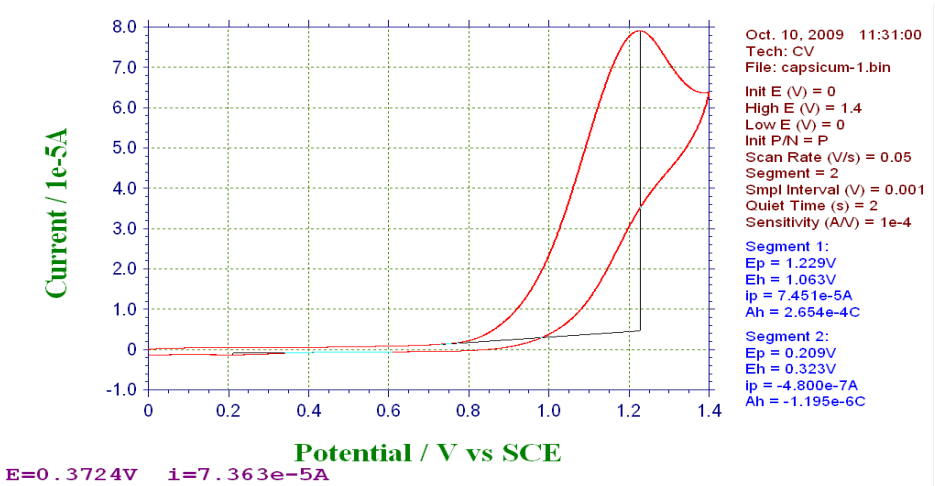


Figure 2. Overlay and calibration of ascorbic acid standards

(A)



(B)



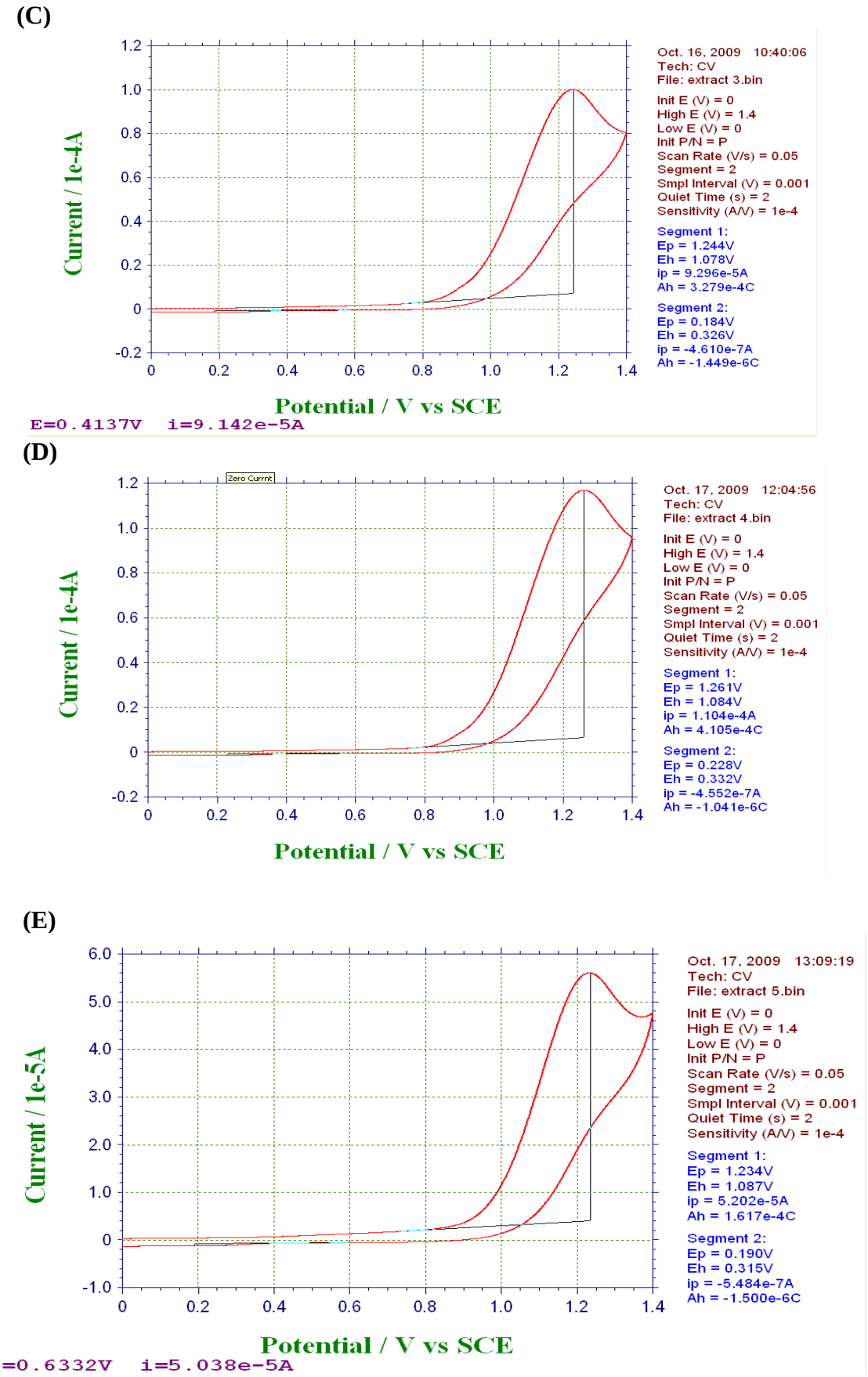


Figure 3. Cyclic voltammograms of chili extracts

As shown in the method to ensure the sensitivity that the peak observed is only for ascorbic acid in the sample extracts after getting its CV 10 µL of 20 µM ascorbic acid was added in voltammetric cell and CV was observed. The same phenomena were observed after the addition of 20 µL, 30 µL and 40 µL of ascorbic acid. The effect on the overlay of the Surkh, which was observed using the above process, is shown in Fig. 4. The result showed that the continuous addition of various volumes of standard 20 µM ascorbic acid, results in continuous increase in anodic peak current. This showed that the peaks observed for samples were only for ascorbic acid. The overall recovery if found to be 98.34±6.43 (36, 40-41). Recovery results are shown in Table 3 and overlay is plotted in Fig. 4.

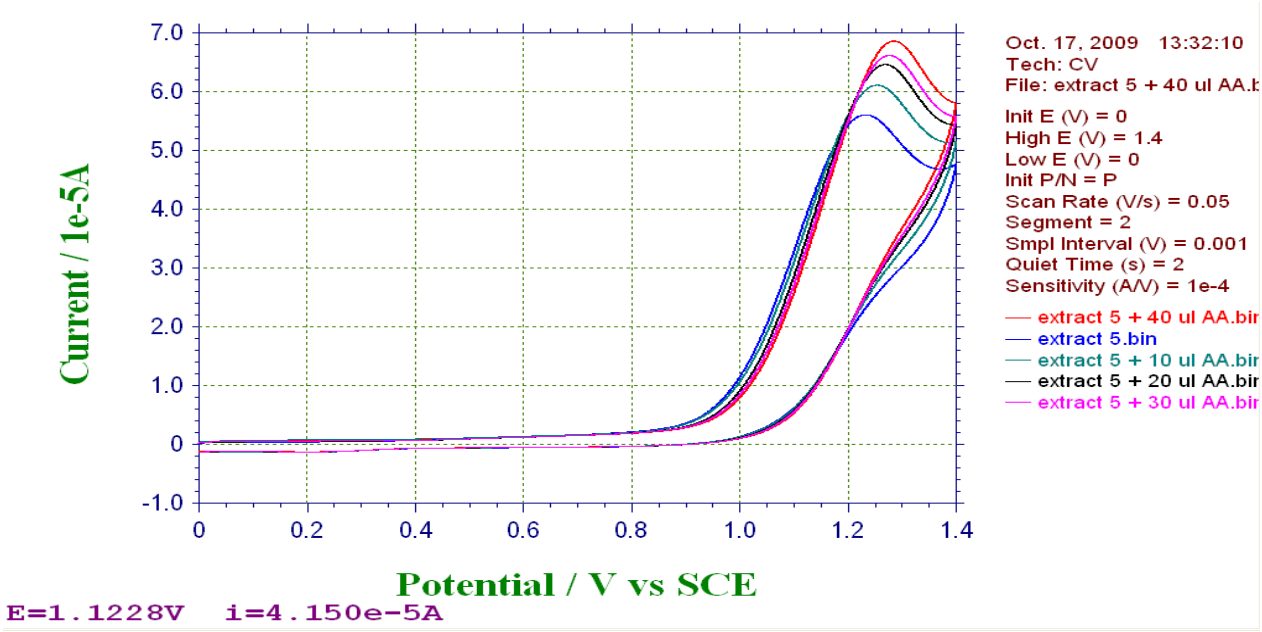


Figure 4. Overlay for recovery estimation

Table 2. AA Concentration and daily intake (mg/d) estimation from chilies

Sample ID	Daily intake (g/day)	Conc. (µM)	Conc. (mg/100 g)	Anodic peak current (µA)	DIA* (mg/d)	RDA (%)**		
						Children	Female	Male
1	12	120.25	2.11	75.37	0.25	1.69	0.34	0.28
2	12	118.16	2.08	74.51	0.25	1.66	0.33	0.28
3	12	175.05	3.08	92.96	0.37	2.46	0.49	0.41
4	12	230.63	4.06	110.4	0.49	3.25	0.65	0.54
5	08	45.66	0.8	52.02	0.06	0.43	0.09	0.07
Mean	11.2	137.95	2.42	81.05	0.28	1.90	0.38	0.32

*Values based on g/person/day ingestion.

**Values were calculated in recommendation of Health Canada [1].

Table 3. Accuracy estimation by % recovery

Extract values			**Recovery values**			
Conc. µM	**Anodic peak current** µA	**Addition** µL	**Sum** µM	**Conc.** µM	**Anodic peak current** µA	**Recovery*** %
45.66	52.02	10	55.66	59.34	60.41	106.6116
		20	65.66	65.67	62.16	100.0152
		30	75.66	71.66	64.12	94.71319
		40	85.66	78.84	65.43	92.03829

*****Mean and SD of recovery (%) were calculated 98.34457 and 6.431445, respectively.

4. CONCLUSION

In conclusion, external standard series calibration method is found to be effective, rapid and low cost for estimation of antioxidants by cyclic voltammeter equipped with glassy carbon electrode. The percentage recovery exposes the high accuracy of this method in chili matrices. Green chilies especially germane showed significant matrix effects of ascorbic acid content, representing relatively bigger size of chilies containing 4.06 mg/100 g of ascorbic acid. Ascorbic acid content among the selected chilies samples varied mainly in each variety. This result also shows ascorbic acid content is in inverse relationship with maturity stages and almost depended on color variations and cultivars. . More and more authors use successfully applied electrochemical techniques in AA determination. Our result is an addition to this information with data for determination of AA in food matrices.

ACKNOWLEDGMENTS
We are grateful to Hafiz A. Shahid and Noreen Fatima for providing the useful material and technical support throughout this research work. All expenses are bear by Department of Chemistry, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi–75300, Pakistan.

References

1. Dietary Reference Intake. Health Canada. http://www.hc-sc.gc.ca/fn-an/nutrition/reference/table/ref_vitam_tbl-eng.php. Accessed July 30, 2010. (2006).
2. C.V. Popa, A.F. Danet, S. Jipa, T. Zaharescu, *Rev. Chim*. 61 (2010) 11.
3. A.M. Pisoschi, M.C. Cheregi, A.F. Danet, *Molecules* 14 (2009) 480.
4. L. Anderson, M.V. Dibble, P.R. Turkki, H.S. Mitchell, H.J. Rynbergen, Nutrition in Health and Disease (17th edn). *Interamericana, Mexico*. (1987) 142.
5. S.J. Padayatty, R. Daruwala, Y. Wang, P. K. Eck, J. Song, W. S. Koh, M. Levine, In: E. Cadenas (Ed) & L. Packer (Ed). Handbook of Antioxidants Second Edition, *Marcel Dekker, New York* (2002).
6. M.T. Parviainen, in: A. Townsend (Ed.). *Encyclopedia of Analytical Science*. Academic Press, London 9 (1995).
7. J.J. Burns, In: L.S. Gilman (Ed) & A. Goodman (Ed). The Pharmacological Basis of Therapeutics Fifth Edition, Macmillan Publishing Co., Inc., New York (1975).
8. B. Halliwell, In: E. Cadenas (Ed) & L.Packer (Ed). Handbook of Antioxidants Second Edition, Marcel Dekker, New York (2002).

9. G. Dryhurst, K. Kadish, F. Scheller, R. Renneberg, Biological Electrochemistry, Academic Press, New York 1 (1982) 256.
10. E.E. Robinson, S.R.J. Maxwell, G.H.G. Thorpe, *Free Radical Research*. 26 (1997) 291.
11. M. Glenville, *Dragons Tale*. 18 (2008) 4.
12. E.B. Dawson, W.A. Harris, M.C. Teter, L.C. Powell, *Ferti Steril*. 58 (1992) 1034.
13. T.P.A. Devasagayam, J.C. Tilak, K.K. Boloor, S.S. Ketaki, S.G. Saroj, R.D. Lele, *JAPI*. 52 (2004) 794.
14. M. Materska, S. Piacente, A. Stochmal, C. Pizza, W. Oleszek, I. Perucka, *Phytochemistry* 63 (2003) 893.
15. A. Hossu, V. Magearu, *Roumanian Biotechnological Lett*. 9 (2004) 1497.
16. AOAC. Official methods of analysis of the Association of Official Analytical Chemists, 15th ed., *Association of Official Analytical Chemists,* Arlington VA. (1990) 1058.
17. J. Yang, C. Tong, N. Jie, G. Zhang, X. Ren, J. Hu, *Talanta*. 44 (1997) 855.
18. M. Hashmi, Assay of vitamins in pharmaceutical preparations. *Wiley Interscience*. Bristol (1973).
19. Y. Hernadez, M.G. Lobo, M. Gonzalez, *Food Chemistry* 96 (2006) 654.
20. B. Albuquerque, F.C. Lidon, E. Leitao, *Gen. Appl. Plant Physiology* 31 (2005) 247.
21. S.P. Arya, M. Mahajan, P. Jain, *Analytical Sciences* 14 (1998) 889.
22. M.A. Farajzadeh, S. Nagizadeh, *Am. J. Anal. Chem*. 58 (2003) 927.
23. M. Ozyurek, K. Guclu, B. Bektasoglu, R. Apak, *Analytica Chimica Acta*. 588 (2007) 88.
24. S.P. Arya, M. Mahajan, P. Jain, *Analytica Chimica Acta*. 417 (2000) 1.
25. L. Casella, M. Gullotti, A. Marchesini, M. Petrarulo, *Journal of Food Science* 54 (2006) 374.
26. A. T. Markas, Gilmartin, J. P. Hart, *Analyst*. 120 (1995) 1029.
27. J. Ballantine, A.D. Woolfson. *Journal of Pharmacy and Pharmacology* 32 (1980) 353.
28. R.C. Barthus, L.H. Mazo, R.J. Poppi, *Journal of Pharmaceutical and Biochemical Analysis* 38 (2005) 94.
29. F. Takahashi, J. Jin, *Annals of Bioanalytical Chemistry* 393 (2009) 1669.
30. D. Skoog, F. Holler, S. Crouch, *Principles of Instrumental Analysis* (2007).
31. K.C. Ock, K. Dae-Ok, S. Nancy, S. David, T.H. Jae, Y.L. Chang, *J. Sci. Food Agric*. 85 (2005) 1715.
32. H. Lund, M.M. Baizer (Eds.) in "Organic Electrochemistry: An Introduction and a Guide," 3rd edition, Marcel Dekker, New York (1991).
33. A.J. Fry in "Laboratory Techniques in Electroanalytical Chemistry," 2nd edition, (P.T. Kissinger and W.R. Heineman, Eds.) Marcel Dekker, New York (1996) 469.
34. C.P Andrieux, J.M. Savéant, *J. Electroanal. Chem. Interfacial Electrochem*. 93 (1978) 163.
35. C.H.V. Hoyle, J.H. Santos, *International Food Research Journal* 17 (2010) 937.
36. W. Okiei, M. Ogunlesi, L. Azeez, V. Obakachi, M. Osunsanmi, G. Nkenchor, *Int. J. Electrochem. Sci.* 4 (2009) 276.
37. M. Ogunlesi, W. Okiei, L. Azeez, V. Obakachi, M. Osunsanmi, Nkenchor, G, *Int. J. Electrochem. Sci*. 5 (2010) 105.
38. I. Perucka, M. Materska, *Acta Sci. Pol. Technol. Aliment.* 6 (2007) 67.
39. A. Marín, F. Ferreres, F.A. Tomás-Barberán, M.I. Gil, *J. Agr. Food Chem*. 52(12) (2004) 3861.
40. A.M. Pisoschi, A.F. Danet, S. Kalinowski, *Journal of Analytical Methods in Chemistry* 2008 (2009) 1.
41. A.M. Pisoschi, A. Pop, G.P. Negulescu, A. Pisoschi, *Molecules*. 16 (2011) 1349.