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Sensitive voltammetric determination of copper in three leafy vegetables using screen printed graphite electrode modified with thin mercury film

Ruuta Tekeraoi, Rajendra Prasad*

School of Applied Sciences, Fiji National University, Lautoka Campus, PO Box 5529, Lautoka, (FIJI)

E-mail: rajendra.prasad@fnu.ac.fj

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ABSTRACT

A thin mercury film electrode was fabricated by *in situ* electrodeposition over screen printed graphite electrode. A paraffin oil paste of 15% $HgCl_2$ in graphite (w/w) was used for the purpose. Copper(II) in acetic acid buffer showed two anodic striping peaks at -0.238 and -0.108 V *versus* Ag/AgCl, of which the peak current for the first peak showed linear concentration dependence. Electrode was used in the determination of copper in three leafy vegetables, *viz.* cabbage (*Brassica rapa* L. ssp. *Chinensis*), lettuce (*Lactuca sativa*) and tabua (*Amaranthus gangeticus*). The results of these determinations were found to be consistent with that obtained from standard AAS method. Cabbage and lettuce were found to contain 4.49 and 3.41 ppm of copper of the dry weight of the leaf, whereas tabua leaves contained signigicantly low amount, *i.e.* 0.31 ppm of the dry weight. Sensitivity and accuracy of the method were found to be comparable to that of standard atomic absorption spectrophotometric (AAS) determinations of copper. Anodic stripping voltammetry (ASV) is known for simultaneous determination of two or metal ions in small volume samples. Coupled with low cost and portability of the instrument, ASV could successfully be employed as a versatile method in the reliable determination of copper and other metals in food and vegetable samples, particularly in the small island countries.

KEYWORDS

Anodic stripping voltammetry;
Mercury film electrode;
Copper;
Electrodeposition;
Trace analysis.

INTRODUCTION

In human body, copper is the third most abundant trace metal after iron and zinc. Being part of about 30 enzymes[1,2], it is an essential micronutrient, responsible for normal functioning of cellular metabolism. Major copper-dependent metalloenzymes of clinical significance include tyrosinase (pigmentation of skin and hair)[3,4], lysyl oxidase (elastin and collagen cross-linking)[5,6], ascorbate oxidase (skeletal development)[7], monoamine oxidase (responsible for pili torti)[8,9], superoxide dismutase (free-radical detoxification)[10,11], dopamine beta-hydroxylase (catecholamine production)[12-14], peptidyl-glycine alpha-amidating mono-oxygenase (bioactivation of peptide hormones)[15], and cytochrome c oxidase (electron transport and possibly

responsible for hypothermia)[16,17].

Copper is relatively non-toxic to mammals, but intake in large quantities or in the event of impairment of copper metabolism, copper toxicity is associated with serious health problems. Its toxicity is generally attributed to the aquo complexed $[Cu(H_2O)_6]^{2+}$ copper(II) ion rather than to its organic or inorganic complex. Impairment of copper excretion metabolism leads to metal accumulation in kidneys, liver, bones and brain causing. The liver excretes excess copper into the bile for elimination from the body. Wilson's disease is a rare genetic disorder and is manifested in unexplained hepatitis, tremors, and personality changes. The copper poisoning generally arise from beverages from vending machines, copper or brass vessels, and sometimes food and water supplies[18]. Thus, the development of a simple, reliable, and low-cost techniques for copper determination is essential.

Several methods for the determination of copper are available, including inductively coupled plasma–mass spectrometry[19,20], neutron activation analysis[21], atomic absorption spectrometry[22,23], ion-selective electrodes[24-26], and emission spectrometry[27]. However, most of them require several time-consuming manipulation steps, sophisticated instruments and special training. It recent years voltammetry has commonly been used to analyse the trace metals in solutions. It refers to the measurement of current that result from the application of potential. There were different voltammetric techniques that were used and each was distinguished by the potential function that is applied to the working electrode to drive the reaction and by the material used as the working electrode. Anodic stripping voltammetry (ASV) is one of the quantitative voltammetric techniques that are very sensitive to detect the trace level of metal ions in the concentration range of 10^{-6}-10^{-12} M and is effective in the simultaneous determination of multi metal ions. Therefore the use of ASV is not only effective but also more advantageous and convenient[28,29]. It is commonly preferred due to the inexpensive and simple instrumentation.

The main focus of the study was to analyse the trace amounts of copper, cadmium and arsenic in the leaves of cabbage, lettuce and tabua, local leafy vegetables in Fiji, using the anodic stripping voltammetry technique. With the availability of low cost portable electrochemical analyzers, ASV technique offers operational advantage in trace level determinations. In this paper results from ASV determinations of total copper are compared with that of from AAS method.

EXPERIMENTAL

Materials and methods

GR grade copper sulfate, zinc sulfate, sodium acetate, mercuric chloride metal salts, graphite powder, paraffin oil, hydrochloric acid and nitric acid were purchased form Loba Chemie (India). Fresh vegetable samples were obtained from the local Suva vegetable market. ASV measurements were carried out using CHI1232A portable electrochemical analyzer. A three electrode assembly was employed for the purpose that consisted of Ag/AgCl reference electrode, platinum wire counter electrode and a 2 mm screen printed graphite working electrode. Mercury films were obtained by depositing approx. 5 mg paste of 15% $HgCl_2$ in graphite and cathodizing the electrode. Atomic absorbances were measured on a Perkin Elmer 3110 Atomic Absorption Spectrophotometer using oxyacetylene flame. Hollow cathode lamp was used as source and flame copper absorbances were measured at 324.8 nm[30].

All glasswares, sample containers, volumetric flasks and cells used were soaked overnight in 3.0 M nitric acid followed by thorough cleaning with distilled-deionised water.

Sample preparation

Vegetable leaves were first cleaned with plenty of tap water followed by deionised water and finally with 5% nitric acid solution. They were dried at 110 ^{0}C for 24 hrs in a hot air oven. Dry mass of leaves was recorded and they were subsequently grounded and an accurately weighed amount of each was burned in the furnace at temperature 400 - 450 ^{0}C for 1 hours. The mass of white residue was recorded after cooling and it was dissolved in a 10M hydrochloric acid. 3 – 5 mL of the acid was used to dissolve each ash sample. The acidic – ash solution was filtered into 10 mL volumetric flask and volume was made upto the volume using distilled-deionised water.

Preparation of the metal ions standard solution

The Copper sulphate ($CuSO_4.5H_2O$) salts was

used to prepare the standard copper solutions. The required concentration for standard solution was of the order 10^{-6} g/L. To prepare this concentration, a stock solution of concentration 1.0 x 10^{-3} mol/L was first prepared by dissolving 0.025 g of $CuSO_4.5H_2O$ in 100 mL of distilled–deionized water. The working standard solutions 1.0, 3.0, 5.0, 7.0 and 9.0 ppm were prepared by appropriate dilution of this stock solution.

Preparation of a supporting electrolyte

3.4 g of hydrous sodium acetate ($CH_3COONa.3H_2O$) was weighed and transferred to the 250 mL volumetric flask. To this was added 1.4 mL of glacial acetic acid (CH_3COOH) followed by 200 mL of distilled-deionised water. Once all salt has dissolved additional volume of distilled-deionised water was added to bring the volume to the 250 mL.

Preparation of the mercury film electrode

The working mercury thin films were obtained by an adoption of in situ electrodeposition in a thin paste[31,32]. A 150 mg of mercury chloride ($HgCl_2$) was ground to form a fine powder. To this added 1.0 g of graphite powder and mixture was further grounded to a fine homogenous powder. About 5mL of paraffin oil was added to the homogenous mixture to obtain a thick black paste that was stored in a clean vial for future use. Thin layer of this paste was applied to carbon electrode and it was cathodized in 1.5 M HCl solution at an electrode potential of -1.1 V *versus* Ag/AgCl for 300 seconds.

Copper ion analysis using ASV

2.0 mL of the supporting electrolyte (buffer solution) was added to the electrochemical cell. Into the same electrochemical cell, were added 0.2 mL of 1.0x10^{-6}M Cu^{2+} standard solution, 2.0 mL of the analyte leaf sample and 7.0 mL of water. The electro deposition of copper was carried out by applying -0.9 V potential to this electrode for 120 seconds, under stirred conditions. The stirrer was turned off and following a 30 s quiet time the potential was ramped between -0.9 to 0.0 V *versus* Ag/AgCl at sweep rate of 150 mV/s. Afterward, an additional 0.2 mL of Cu^{2+} standard solution was added carefully and the same steps of electrodeposition and stripping were carried out in succession as done earlier. The procedure was repeated with the incremental addition of the 0.2 mL of standard Cu^{2+} solution bringing total added volume to 0.6, 0.8 and 1.0 mL. All analyses were carried out in duplicate.

RESULTS AND DISCUSSION

The anodic stripping of standard copper sulfate solutions is shown in Figure 1. It is seen that under the study conditions, in acetate buffer copper exhibited two well resolved stripping peaks at -0.238 and -0.108 V *versus* Ag/AgCl, both of which showed concentration dependence. It is an important feature of ASV analysis that only the labile metal species that undergo deposition, yield analytical stripping peaks. In the standard solution Cu^{2+} is the main labile species of copper undergoing deposition, the two peaks must be arising due to stripping of copper into two different chemical forms. This could arise due to Cu/Cu^{2+}, $Cu/Cu^{2+}(CH_3COO^-)_x$ or Cu/Cu^+ processes. The half width of 0.039 and 0.042 V of these peaks respectively indicated their origin in 2 electron processes. Pinchin and Newham had also observed two stripping peaks, but their apparent *n* values were non-integral and the two stripping reactions were not resolved in their experiment[33], possibly due to presence of competing chloride ions in the acetate buffer to bind with stripped copper(II) ions.

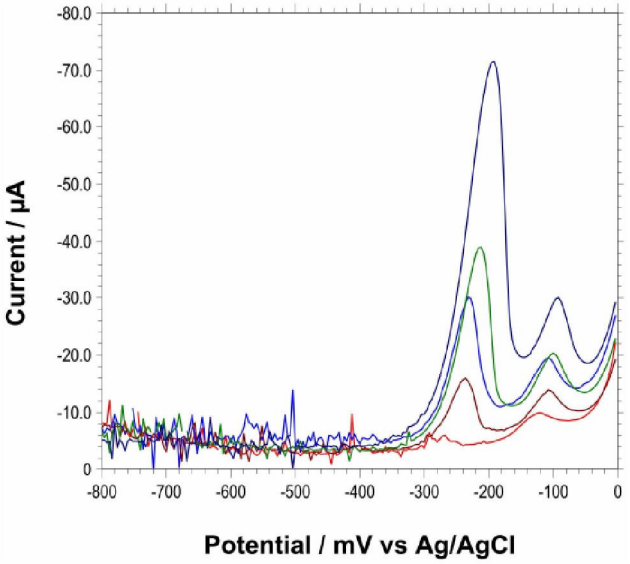

Figure 1 : Anodic stripping of standard copper solutions over mercury paste electrode (Concn. bottom to top: 1.0 x10^{-6}, 3.0 x10^{-6}, 5.0 x10^{-6}, 7.0 x10^{-6} and 9.0 x10^{-6} mol/L)

The peak current and peak area for the copper stripping peak at -0.238 V was found to exhibit linear dependence with solution Cu^{2+} concentration (TABLE

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1 and Figure 2) and hence it was used for the determination of copper in unknown solution.

TABLE 1 : Variation of ASV peak current and peak area with solution Cu^{2+} concentration

Concentration (mol/L)	Peak Current	Peak Area
1.0 x10^{-6}	2.1x10^{-6}	0.78x10^{-7}
3.0 x10^{-6}	10.3x10^{-6}	4.94x10^{-7}
5.0 x10^{-6}	20.9x10^{-6}	10.4x10^{-7}
7.0 x10^{-6}	29.9x10^{-6}	16.3x10^{-7}
9.0 x10^{-6}	55.7x10^{-6}	23.0x10^{-7}

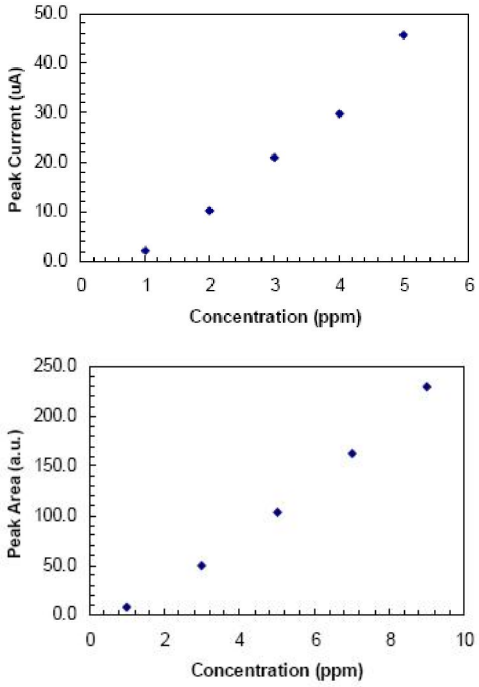

Figure 2 : Dependence of peak current and peak area over solution Cu^{2+} concentration.

Standard addition method was used in the determination of concentrations in the leaf solutions. In the ASV measurements, to the mixture of 2.0 mL supporting electrolyte, 2.0 mL of the analyte sample and 7.0 mL of water, addition of 0.2, 0.4, 0.6, 0.8, and 1.0 mL of 1.0x10^{-6} M standard copper solutions yielded 1.1, 2.2, 3.3, 4.3 and 5.3 ppm of the copper solutions respectively. Figure 3 shows stripping voltammograms of cabbage and lettuce samples with added 2.2 and 4.3 ppm copper(II) ions. Since the sample solution consisted of whole ash, other strippable metal ions[34], *viz.* Cd and Pb were also present in it. However copper peaks were observed distinctly apart, at more positive potentials and hence it was analyzed without intereference and without requiring any masking agent[35,36].

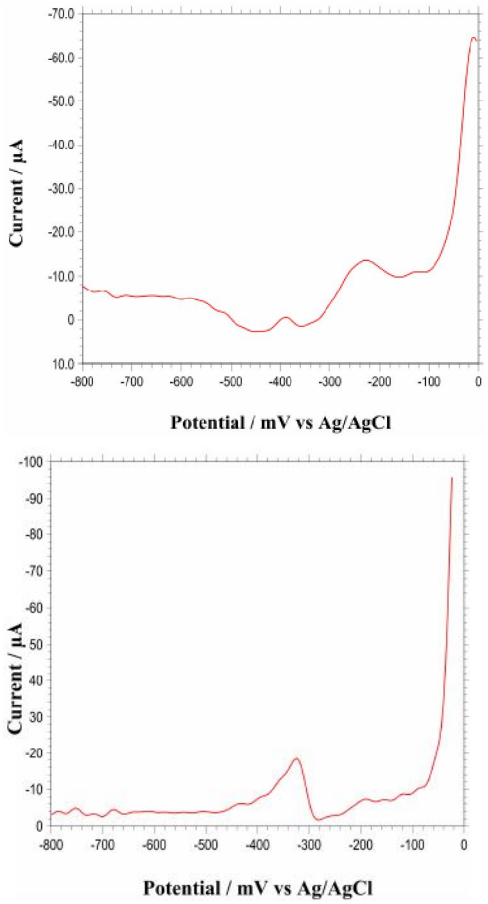

Figure 3 : ASV of tabua leaf (left) and lettuce leaf (right) sample with 2.2 and 4.3 ppm of added Cu^{2+} ions.

Although ASV is prone to give concentration of labile copper in the solution should there be speciations, it has given total copper concentrations in these determinations. The ashing method used in the sample preparation destroyed all other organic forms of copper and yielded inorganic copper (II) only in the acid extract. As the vegetable ashes were complex mixture of various metal oxides, the concentration axis intercepts of the standard addition graphs gave the concentration of the analyte that were obtained using regression analysis (TABLE 2-4 and Figure 4).

TABLE 2 : ASV measurement of tabua leaves

Added volume (mL)	Total Volume	Peak Height (x10^{-6} A)	Dilution factor	Corrected peak height (10^{-6} A)
0.2	11.2	1.9	1.05	2.0
0.4	11.4	4.2	1.04	4.4
0.6	11.6	4.7	1.02	4.8
0.8	11.8	7.1	1.00	7.1

TABLE 3 : ASV measurement of Lettuce leaves

Added volume (mL)	Total Volume	Peak Height ($x10^{-5}$A)	Dilution factor	Corrected peak height (10^{-6} A)
0.2	11.2	0.65	1.07	0.70
0.4	11.4	1.0	1.05	1.05
0.6	11.6	1.2	1.03	1.24
0.8	11.8	1.5	1.02	1.53
1.0	12.0	1.8	1.00	1.80

TABLE 4 : ASV measurement of cabbage leaves

Added volume (mL)	Added Concentration (mol/L)	Peak Height ($x10^{-5}$ A)	Dilution factor	Corrected Peak height (A)
0.2	11.2	0.54	1.07	0.58
0.4	11.4	0.65	1.05	0.68
0.6	11.6	0.75	1.03	0.78
1.0	11.8	0.98	1.02	1.00
1.2	12.0	1.19	1.00	1.19

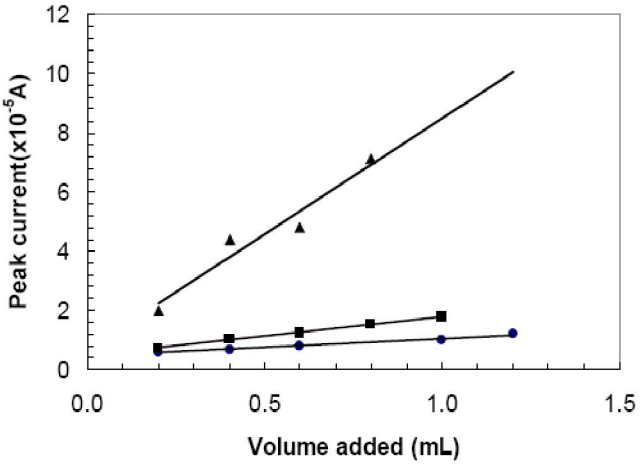

Figure 4 : Standard addition plots for Cu^{2+} determination in cabbage (–●–), lettuce (–■–) and tabua (–▲–) leaf samples by ASV.

TABLE 5 : AAS/ASV measurement of standard solutions and leave samples

	Standard solution					Leave samples		
						Tabua	Lettuce	Cabbage
Cu^{2+} Concentration by AAS (in ppm)	1.0	3.0	5.0	7.0	9.0	1.0	3.1	5.3
Absorbance	0.002	0.006	0.007	0.010	0.013	0.002	0.005	0.008
Cu^{2+} Concentration by ASV (in ppm)			---			0.31	3.41	4.49

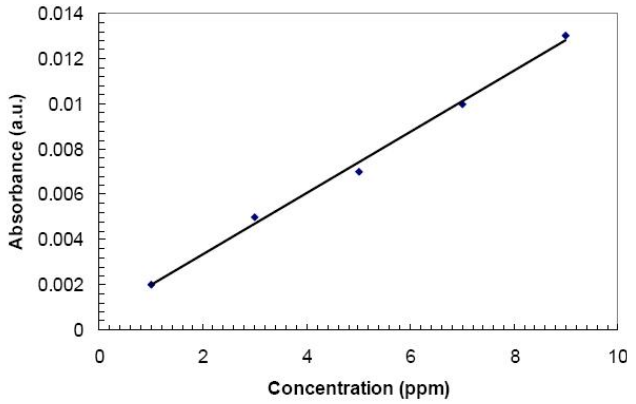

Figure 5 : Calibration plot for copper absorbance at 324.8 nm.

Althouth slopes of the standard addition plots varied from sample to sample, it has given consistency in concentration of copper in a given analyte sample in the replicate analysis. One major cause of this variation has been the variable thickness and consequent resistivity of the carbon paste over the electrode that was applied manually. However, as the time and conditions for the electrodeposition of mercury film were invariant, it is presumed that same thickness of the mercury film were deposited over the screen printed graphite electrode all the time. Variable thckness of the graphite layer lead to difference in conductances and hence the peak currents.

TABLE 6 : Concentrations of copper in different leave samples as determined by anodic stripping voltammetry.

Leaf sample	Mass of dried leaf sample (g)	Concentration of Cu in analyte (ppm)	Mass of Cu in 10 mL extract	Mass of Cu per g of dried leaf
Tabua	0.82	25.5	$2.55x10^{-7}$	$3.11 x10^{-7}$
Lettuce	0.32	109.0	$1.09 x10^{-6}$	$3.41 x10^{-6}$
Cabbage	0.53	238.0	$2.38 x10^{-6}$	$4.49 x10^{-6}$

The concentrations determined by ASV were also verified using standard AAS method (Figure 5). The amount of copper in different vegetable leaf samples is shown in TABLE 5. It is seen that the results obtained by both methods are in good agreement. Cabbage and lettuce were found to contain nearly comparable amounts of 4.49 and 3.41 ppm of copper relative to dry weight of the leaf, but it is present in signigicantly lower amount (0.31ppm) in tabua leaves.

CONCLUSIONS

The anodic stripping voltammetry in acetic acid buffer has been successfully used in the determination of

copper in three vegetable leaf samples. Despite of its simplicity it was highly sensitive and accurate and produced results that were comparable to standard AAS method, for the determination of copper. Also this method possesses inherent advantage of simultaneously determination of two or metals in a given solution. Given the cost and portability of the instrument, ASV could readily be used as a reliable substitute of AAS in the determination of copper and other metals in food and vegetable samples. Method could be useful in small island countries, limited with maintenance and supplies for AAS..

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