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ORIGINAL PAPER

Pulse anodic stripping voltammetric determination of copper with an amoxicillin–nafion modified glassy carbon electrode

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Abstract The analysis of Cu^{2+} by pulse anodic stripping voltammetry using a Nafion-modified glassy Carbon electrode incorporated with Amoxicillin is described. A significant increase in the voltammetric response was achieved at the modified electrode compared to a bare glassy carbon electrode. Cu^{2+} was accumulated in HAc–NaAc buffer (pH 3.6) at a potential of −0.7 V (vs. Ag/AgCl) for a certain time and then determined by pulse anodic stripping voltammetry. Parameters and conditions, such as the mass of Nafion, the concentration of Amoxicillin, the pH of medium, the accumulation potential, and the accumulation time were optimized. Under the optimum conditions, the calibration curve was linear in the range 8×10^{-10}–2×10^{-8} M with a correlation coefficient of 0.9998 and relative standard deviation 4.87% ($n = 5$). The detection limit was 1.3×10^{-10} M. A study of interfering substances was also performed and the analytical utility of the method was demonstrated by applying to various pharmaceutical products.

Keywords Modified electrode · Pulse anodic stripping voltammetry · Copper determination

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1 Introduction

Copper is an important element in biological systems. It is an essential micronutrient present in at least thirty enzymes. On the other hand, it is toxic above a certain concentration. Wilson's disease is a prototypical disease caused by copper toxicity, affecting first the liver and later the central nervous system, kidneys and eyes. The World Health Organization (WHO) and the European Water Quality Directive recommends that the concentration in drinking water does not to exceed 2 mg L^{-1}. Copper (II) is found in waste water from electroplating and other metal processing industries as well as water supply systems due to corrosion of copper in pipes. The concentration of copper is low in natural, environmental, biological and other real samples. A sensitive method for reliable detection of copper is of great interest [1–4].

Several methods for the determination of copper are available, such as inductively coupled plasma-mass spectrometry (ICP-MS), neutron activation analysis (NAA), atomic absorption spectrometry (AAS), ion-selective electrode (ISE) and emission spectrometry. However, most of them require several time consuming manipulation steps, sophisticated instruments and special training. A common method for the determination of copper is anodic stripping voltammetry (ASV). ASV is one of the most sensitive methods for the determination of traces of numerous ions because of its remarkably low detection limit. Other advantageous features of ASV include relatively low cost instrumentation and the capability for simultaneous multi-element determination [1, 5–10].

Chemically modified electrodes (CME) have been recognized as being one of the desirable alternatives. CME can easily and conveniently be fabricated and have some unique advantages in enhancing the selectivity and sensitivity due to the chemical nature and microstructure of the modified electrode surface. Therefore, CME are already widely used in analytical chemistry [11–14].

In recent years, various chemically modified electrodes have been used to determine copper. Many substances, such as Alga, *N*-phenylcinnamohydroxamix acid, *N*-acetyl-1-cysteine, 10-phenan-throline, Cysteine, Natural ionic polysaccharides, Sphagnum peat moss, 1,2-bis-methyl (2-aminocyclopentene-carbodithioate) ethane, Salicylideneamino-2-thiophenol, Diquinolyl-8,8′-disulfide, polypyrrole pyrocatechol violet, *N*-(2-hydroxybenzyl) chitosan, *p*-tert-Butylthiacalix(4)arene, Calix(4)arene, Hexacyanoferrate, 2-aminothiazole organofunctionalized silica, Tetraethyl thiuram disulfide and Tetraphenylporphyrin have been used to fabricate CME for the determination of copper [12–29].

The aim of this work is to develop a new glassy Carbon electrode modified with Amoxicillin–Nafion for the determination of copper by pulse stripping voltammetry. This study has led to the development a new and sensitive voltammetric method for the determination of copper and has been applied in various pharmaceutical products.

2 Experimental

2.1 Reagents

Nafion perfluorinated ion-exchange resin (5%) was purchased from Aldrich. Amoxicillin was from Aurobindo to India. A stock solution (1×10^{-2} mol L^{-1}) of Cu^{2+} was prepared using deionized water. A working solution for voltammetric investigations was prepared by dilution of the stock solution with deionized water. An HAc–NaAc buffer solution was used to control the pH. All other reagents were of analytical grade. All solutions were prepared using deionized water. All results were obtained using calibration curves.

2.2 Apparatus

A polarograghic analyser, model PRG-5 (Tacussel), with increasing amplitude pulses was used for differential detection of current and for superimposing constant amplitude pulses of negative or positive polarity and pulses of linearly increasing amplitude as the source of scanning voltage. A programmer model POLARMAX-78, and a recorder model ECOSRIPT (Tacussel) were also used. A RDGCE model ED165-14 was used as a working electrode. The reference electrode was Ag/AgCl model BJC. The solution was stirred with a rotating electrode and was kept in a thermostat at 25 °C. The diluter pipette model DIP-1 (Shimadzu), having 100 μL sample syringe and five continuously adjustable pipettes covering a volume range from 2 to 5,000 μL (model PIPTMAN P, GILSON), were used for preparation of the experimental solutions.

2.3 Preparation of modified glassy carbon electrode

Prior to each experiment, the glassy Carbon electrode was first polished, rinsed with deionized water and ultrasonicated successively in a 1:1 aqueous solution of Nitric acid and an Ethanol solution each for 2 min. It was then dried. A modified glassy carbon electrode was prepared by placing 5 μL 2% (v/v) Nafion–Ethanol solution which contained 0.21 mg mL^{-1} Amoxicillin onto the dry electrode with a micro syringe. The electrode was dried to evaporate the solvent and rinsed with deionized water.

2.4 Determination of Cu^{2+}

A 10 mL volume of a solution containing an appropriate concentration of Cu^{2+} and HAc–NaAc buffer (pH 3.6) was transferred into an electrochemical cell. The accumulation potential (−0.7 V) was applied to the modified electrode for a certain time. The potential was then scanned from −0.5 to 0.15 V by pulse stripping voltammetry using the auto-scan facility. The peak height was measured at 0.0 V.

3 Results and discussion

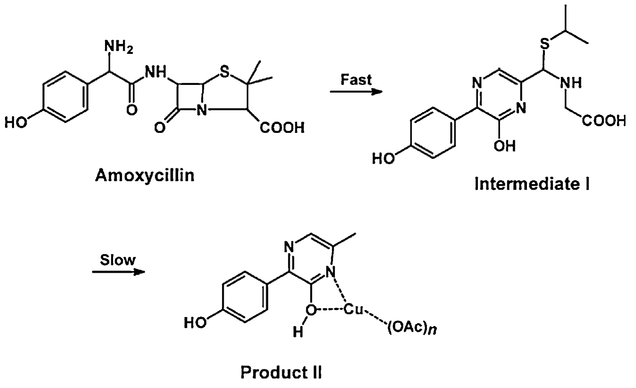
3.1 Voltammetric behavior

The anodic pulse stripping voltammograms using the procedure described above with a bare glassy electrode and an electrode modified with Amoxicillin–Nafion show that the peak potential shifted slightly from −0.05 V to 0.0 V and the sensitivity increased when the Amoxicillin was introduced to modify the coating (Fig. 1).

3.2 Effect of modified electrode composition

The effect of the Nafion concentration on the peak current was studied, and the results are shown in Fig. 2. The peak current reached its maximum when the concentration of Nafion, which contained 0.21 mg mL^{-1} Amoxicillin, was 2%. When the concentration of Nafion exceeded 2%, the film became thicker and the electrical resistance became higher. The function of Nafion was to fix Amoxicillin on the glassy carbon electrode, and also to serve as a barrier to prevent interferents from reaching the electrode surface.

The influence of the Amoxicillin concentration on the peak current was investigated (Fig. 3). The peak current reached a maximum when the concentration of Amoxicillin was 0.21 mg mL^{-1}. This is due to the formation of a complex between Amoxicillin and Cu (II) according to the equation [30]:



3.3 Effect of pH

The voltammetric response of Cu^{2+} in various supporting electrolytes, such as HCl, NaCl and HAc–NaAc buffer, was investigated. It was found that a well-defined and sensitive anodic stripping peak current appeared at 0.0 V when HAc-NaAc buffer was used as the electrolyte. The effect of different pH values (adjusted with HAc–NaAc buffer) on a peak current of 4×10^{-6} M were examined (see Fig. 4). The pH value 3.6 was selected as optimum.

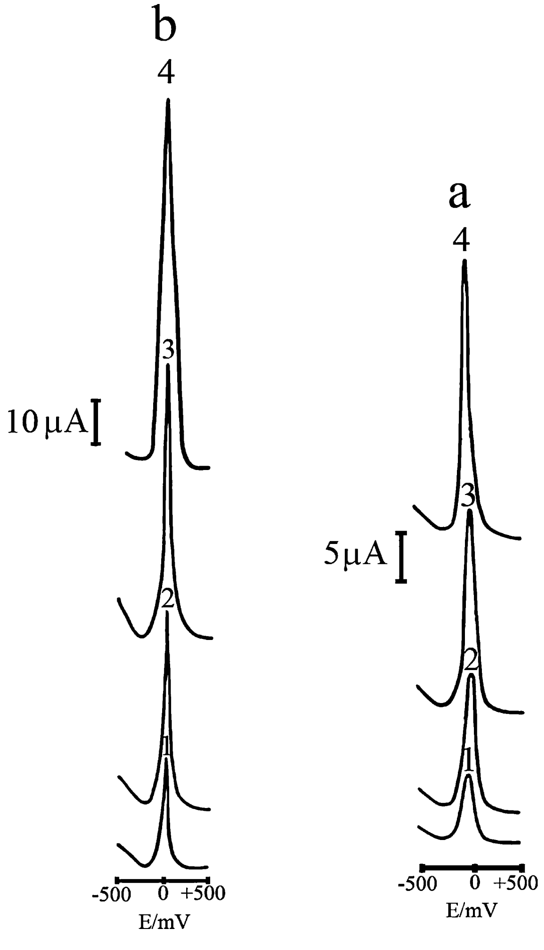


Fig. 1 Pulse anodic stripping voltammograms of 2×10^{-6} M (1), 4×10^{-6} M (2), 6×10^{-6} M (3) and 8×10^{-6} M (4) of Cu^{2+} at (**a**) bare glassy carbon electrode, (**b**) modified glassy carbon electrode (accumulation time 60 s)

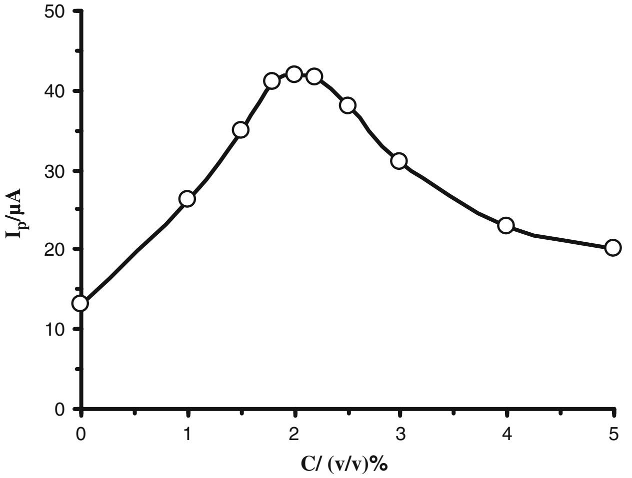


Fig. 2 Effect of the Nafion concentration on the anodic stripping peak current (Cu^{2+} concentration 4×10^{-6} M, Amoxicillin concentration 0.21 mg mL^{-1}, HAc-NaAc buffer pH 3.6, accumulation potential −0.7 V, accumulation time 60 s)

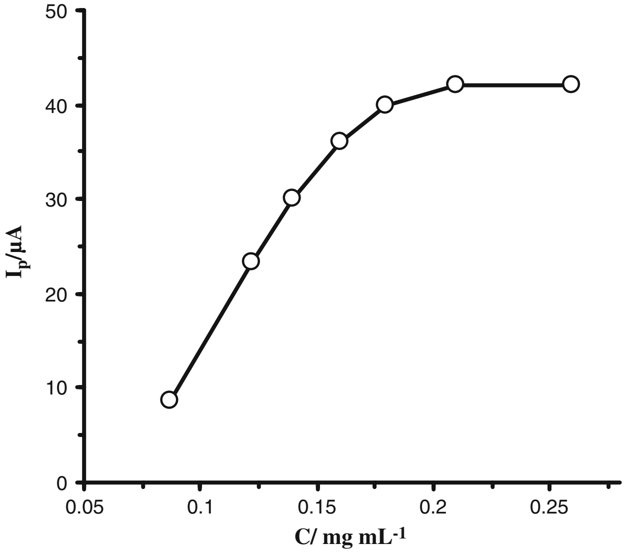


Fig. 3 Effect of the Amoxicillin concentration on the anodic stripping peak current (Cu^{2+} concentration 4×10^{-6} M, Nafion concentration 2% (*v*/*v*), HAc–NaAc buffer pH 3.6, accumulation potential −0.7 V, accumulation time 60 s)

3.4 Effect of accumulation potential

The dependence of the differential pulse anodic stripping peak current on the accumulation potential was examined. It was found that the maximum response for copper occurs

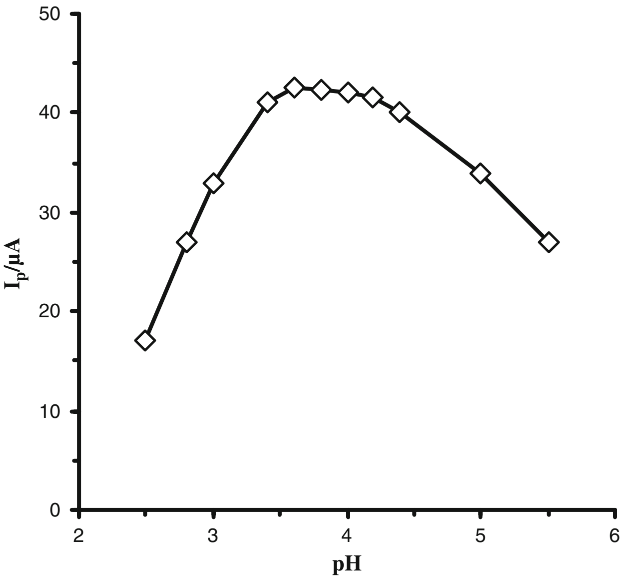


Fig. 4 Effect of the pH on the anodic stripping peak current (Cu^{2+} concentration 4 × 10^{-6} M, Nafion concentration 2% (*v*/*v*), Amoxicillin concentration 0.21 mg mL^{-1}, HAc-NaAc buffer pH 3.6, accumulation potential −0.7 V, accumulation time 60 s)

with potentials equal to, or more negative than, −0.7 V. Therefore, −0.7 V was chosen as the accumulation potential (Fig. 5).

3.5 Effect of accumulation time

The dependence of the peak current on the accumulation time for three different copper concentrations was studied. The peak current increases with increasing accumulation time. At copper concentrations of 4 × 10^{-6}, 4 × 10^{-7} and 4 × 10^{-8} M, the current is nearly linear from 10 to 100 s, 60 to 200 s, 90 to 300 s, respectively; the linear equation for each concentration is $i = 1.157 + 0.6865t$, $i = -0.0907 + 0.1009t$ and $i = 0.0266 + 0.029t$ respectively (i/μA, t/s).

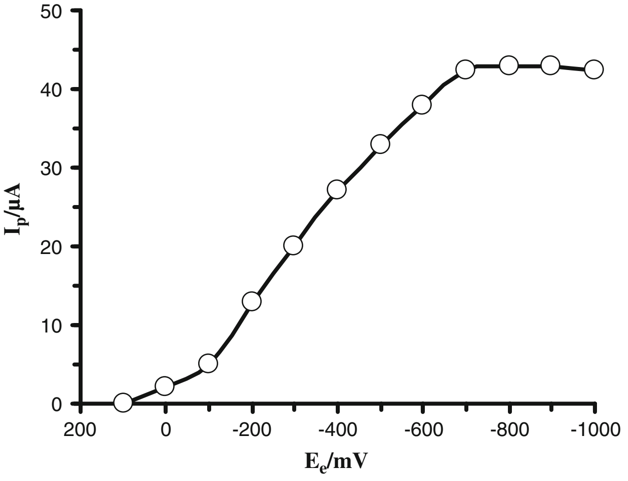


Fig. 5 Effect of the accumulation potential on the anodic stripping peak current (Cu^{2+} concentration 4 × 10^{-6} M, Nafion concentration 2% (*v*/*v*), Amoxicillin concentration 0.21 mg mL^{-1}, HAc-NaAc buffer pH 3.6, accumulation potential −0.7 V, accumulation time 60 s)

The factors affecting peak current were studied. It was found that the most suitable conditions for the modified electrode are as follows:

- Modified electrode composition: 2% (v/v) Nafion–Ethanol solution which contained 0.21 mg mL^{-1} Amoxicillin.
- Supporting electrolyte HAc–NaAc (pH 3.6).
- Stripping potential −700 mV.
- Pulse amplitude 100 mV.
- Deposition time was 60 s for the concentration range 4 × 10^{-7}–4 × 10^{-6} M, 120 s for the concentration range 4 × 10^{-8}–4 × 10^{-7} M and 180 s for the range 8 × 10^{-10}–2 × 10^{-8} M.
- Rotation speed 1,000 rpm.

3.6 Effect of Interference

Zn^{2+}, Fe^{3+}, As^{3+} and Pb^{2+} do not cause any interference in the concentration ratio 1:1 to copper. However Cd^{2+} and Hg^{2+} interfere, as shown in Table 1.

4 Analytical results

The peak current (I_p) was linear with copper concentration in the range 4 × 10^{-7}–4 × 10^{-6} M, 4 × 10^{-8}–4 × 10^{-7} M and 8 × 10^{-10}–2 × 10^{-8} M (See Figs. 6–8).

In this method a very low concentration (8 × 10^{-10} M) of copper (II) was determined on the modified electrode in the presence of HAc–NaAc with relative standard deviation not exceed ±4.87% (n = 5), (see Table 2). This

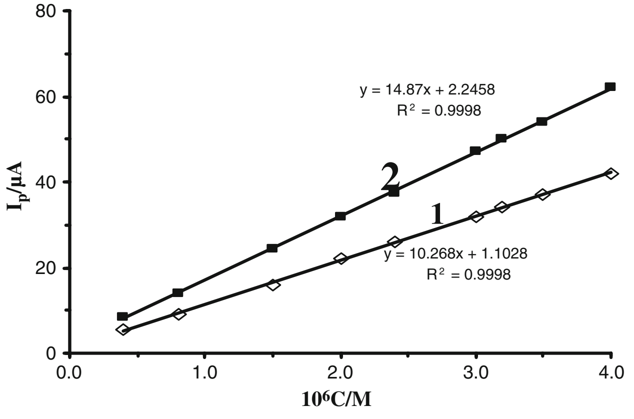


Fig. 6 Calibration plot of the anodic peak current versus the concentration of Cu(II) (Accumulation time: 1. 60, 2. 90 s)

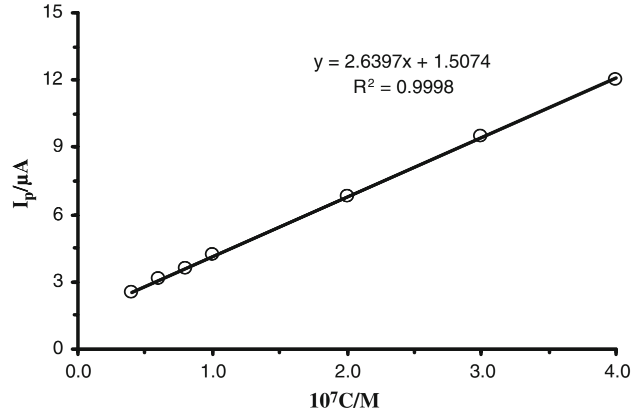


Fig. 7 Calibration plot of the anodic peak current versus the concentration of Cu(II) (Accumulation time 120 s)

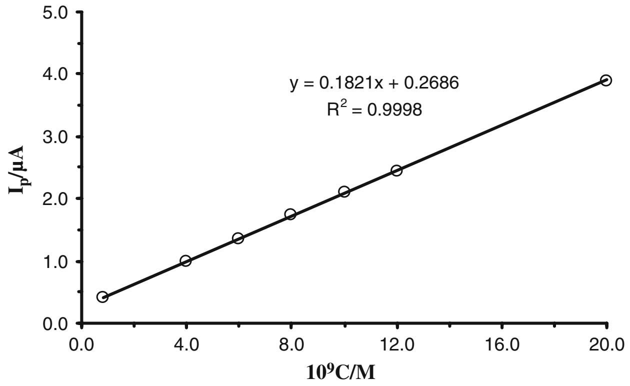


Fig. 8 Calibration plot of the anodic peak current versus the concentration of Cu(II) (Accumulation time 180 s)

method gives accurate and sensitive results compared with other work [1, 11, 31–33].

5 Application

The modified electrode was tested by the determination of Cu^{2+} in various pharmaceutical products as follows:

- *Centaraz* product Razi company-Syria, containing: 2 mg Cu, 18 mg Fe, 162 mg Ca, 125 mg P, 10 μg Si, 10 μg V, 100 mg Mg, 15 mg Zn, 2.5 mg Mn, 40 mg K, 25 μg Cr, 25 μg Mo, 25 μg Se, 5 μg Ni and 10 μg Sn.
- *Adavit Silver* product Adamco company-Syria, containing: 2 mg Cu, 120 mg Ca, 100 mg Mg, 105 mg Se, 94 μg Mo, 37.5 mg K and 22.5 mg Zn.
- *Supradyn* product MPI company-Syria, containing: 900 μg Cu, 120 mg Ca, 25 μg Cr, 8 mg Fe, 45 mg Mg, 1.8 mg Mn, 45 μg Mo, 126.3 mg P, 20.4 mg K, 55 μg Se and 8 mg Zn.
- *Ophtavite* product Medipharm company-Syria, containing: 2 mg Cu, 40 mg Zn and 40 μg Se.
- *Supravit Forte* product Amrit company-Syria, containing: 398.1 μg Cu, 2 mg Mn, 4.9 mg Zn, 100 mg Fe(II) and 20 mg Mg.
- *Cenvite* product Pharmasyr company-Syria, containing: 2 mg Cu, 2.5 mg Mn, 15 mg Zn, 40 mg K, 25 μg Cr, 25 μg Se, 25 μg Mo and 100 mg Mg.

Table 1 Interference of other cations (4×10^{-6} M) for determination of 4×10^{-6} M of copper

Cation	The peak current value after addition of other cations/μA	Recovery/%
Cu^{2+}	42	100
Zn^{2+}	42	100
Fe^{3+}	42	100
As^{3+}	42	100
Pb^{2+}	42	100
Cd^{2+}	22	52.4
Hg^{2+}	54	128.6

Twenty tablets (or capsules) were accurately weighed and powdered. A portion equivalent to the weight of one tablet (or capsule) was put into a platinum crucible and placed in a furnace at 600 °C for 4 h. When ashing was completed, the residue was dissolved in a small amount of concentrated HCl and then evaporated to dryness on a hot plate. The residue was dissolved again in HNO_3 (1:10). Any remaining residue was filtered off and the effluent was collected in a volumetric flask and diluted to the mark with the deionized water. This solution was then used for the analysis.

The results obtained by this method and compared with flame atomic absorption spectrometry [34] are summarized in Table 3. Agreement between the two methods is seen to be excellent.

Table 2 Determination of Cu(II) by pulse stripping voltammetry on Amoxicillin–Nafion modified glassy carbon electrode in presence of HAc–NaAc (Accumulation time 180 s)

$10^9 C_{Cu^{2+}}$/ M (taken)	$10^9 \bar{C}$/M (found)	10^9 SD/M	$10^9 \frac{SD}{\sqrt{n}}$/ M	10^9 ($\bar{x} \pm \frac{t.SD}{\sqrt{n}}$/M)	RSD/ %
0.80	0.78	0.038	0.017	0.78 ± 0.047	4.87
1.00	1.03	0.043	0.019	1.03 ± 0.053	4.17
2.00	2.02	0.078	0.035	2.02 ± 0.097	3.86
4.00	3.97	0.141	0.063	3.97 ± 0.175	3.55
6.00	6.00	0.200	0.089	6.00 ± 0.247	3.33
8.00	8.00	0.257	0.115	8.00 ± 0.319	3.21
10.00	10.03	0.301	0.135	10.03 ± 0.375	3.00
20.00	19.98	0.601	0.269	19.98 ± 0.748	3.01

$n = 5$

Table 3 Determination of Cu(II) in studied pharmaceuticals products by pulse stripping voltammetry on Amoxicillin–Nafion modified glassy carbon electrode in presence of HAc–NaAc

Commercial name	Voltammetric method			Flame atomic absorption spectrometry method [34]		
	$\bar{x}$	RSD/%	Recovery/%	$\bar{x}$	RSD/%	Recovery/%
Centaraz, tablet 2 mg/tablet of copper (Razi Laboratories—Aleppo, Syria)	2.06 mg/tablet	2.5	103.0	2.05 mg/tablet	2.7	102.5
Adavit Silver, tablet 2 mg/tablet of copper (Adamco co.—Damascus, Syria)	2.05 mg/tablet	2.4	102.5	2.05 mg/tablet	2.6	102.5
Supradyn, tablet 900 µg/tablet of copper (MPI—Damascus, Syria)	933 µg/tablet	2.6	103.7	933 µg/tablet	2.7	103.7
Ophtavite, tablet 2 mg/tablet of copper (Medipharm—Damascus, Syria)	2.07 mg/tablet	2.3	103.5	2.07 mg/tablet	2.7	103.5
Supravit Forte, capsule 398.1 µg/capsule of copper (Amrit—Aleppo, Syria)	398 µg/capsule	2.7	99.9	398 µg/capsule	2.7	99.9
Cenvite, tablet 2 mg/tablet of copper (Pharmasyr— Damascus, Syria)	2.04 mg/tablet	2.3	102.0	2.04 mg/tablet	2.6	102.0

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References

1. Dai X, Compton RG (2005) Electroanalysis 17:1835
2. Solomons NW (1995) J Am Coll Nutr 4:83
3. Forstner U, Wittmann GT (1981) Metal pollution in the aquatic environment. Springer, Berlin
4. European Commission (1998) Water Quality Directive 98/83/EU, Brussels
5. Steinberg IM, Lobnik A, Wolfbeis OS (2003) Sensor Actuators B 90:230
6. Mahendra N, Gangaiya P, Sotheeswaran S, Narayanaswamy R (2002) Sensors Actuators B 81:196
7. Reis BF, Knochen M, Pignalosa G, Cabrera N, Giglio J (2004) Talanta 64:1220
8. Nelms S (2003) Am Biotechnol Lab 21:3–9
9. Herzog G, Arrigan DWM (2003) Anal Chem 75:319
10. Honeychurch KC, Hawkins DM, Hart JP, Cowell DC (2002) Talanta 57:565
11. Li NB, Luo HQ, Chen GN (2004) Anal Sci 20:825
12. Torresday JG, Damall D, Wang J (1988) Anal Chem 60:72
13. Alemu H, Chandravanshi BS (1998) Anal Chim Acta 368:165
14. Zeng B, Ding X, Zhao F, Yang Y (2002) Anal Lett 35:2245
15. Prabbu SV, Baldwin RP, Kryger L (1987) Anal Chem 59:1074
16. Bai Y, Ruan X, Mo J, Xie Y (1998) Anal Chim Acta 373:39
17. Wang J, Taha Z, Naser N (1991) Talanta 38:81
18. Wang J, Naser N, Damall D, Torresday JG (1992) Electroanalysis 4:71
19. Safavi A, Pakniat M, Maleki N (1996) Anal Chim Acta 335:275
20. Sugawara K, Tanaka S, Taga M (1992) Fresenius J Anal Chem 342:65
21. Sugawara K, Tanaka S, Taga M (1991) Analyst 116:131
22. Nateghi MR, Bagheri A, Massoumi A, Kazemeini MH (1998) Synthetic Met 96:209
23. Kurauchi Y, Tsurumori E, Ohga K (1989) Bull Chem Soc Jpn 62:1341
24. Zheng H, Dong H, Yan Z, Wen L, Zhang S, Ye B (2006) Electroanalysis 18:2115
25. Canpolat E, Sar E, Coskun N, Cankurtaran H (2007) Electroanalysis 19:1109
26. Yu H, Zheng JB (2007) Chinese J Chem 25:503
27. Takeuchi RM, Santos AL, Padilha PM, Stradiotto NR (2007) Talanta 71:771
28. Gismera MJ, Hueso D, Procopio JR, Sevilla MT (2004) Anal Chim Acta 524:347
29. Zhang Y, Lu X, Zhu K, Wang Z, Kang J (2002) Anal Lett 35:369
30. Reiriz AG, Damiani PC, Olivieri AC (2007) Talanta 71:806
31. Rahman MA, Park DS, Park MS, Shin YB (2004) Electroanalysis 16:1366
32. Shiu KK, Song FY (1998) Electroanalysis 10:256
33. Sayen S, Gerardin C, Rodehuser L, Walcarius A (2003) Electroanalysis 15:422
34. Valfredo AL, Douglas GDS, Anaildes LDC, Debora DAS, Geisiane DSN, Adenilde SDP (2006) Microchem J 84:14