

Copper and Lead Ions Anodic Stripping Voltammetry Quantitative Determination in Healthy and Cancerous Female Serum Samples

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Abstract

Metals can contaminate the environment and increasingly affect ecological systems and public health worldwide. Cu(II) and Pb(II) ions play a significant part in increasing the cancer rate, since their amounts are higher in cancerous patients than in healthy individuals. Electrochemical methods have been extensively used for analyzing biological, beverages and food samples to estimate Cu(II) and Pb(II). The present study has employed LSASV for Cu(II) and Pb(II) determination in female patients (suffering from breast-cancer) cancerous blood serum samples. Optimized conditions for analysis have required: Gc, with an e_{acc} of -0.2 V and a t_{acc} of 400 s, as WE; 0.1 M AB (pH 5.1) as SE, at a stirring rate of 9 rpm and SR of 100 mV/s⁻¹; Ag/AgCl as RE; and a Pt wire as CE. Under the optimized conditions, the cost-effective procedure was successfully applied for Cu(II) and Pb(II) determination in cancerous human serum. LOD was in the range of ppb. The analysis indicated higher Cu(II) and Pb(II) levels in cancerous female patients than in the healthy ones. Therefore, a strictly controlled diet is recommended.

Keywords: biological sample; Cu(II); healthy and cancerous serums; LSASV; Pb(II); public health.

Introduction*

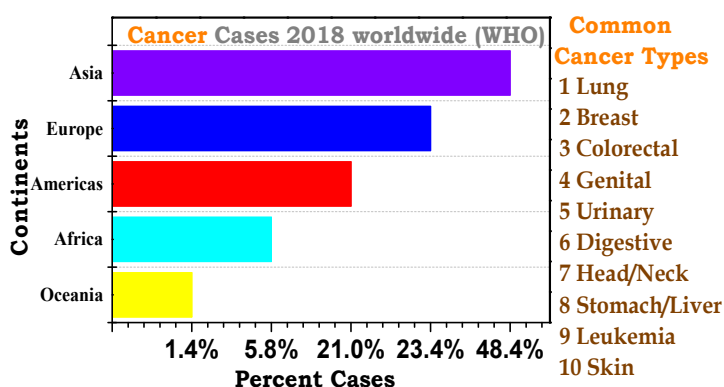
Cancer is well-recognized as a malignant neoplasm with wide-range of several diseases linked to an uncontrolled cell-growth process (Scheme 1). Most cancer cases have been linked to environmental and genetic problems. Tobacco (25-30%), nutrition and obesity (30-35%), various infectious disorders (15-20%), exposure to radiations (10%) and environmental pollution were identified as factors that play an important role in causing cancer [1]. HM also contribute to increase the cancer rate. HM are those with a density greater than $5 \times 10^3 \text{kg/m}^3$, such as Pb, Hg, Cd and As [2].

*The abbreviations and symbol definitions lists are in pages 56-57.

Through different pathogenetic links, HM stimulate the development of breast cancer [3]. Many epidemiological studies have stated that exposure to HM has toxic and carcinogenic effects on animals and humans. Prostate cancer mortality rate was found to be influenced by Cd, followed by Cr and Zn [4].

Cu(II) inside living organisms is able to initiate oxidative-stress in the cells, by ROS directly formed through a Fenton like reaction [5-6]. An increased Cu(II) exposure may also lead to the decline in glutathione levels [7-10].

Ionic Pb(II) is toxic to humans, since it inhibits and replaces Ca(II) ion absorption mechanism, interacting with the proteins through sulfhydryl and amide groups mutations, which result in health decline. Pb(II) can exchange essential metal cations at the binding sites, inhibiting the enzyme activity, which disrupts the cations transportation [11].



Scheme 1. Cancer common types and distribution.

HM toxicity through contaminated air, H₂O and food is a worldwide threat to human health. HM quantification has been reported by ICP-MS, GS/MS, GC, HPLC, AAS and FTIR techniques [12].

Electrochemical methods have numerous advantages, due to their low-cost, high selectivity and fast response. SV is a precise, significant and selective electroanalytical method [13] capable of simultaneously detecting and estimating different metals in various matrices. SV is a highly sensitive and fast electrochemical method that has been widely used for HM detection [14]. It requires none or minimum sample alterations, thus dramatically reducing the chances of contamination by reagents, or of losses by their adsorption onto containers [15].

[16] reported Cu(II), Cd(II), Pb(II) and Zn(II) simultaneous SV detection. In this electrochemical measurement, HM ions are oxidized or reduced on the WE surface, by losing or gaining electrons. The chemical signals are transformed into electrical signals, and the data from HM ions C with i are obtained [17].

Electrochemical methods are more fast, portable and cost-effective than traditional methods, and they can be adapted to different on-site inspections [18]. Previous researches have measured Cu(II) and Zn(II) in blood serum, for comparing their ions

C in the lungs, breasts, gastrointestinal tract and reproductive system of cancerous patients with multiple disorders [19].

[20] reported AAS analysis for Cu, Mg, Zn and Se ions C determination in the blood serum and colon tissue samples from twenty patients with colorectal cancer. [21] used AAS to determine Zn, Se and Cu ions levels in the serum samples of forty male and female patients with bladder cancer, and twenty healthy controls. [22-24] determined Cu ions in the whole blood, by using DPASV and ASV. [25] described Sb(III) and As(III) trace metals separation in biological and environmental samples, using MHPLC packed with CNF-APDC. [26] analyzed Ni, Mn, Co, As, Zn, Cd, Pb, Fe, Cr and Cu C by ICP-MS, for assessing gluten-free foods samples sold and consumed by celiac patients in Saudi Arabia. [27] determined Ni, Mn, Cu, Fe and Zn C in the snack samples sold at Turkey, by flame AAS.

In this study, Cu(II) and Pb(II) C in cancerous serums of female patients suffering from breast-cancer, and in healthy ones, were determined. To the best of the authors knowledge, this is the first report on optimal parameters for Cu(II) and Pb(II) quantitative determination in cancerous and healthy female patient serums by ASV.

Materials and methods

Reagents and chemicals

All reagents and chemicals used were of analytical grade. AB of different pH values were prepared with NaOH and CH₃COOH (both from Merck, Germany). 1000 ppm each of Cu(II) and Pb(II) standard solutions (Merck, Germany) were used in electroanalytical experiments. All solutions were prepared in deionized H₂O.

Apparatus

ASV experiments were carried out by CHI-760D electrochemical workstation (CH Instrument Inc. Austin, USA). Electrochemical experiments were carried out in stirred solutions with: GC WE, Ag/AgCl RE saturated with 0.1 M KCl, and a Pt wire CE, respectively. During accumulation, a magnetic stirrer was used to provide the electrons feasible transport towards the WE. Pure N gas (99.99%) (National Gas Company, Karachi, Pakistan) was used to achieve the inert atmospheric conditions. To avoid materials adherence, the WE was polished with α -Al₂O₃ powder (0.3 μ), prior to each run.

Blood samples collection and preparation

Cancerous female patients' (from 30 to 75 years (n = 50)) blood serum samples were collected from different hospitals of Karachi City, Sindh Province, Pakistan, after the permission from health administrative authorities. A questionnaire was used to record their medical and personal history. The blood serum samples of healthy subjects (n = 50) were also collected. Clean vacutainers were used for the blood samples collection and storage in laboratory. The healthy and cancerous blood samples digestion was performed in 5 mL HNO₃ (65%) and H₂O₂ (1-2 mL). The mixture was refluxed and, then, the digested samples were left to cool down at room T. Afterwards, they were analyzed.

Electrochemical measurements

For the electrochemical experiments, an AB was used as SE. After adjusting the baseline, the digested cancerous blood sample (5 μL) was transferred into a 5 mL volumetric flask. Then, 1 μL of each standard Cu(II) and Pb(II) solutions was added, and the volume was made up with deionized H_2O . Four solutions with different volumes of Cu(II) and Pb(II) standard solutions (2-5 μL) were prepared. For the optimized experimental conditions, the solutions were purged with pure N_2 , for 5 min. Then, the samples were run on CHI-760D electrochemical workstation, to determine Cu(II) and Pb(II) C, by using the standard addition method.

Results and discussion

This work focused on Cu(II) and Pb(II) determination in healthy and cancerous female patient's serums by electroanalysis, using ASV. ASV i_p was remarkably dependent on the variables, i.e., t_{acc} , e_{acc} , stirring rate (SE) and C. So, as to obtain good reproducible analyses, these parameters had to be optimized. Therefore, herein it is explained in detail how to obtain optimized parameters, and the electroanalysis is described based on them.

Electrode selection

The WE is an important tool to identify the analyte. Therefore, it requires a careful selection. Gc, Au and Pt wire electrodes (Fig. 1) were used, and the best analyte current (μA) response for Gc electrode was found.

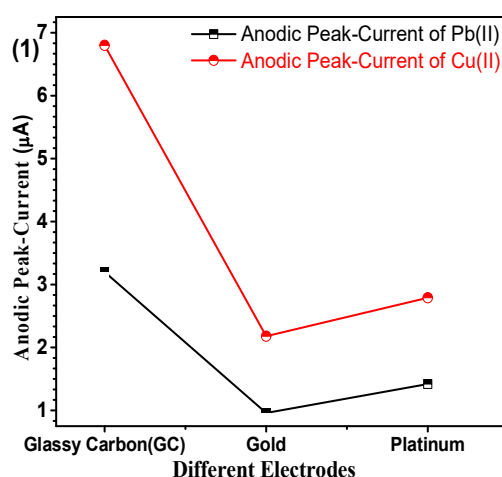


Figure 1: Plot of Pb(II) and Cu (II) μA responses vs Gc, Au and Pt electrodes.

μA optimization at different AB pH values

[24] reported that AB gave a good μA response for analytes. Generally, the analyte solubility may depend on the BS pH. Therefore, Pb(II) and Cu (II) were used with different AB pH ranges (from 4.0 to 5.4), to identify the better μA response for the analytes. AB with pH 5.1 showed the best reproducible μA response (Fig. 2).

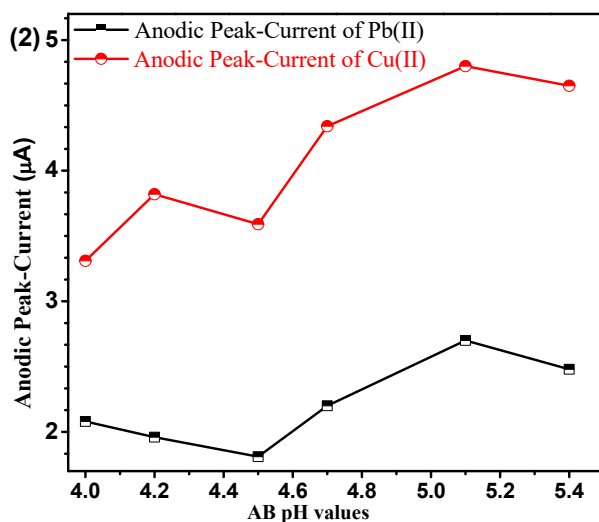


Figure 2: Plot of Cu(II) and Pb(II) μA responses in AB with various pH values, at 30 ± 1 °C.

C of SE

Cu(II) and Pb(II) μA responses using Gc as WE were measured at variable AB C (from 0.01 to 0.3 M), and at pH of 5.1. A non-linear trend was observed (Fig. 3) for the analytes μA responses vs. AB C, which allowed to select optimum C of SE (0.1 M) for further experiments.

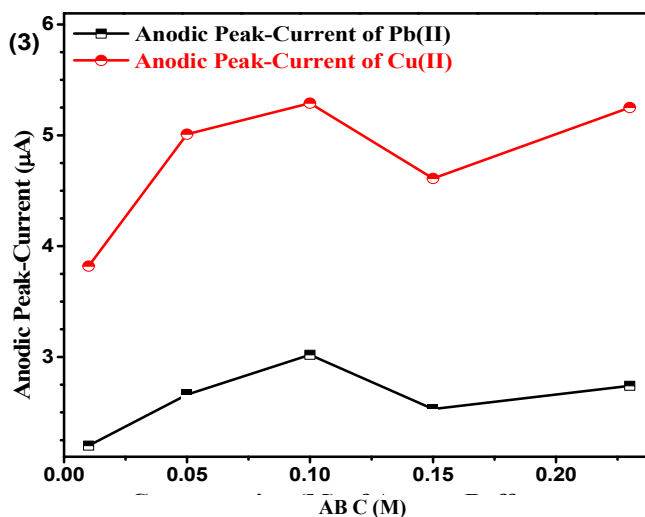


Figure 3: Cu(II) and Pb(II) μA responses using Gc as WE vs different AB (pH 5.1) C (M), at 30 ± 1 °C.

Estimation of Cu(II) and Pb(II) t_{acc} on Gc electrode

In SV analysis, analytes t_{acc} is an imperative aspect, due to LOD. After prolonging t_{acc} from 15 to 800 s, Cu(II) and Pb(II) μA gradually increased (Fig. 4).

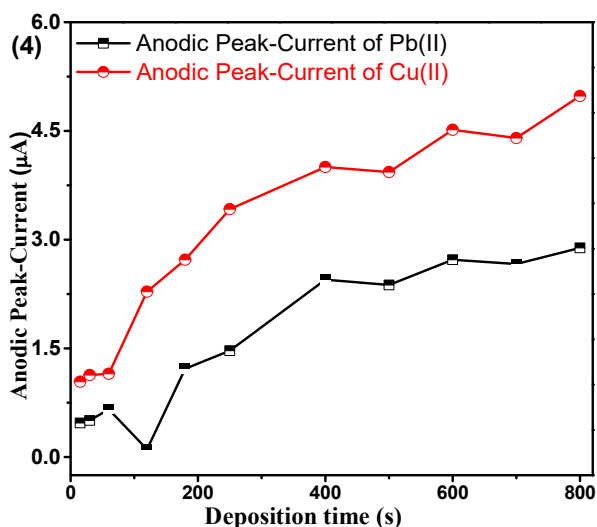


Figure 4: Cu(II) and Pb(II) μA responses vs. their t_{acc} on the WE (Gc) in AB (SE) with pH of 5.1, at 30 ± 1 °C.

After 400 s, the gradual increase in μA stopped for Cu(II) and Pb(II). Therefore, the 400 s t_{acc} was chosen for further analyses [15, 16].

e_{acc} optimization

SV investigated the analytes t_{acc} , in order to check their sensitivity and LOD (Fig. 5).

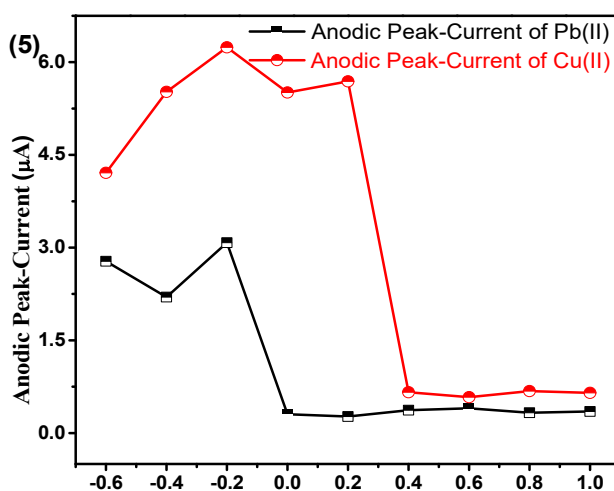


Figure 5: Cu(II) and Pb(II) μA responses vs e_{acc} (V) with Gc as WE and AB (pH = 5.1) as SE, at 30 ± 1 °C.

In order to determine Cu(II) and Pb(II) e_{acc} , experiments were performed in the potential range from -0.6 to 1.0 V, which resulted in the highest sensitivity at -0.2 V, due to the maximum μA response.

SR effect on Cu(II) and Pb(II) μA and V

Fig. 6(a) and 6(b) show the SR (0-500 mV/s^{-1}) effect on Cu(II) and Pb(II) V and μA , respectively.

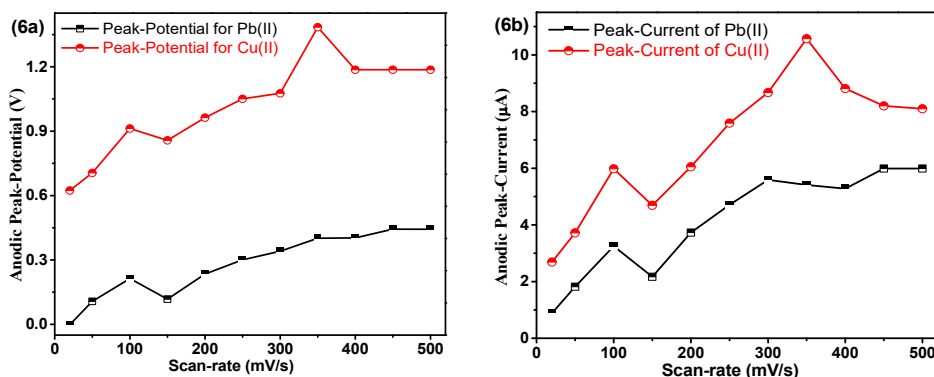


Figure 6: Plots of Cu(II) and Pb(II) (a) V vs. SR and (b) μA vs. SR, with Gc as WE and AB (pH = 5.1) as SE.

When the SR was increased from 0 to 500 mV/s^{-1} , μA increased. Both μA and V decreased slightly after 100 mV/s^{-1} . Therefore, SR of 100 mV/s^{-1} was used for all further experiments.

Stirring rate effect

Since stirring promotes the mass-transfer process, the accumulation efficiency requires optimization. The results (Fig. 7) showed enhanced μA with an increased stirring rate. Therefore, 9 rpm stirring rate was selected and used for further analyses [16].

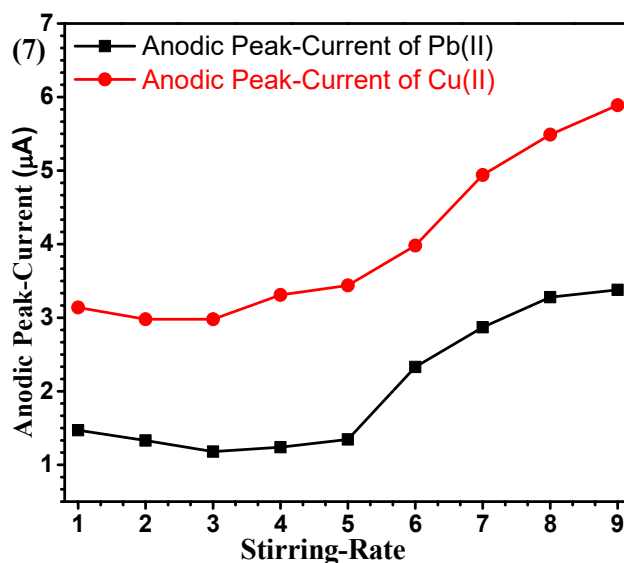
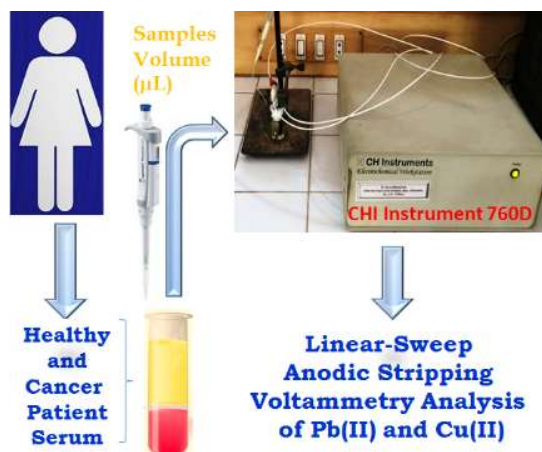


Figure 7: Plot of V vs. stirring rate, with Gc as WE and AB (pH = 5.1) as SE.

Voltammograms comparison between healthy and cancerous serums in AB as SE and metals ions standard solutions

Scheme 2 shows the general procedure for females' serums analyses.



Scheme 2: General procedure for healthy and cancerous females' serums analyses.

There were no voltammogram peaks without analytes, with AB (SE) pH of 5.1, Gc as WE and Pt wire as CE, which indicates the BS electrochemically inert nature (Fig. 8). The voltammograms comparison (Fig. 8) between healthy (H₁-H₁₁) and cancerous blood serums, in AB and Pb(II) and Cu(II) standard solutions, showed no peaks for the first, and smaller peaks for the second than those for the third one. (Fig. 9).

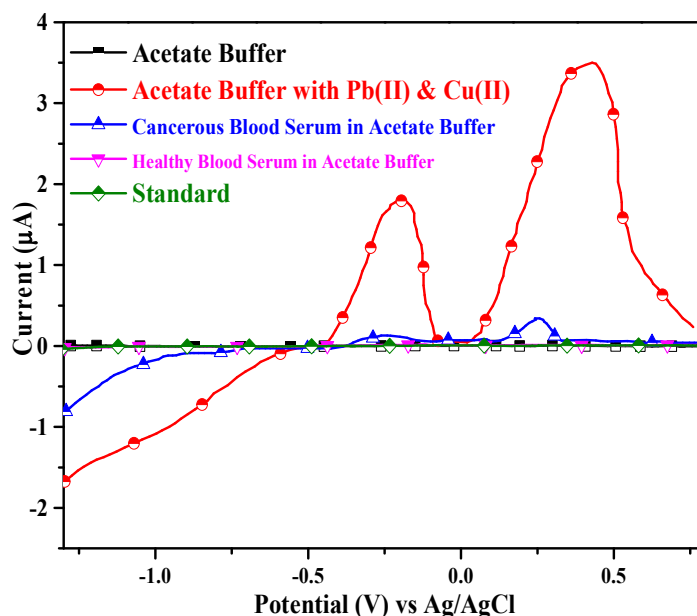


Figure 8: Voltammograms comparison between: AB (Ph = 5.1); AB with Pb(II) and Cu(II); healthy and cancerous serums in AB; Pb(II) and Cu(II) standard solutions. Gc was the WE.

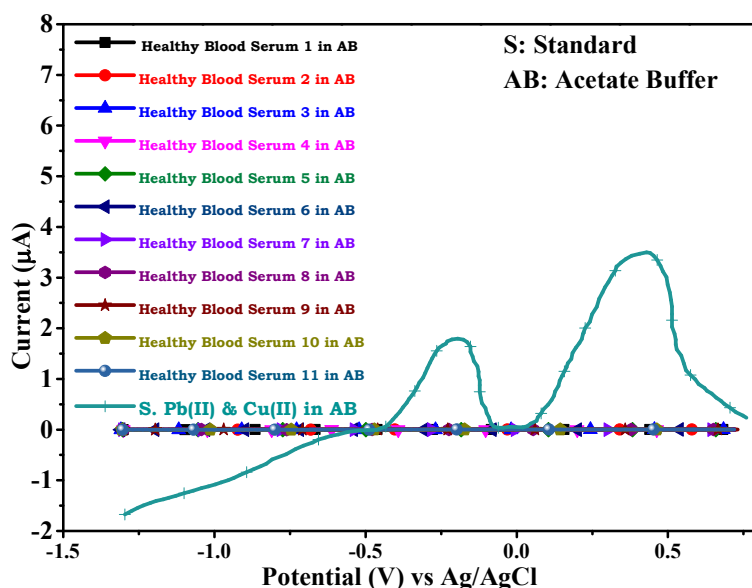


Figure 9: Voltammograms of healthy blood serums (H1-H11) in AB (SE) with pH of 5.1, and of Cu(II) and Pb(II) standard solutions in AB, using Gc as WE.

Cancerous serum electrochemical determination

For data comparison, five voltammograms of six serum samples each of the female patients suffering from breast cancer are shown in Figs. 10-14.

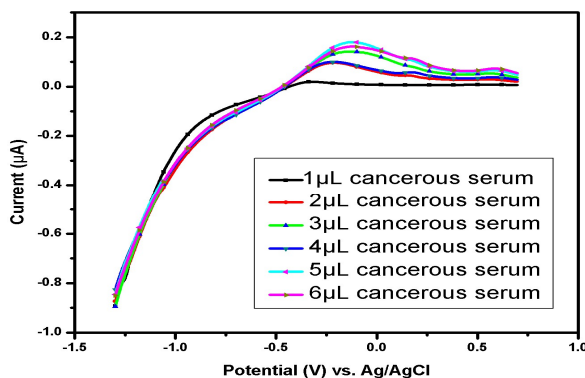


Figure 10: Voltammogram of cancerous serums containing Cu(II) and Pb(II) in the AB (SE) of pH 5.1, at 30 ± 1 °C, using Gc as WE.

Fig. 10 shows the voltammogram of 6 different samples of one cancerous serum of a female patient (-1.5 from 1 V). Broad (0.05-0.5 V) and small peaks (0.003-0.029 V) for Pb(II) and Cu(II), respectively, were observed. All the samples showed weak peaks at 1 μL , but, as the analyte volume increased from 2 to 5 μL , a significant μA response was observed, thereby confirming Pb(II) and Cu(II) strong presence. Similar observations were found in the serum samples of other female (Fig. 11), while for samples in Figs. 13 and 14, Cu(II) μA responses were higher than those from the

samples in Figs. 10, 11 and 12. This indicates Cu(II) higher and variable C among different female cancer patients, which, hence, required a strictly controlled diet.

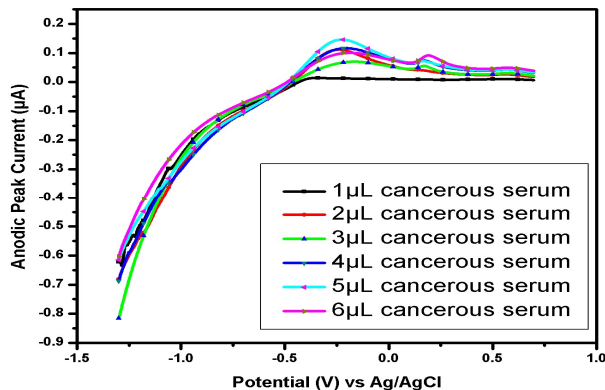


Figure 11: Voltammogram of cancerous serum with Cu(II) and Pb(II) in the AB (SE) of pH 5.1, at 30 ± 1 °C, using Gc as WE.

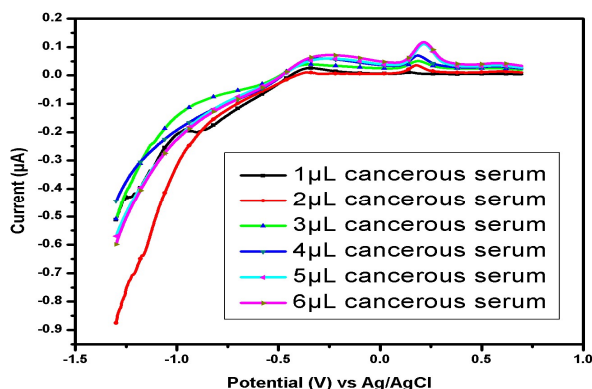


Figure 12: Voltammogram of cancerous serum containing Cu(II) and Pb(II) in the AB (SE) of pH 5.1, at 30 ± 1 °C, using Gc as WE.

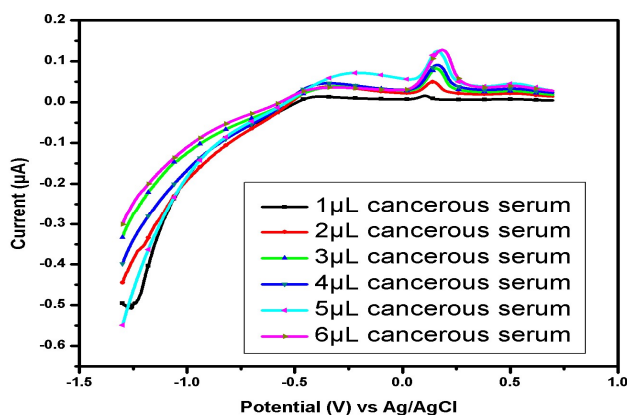


Figure 13: Voltammogram of cancerous serum containing Cu(II) and Pb(II) in the AB (SE) of pH 5.1, at 30 ± 1 °C, using Gc as WE.

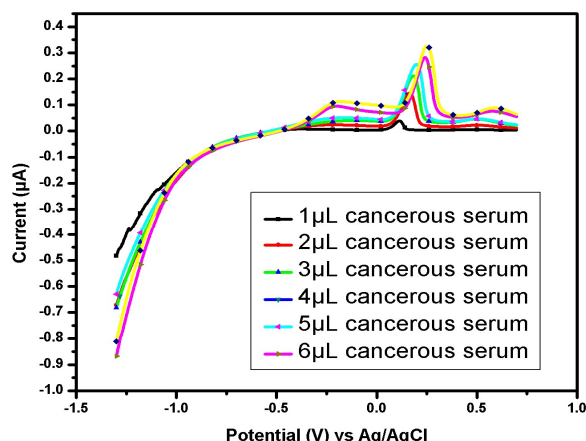


Figure 14: Voltammogram of cancerous serum containing Cu(II) and Pb(II) in the AB (SE) of pH 5.1, at 30±1 °C, using Gc as WE.

Estimation of Cu(II) and Pb(II) C

ASV was used for Pb(II) and Cu(II) determination in the healthy and cancerous serum (10 each) samples of females suffering from breast-cancer. The results are given in Tables 1 and 2.

Table 1: Cu(II) and Pb(II) C in healthy and cancerous serum samples (10 each).

Cu(II) measured C in cancerous serum (µg/L) Mean±SD	Cu(II) measured C in healthy serum (µg/L) Mean±SD	Pb(II) measured C in cancerous serum (µg/L) Mean±SD	Pb(II) measured C in healthy serum (µg/L) Mean±SD
3.1775±0.0002	1.9065±0.0001	2.4864±0.0020	1.2432±0.0020

The µA was measured by the standard addition method. I graphs versus standard solutions volumes were plotted. Pb(II) and Cu(II) C were determined by the calibration curves in the cancerous and healthy serums (Table 1). The method sensitivity is given in Table 2.

Table 2: Regression statistics and sensitivity of the proposed method.

Metal	Regression equation	Slope (S)	R ₂	LOD	LOD
Cu(II)	y=10.08x+2.542	10.080	0.903	133.900	44.100
Pd(II)	y=33.7x+3.549	33.700	0.957	39.000	13.000

Conclusion

In the current study, Cu⁺² and Pb⁺² C were examined in female serums (healthy and cancerous) by ASV, via standard addition method and calibration curve. The reported method was cost effective and required less time of analysis. The analyses indicated that female cancer patients had higher C of Cu(II) and Pb(II) than those of the healthy ones. Therefore, we recommend that female cancer patients practice strict diet-control, and monitor their intake of trace metals via different sources of food.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Authors' contributions

Kousar Yasmeen Hafsa: conceived and designed the analysis; collected the data; inserted data or analysis tools; performed the analysis; wrote the paper; other contributions: collected blood samples from patients. **Atya Hassan:** other contributions. **Haji Muhammad:** conceived and designed the analysis; inserted data or analysis tools; wrote the paper; other contributions: corresponding author. **Sobia Tahir:** wrote the paper; other contributions. **Iftikhar Ahmed Tahiri:** inserted data or analysis tools; other contributions. **Atta-ur-Rehman:** collected the data; inserted data or analysis tools; performed the analysis.

Abbreviations

AAS: atomic absorption spectrophotometry

AB: acetate buffer

Ag/AgCl: silver/silver chloride

APDC: ammonium pyrroinedithiocarbamate

ASV: adsorptive stripping voltammetry

BS: buffer solution

C: concentration

CE: counter electrode

CH₃COOH: acetic acid

CNF: carbon nanofibers

DPASV: differential pulse anodic stripping voltammetry

e_{acc}: accumulation potential

FTIR: Fourier transform infrared spectroscopy

Gc: glassy-carbon

GC: gas chromatography

GS: gas spectroscopy

H₂O₂: hydrogen peroxide

HM: heavy metals

HNO₃: nitric acid

HPLC: high performance liquid chromatography,

ICP-MS: inductively coupled plasma mass spectroscopy

i_p: response current

KCl: potassium chloride

LOD: limit of detection

LSASV: linear-sweep anodic stripping voltammetry

MHPLC: microcolumn high-performance liquid chromatography

NaOH: sodium hydroxide

ppb: parts per billion

r²: coefficient of determination

RE: reference electrode

ROS: reactive oxygen species

SE: supporting electrolyte

SR: scan-rate

SV: stripping voltammetry

T: temperature

t_{acc}: deposition time

WE: working electrode

Symbols definition

α-Al₂O₃: alpha alumina

μA: anodic peak current

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