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Diagnostic Assay of Toxic Zinc in an *Ex Vivo* Cell Using Voltammetry

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Voltammetric detection of the toxic Zn ion was investigated using a fluorine-doped graphite pencil electrode (FPE). It is notable from the study that pencils were used as reference and working electrodes. In all the experiments, a clean seawater electrolyte solution was used to yield good results. The analytical working range was attained to 10 μgL^{-1}. The optimized voltammetric condition was examined to maximize the effect of the detection of trace Zn. The developed sensor was applied to an earthworm's tissue cell. It was found that the methods can be applicable to *in vivo* fluid or agriculture soil and plant science.

***Key words*:** Diagnosis, Toxic zinc, *Ex vivo*, Cell, Voltammetry

INTRODUCTION

Among *in vivo* cell systems, the Zn(II) ion is essential in the human body for the implementation of multiple functions in the organisms. However can be toxic harmful at *in vivo* high concentration (Zheng and Hu, 2007). Zn is also related to the Wilson's disease, Alzheimer's, Blackfoot and cutaneous diseases (Santon *et al*., 2003; Miller *et al*., 2006; Horng and Lin, 1997; Fabris *et al*., 2006). Here *in vivo* detections are demand for there control and diagnostics. Recently many detection methods have been adopted such as Chelating Fluorescent Protein Chimeras, high-performance liquid chromatography, online ICP MS detection, thermospray flame furnace atomic, low-level g-ray spectrometry, two-column ion exchange, slurry sampling electrothermal vaporization, atomic absorption spectrometric, isotope dilution thermal ionization, FAAS high performance nebulizer, flow injection diode array, ICP-AES, and X-ray fluorescence spectrometry (Evers *et al*., 2007; Capitan-Vallvey *et al*., 2002; Zheng and Hu, 2007; Nascentes *et al*., 2004; Kohler *et al*., 2000; Pohi and Prusisz, 2007; Lu and Jiang, 2001; Zaporozhets *et al*., 1999; Ayoub *et al*., 2002; Zareba *et al*., 2005; Azubel *et al*., 1999; Bianchi *et al*., 2007; Marcó *et al*., 2001). Some these methods are complicated, expensive, and unusable for *in vivo* diagnosis, but simple and inexpensive voltammetric methods have been developed for these purpose such as film electrodes, bismuth poly film electrodes, hanging mercury drop electrodes and stationary mercury electrodes (Kefala *et al*., 2003; Wu *et al*., 2008; Nedeltcheva *et al*., 2005; Locatelli and Torsi, 2000). There are still toxic and complicated modification techniques. However in this study, a non-toxic and sensitive fluorine modification method was used to make a working electrode. It was also low-cost as it used pencils for the auxiliary, reference and working electrodes. Moreover, clean seawater was used for the electrolyte solution to yield good results in all the experiments. This developed prove was applied to an earthworm's cell and good results were obtained.

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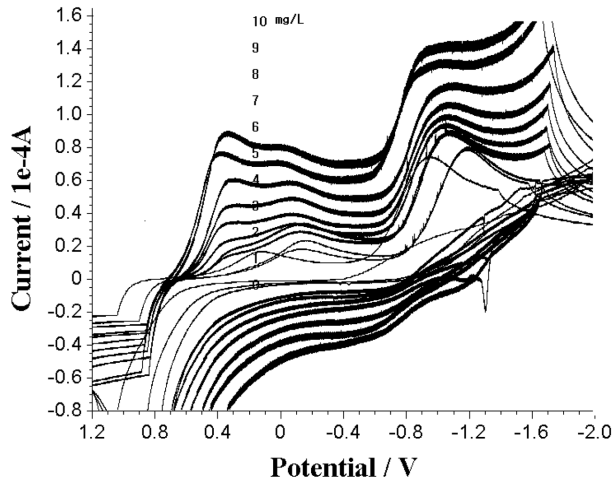
MATERIALS AND METHODS

Systems, reagents, electrode preparation, and procedure. The voltammetric systems were carried out using the Bioelectronics-2 circuit, which was constructed by the authors' institution. Its second version was fabricated to a computerized handheld voltammetric system with a 2.4 V potential window, a pico A measuring current, a rechargeable battery or an external power supply, and a USB interface with a PC. The instrument's size was that of a typical cellular phone.. The FPE fluorine coating was performed using conc-HF solution, a 10-cycle scan with a 1.0 V initial potential, a 1.0 V switching potential, and a 0.5 Vs^{-1} scan rate. Other two pencils each served as the reference and auxiliary electrodes. The supporting electrolyte was prepared with clean seawater. All the other reagents were of analytical grade. The voltammetry was carried out at an open circuit.. The initial analytical SW conditions were used with a 0.06 V amplitude, 25 Hz frequency, 170-sec deposition time,

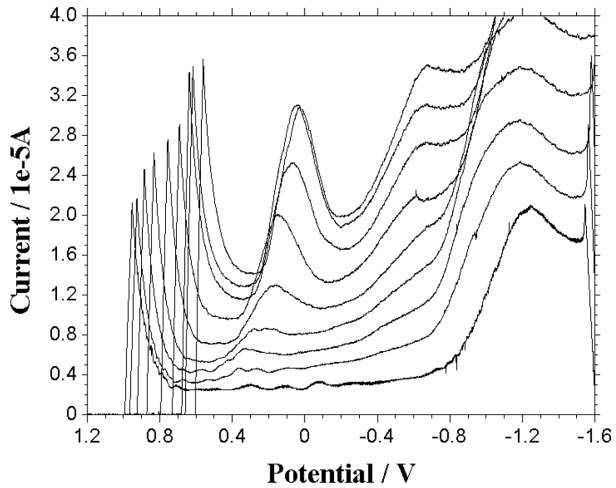
and −1.3 V initial potential. All the experiments were performed at room temperature and without oxygen removal. Under these conditions, cyclic peak potentials were examined.

RESULTS AND DISCUSSION

First, a high concentration of Zn was investigated with cyclic voltammetry using FPE in clean seawater electrolyte. Pencils served as auxiliary and reference electrodes. Fig. 1(A) shows the CV results that ranged from 1 to 10 mg/*l* variations. The peak current reached 12.427×10^{-5} A in a 1 mg/*l* spike via an oxidation scan for the 0.3 V peak potential. It increased quickly to 34.15×10^{-5} A, then dropped slightly at 5 mg/*l* before it increased again and approached

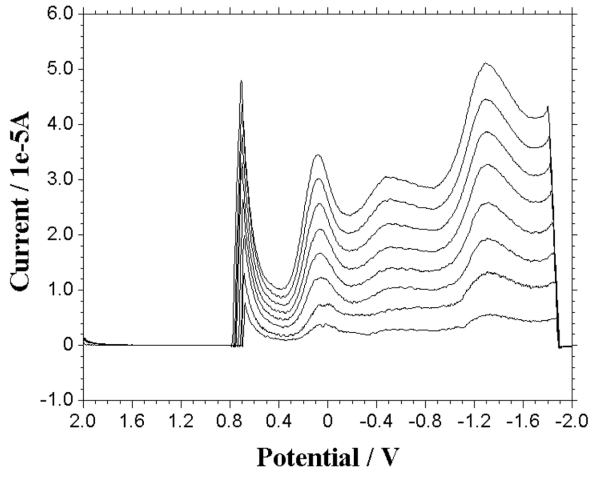


(A)

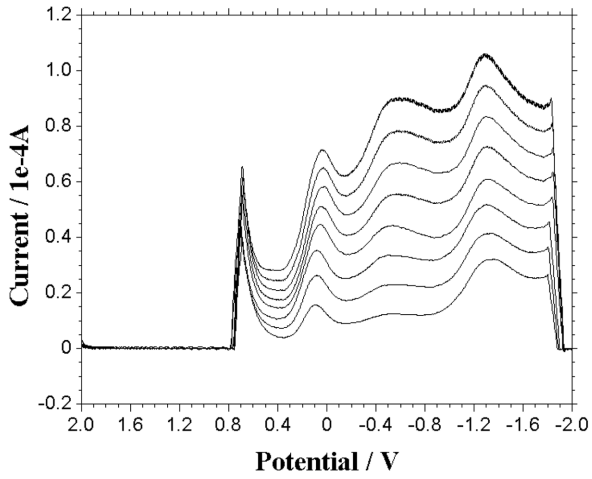


(B)

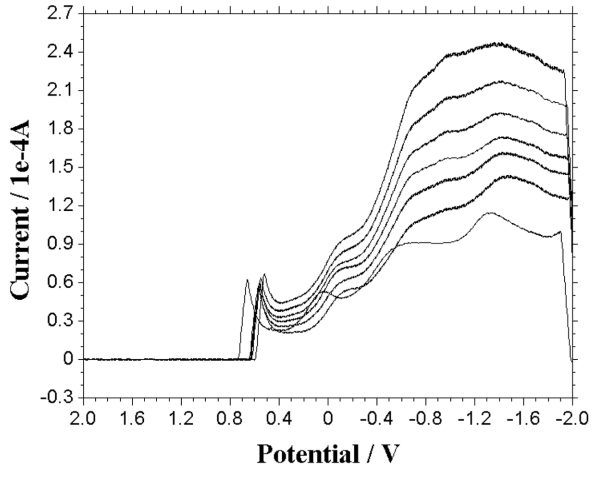
Fig. 1. (A) Cyclic voltammetry for the concentration effects of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/*l* of Zn(II) in seawater electrolyte, -2.0V initial potential, 1.2 V switching potential, and 0.5 mV/sec scan rate. (B) Examination using an anodic SW range for 1, 2, 3, 4, 5, 6, 7, and 8 mg/*l* of Zn(II) with optimum conditions.



(A)



(B)



(C)

Fig. 2. (A) The SW amplitude variations for 0.005, 0.01, 0.015, 0.02, 0.025, 0.03, 0.035, and 0.04 V. (B) The SW frequency variations of 5, 10, 15, 20, 25, 30, 35, and 40 Hz. (C) The SW accumulation time variations of 0, 30, 60, 90, 120, 150, 180, 210, and 240 sec for the 80 mg/*l* Zn(II) ion spike in the clean seawater electrolyte.

84.28×1^{-6} A. The reduction peak was not reached, though. Under these conditions, the anodic and cathodic SW conditions were examined. Fig. 1(B) shows the cathodic results in the high concentration. The anodic SW results were not obtained. The cathodic values of the 1, 2, 3, 4, 5, 6, 7, and 8 mg/*l* of Zn(II) were spiked. The bottom curve shows the result of the blank electrolyte and the simple. At 1 mg/*l*, it started to have a small peak, and the peak increased linearly. It became sharp, and the peak currents increased from 1.079×10^{-6} A to 13.93×10^{-6} A, the linear equation was y = 4.2946x – 7.6953, and the statistic relative standard deviation was R2 = 0.9936. These results can be used for SW optimization.

SW optimizations of FPE. The optimum SW conditions were examined to obtain sensitive Zn detection results. First, the SW amplitude variations were studied by maintaining the 80 mg/*l* Zn spike in the 10ml seawater electrolyte. Fig. 2(A) shows the results that ranged from 0.005 V to 0.04 V amplitude. They increased from 2.109 to 71.680×10^{-6} A without a decline, due to which 0.04 V amplitude was chosen as the optimum condition. Under this condition, Fig. 2(B) shows the variations of the SW frequency of 5, 10, 15, 20, 25, 30, 35, and 40 Hz. The width of the curve became narrow, and the peak current increased from 9.594 to 25.080×10^{-6} A. The maximum current was attained at 40 Hz. Under there conditions, Fig. 2(C) shows the SW accumulation time variation. At 30 sec, the peak current was 1.013×10^{-5} A, and it increased to 1.236×10^{-5} A at a 60-sec accumulation time. After that, no current bigger than 1.236×10^{-5} A was obtained. Therefore, 60 sec was chosen as the optimum condition. Under this condition, the analytical working range and application were performed using the seawater electrolyte.

Analytical working range and statistics. Under the optimum SW conditions, the analytical working range was examined using cathodic. Fig. 3(A) shows the chronoamperometric results according to the time variations spiking. Every 30 seconds, the 100-mg/*l* concentration was added. The linear curve increased from 100 to 700 mg/*l*, and the linear equation was y = 0.0959x – 5.1363, with R^2 = 0.9859. The peak currents can be used for mili ranges. Under these conditions, Fig. 3(B) shows the SW milli ranges of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg/*l*. The peak current was attained at 5.846×10^{-6} A, and the linear equation was y = 24.512x + 2.6231, with R^2 = 0.976. can be used for low ranges, but Fig. 3(C) shows the results of more sensitive micro ranges. The concentrations from 10 μg/*l* to 80 μg/*l* were spiked in order. The small peak of 3.162×10^{-7} A was attained at 10 μg/*l*. The bigger currents started to be obtained, and reached 7.513×10^{-7} A. These results can be used for trace detection. Moreover, seawater electrolyte can help yield good results. Under these condi-

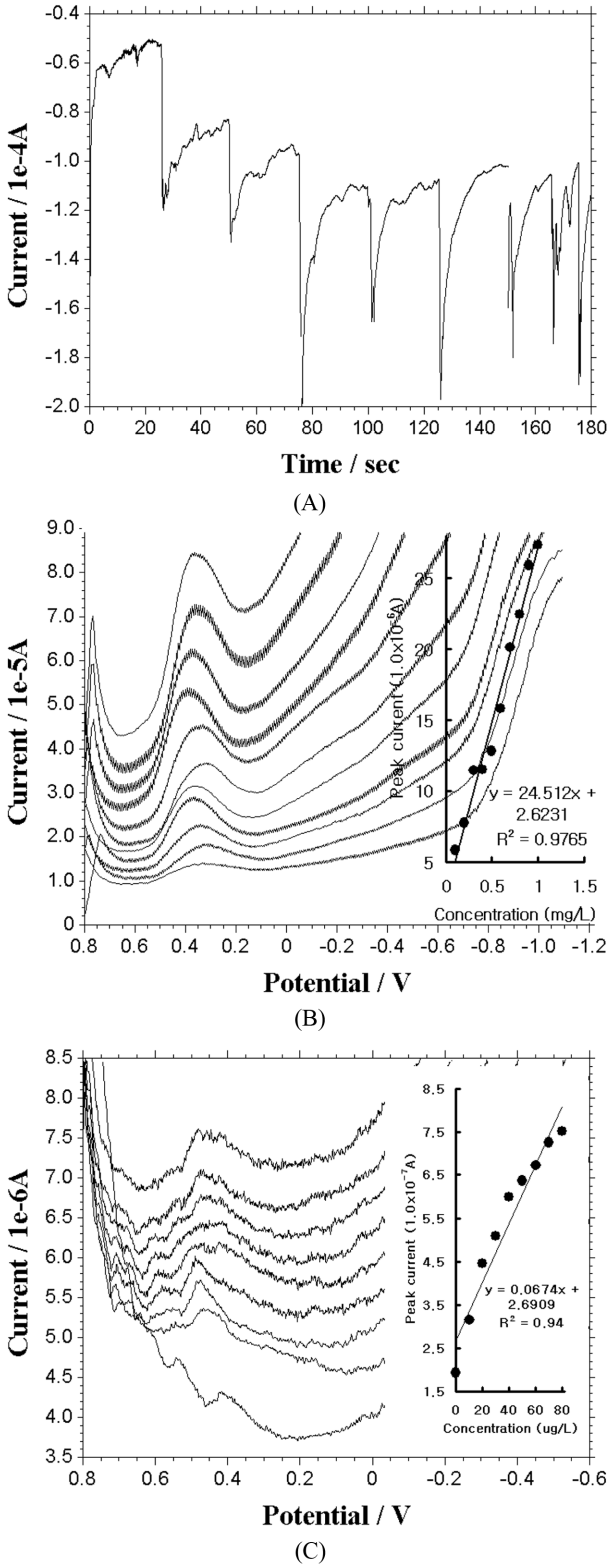


Fig. 3. (A) The chronoamperometry, each spiking at 100 mg/*l* of Zn(II) every 30 sec seven times. (B) The SW working ranges of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mg/*l* Zn(II) using FPE. (C) The low values of 10, 20, 30, 40, 50, 60, 70, and 80 μg/*l* of Zn(II) in seawater electrolyte with optimum parameters.

tions, the analytical detection limit was calculated as 10 μg/*l*. These results can be used for the biological *in vivo* and vitro diagnostics.

In vivo applications. The diagnostic application was performed in the earthworm's cell using contaminated soil. Fig. 4(A) shows the results of the standard addition method in the range of microgram additions. The first curve represents the blank solution, in which no signal was obtained. Then the 0.1 m*l* cell solution (Here the 1.0 g earthworm cell was dissolved in a 1.0 m*l* nitrate concentrate and diluted with 100 m*l* distilled water) was spiked and obtained a 9.114×10^{-6} A peak current within a 90-sec accumulation time, which is zinc's peak potential. Thus, the 0.05 m*l* Zn standard was added with three points, and the peak currents linearly increased from $12.2Xe^{-6}$ to $16.44Xe^{-6}$ A, and y = 2.4468x + 4.5472 and R2 = 0.9853. Thus, 1.86-μg/m*l* Zn(II) was obtained. The results can be used for medicinal diagnosis, specifically for *in vivo* fluid diagnosis of live organs. Fig. 4(B) shows the results of the CV voltammograms. The same peak potential and the same results were obtained.

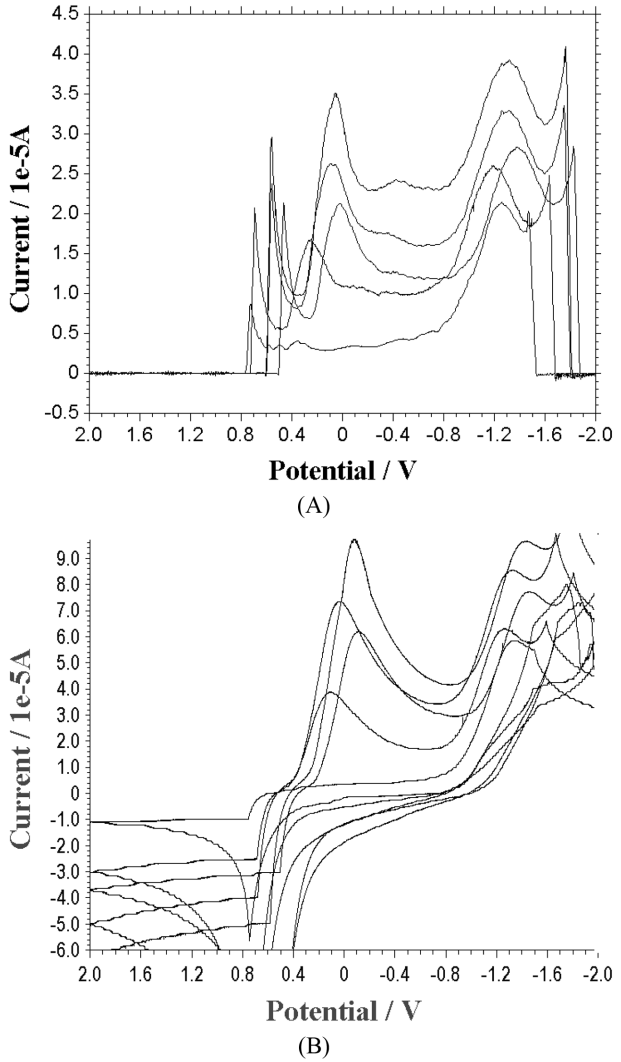


Fig. 4. (A) Detection of Zn(II) in an earthworm's cell using standard addition methods by SW and (B) CV with optimum conditions. The first curve represents the electrolyte blank. The second curve represents the result of the solution in which the earthworm's cell was dissolved. After that, the 0.05 μg/*l* Zn standard spiked three times based on optimum conditions.

The novel fluorine-doped FPE sensor described in this study is for the diagnosis of trace Zn ions in *in vivo* fluids. The optimized conditions that were examined in this study consisted of a 0.04 V amplitude, a 25 Hz frequency, and a 60-sec accumulation time. Under these conditions, the developed sensor approached the detection limit of 10 $\mu g l^{-1}$. The application in the earthworm's cell was performed using a standard addition method. The results of the application showed that the developed method can be used for any *in vivo* or *in vitro* diagnostic application. It can also be applied in other fields that require medicinal diagnosis of humans.

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REFERENCES

Ayoub, A.S., McGaw, B.A. and Midwood, A.J. (2002). Determination of Cd and Zn by isotope dilution-thermal ionisation mass spectrometry using a sequential analysis procedure. *Talanta*., **57**, 405-413.

Azubel, M., Fernandez, F.M., Tudino, M.B. and Troccoli, O.E. (1999). Novel application and comparison of multivariate calibration for the simultaneous determination of Cu, Zn and Mn at trace levels using flow injection diode array spectrophotometry. *Analytica Chimica Acta*, **398**, 93-102.

Bianchi, F., Maffini, M., Mangia, A., Marengo, E. and Mucchino, C. (2007). Experimental design optimization for the ICP-AES determination of Li, Na, K, Al, Fe, Mn and Zn in human serum. *J. Pharm. Biomed. Anal*., **43**, 659-665.

Capitan-Vallvey, L.F., Titos, A., Checa, R. and Navas, N. (2002). High-performance liquid chromatography determination of Zn-bacitracin in animal feed by post-column derivatization and fluorescence detection. *J. Chromatogr. A*, **943**, 227-234.

Evers, T.H., Appelhof, M.A., de Graaf-Heuvelmans, P.T., Meijer, E.W. and Merkx, M. (2007). Ratiometric Detection of Zn(II) Using Chelating Fluorescent Protein Chimeras. *J. Mol. Biol.* **374**, 411-425.

Fabris, C., Soncin, M., Miotto, G., Fantetti, L., Chiti, G., Dei, D., Roncucci, G. and Jori, G. (2006). Zn(II)-phthalocyanines as phototherapeutic agents for cutaneous diseases. Photosensitization of fibroblasts and keratinocytes. *J. Photochem. Photobiol. B*, **83**, 48-54.

Horng, C.J. and Lin, S.R. (1997). Determination of urinary trace elements (As, Hg, Zn, Pb, Se) in patients with Blackfoot dis-

ease. *Talanta*., **45**, 75-83.

Kefala, G., Economou, A., Voulgaropoulos, A. and Sofoniou, M. (2003). A study of bismuth-film electrodes for the detection of trace metals by anodic stripping voltammetry and their application to the determination of Pb and Zn in tapwater and human hair. *Talanta*., **61**, 603-610.

Kohler, M., Harms, A.V. and Alber, D. (2000). Determination of Zn in high-purity GaAs with neutron activation analysis. *Appl. Radiat. Isot*., **53**, 197-201.

Locatelli, C. and Torsi, G. (2000). Determination of Se, As, Cu, Pb, Cd, Zn and Mn by anodic and cathodic stripping voltammetry in marine environmental matrices in the presence of reciprocal interference. Proposal of a new analytical procedure. *Microchemical Journal*, **65**, 293-303.

Lu, H.H. and Jiang, S.J. (2001). Organic acids as the modifier to determine Zn, Cd, Tl and Pb in soil by slurry sampling electrothermal vaporization inductively-coupled plasma mass spectrometry. *Analytica Chimica Acta*, **429**, 247-255.

Marcó, L.M., Jiménez, E., Hernández, E.A., Rojas, A. and Greaves, E.D. (2001). Determination of ZnCu ratio and oligoelements in serum samples by total reflection X-ray fluorescence spectrometry for cancer diagnosis. *Spectrochimica Acta Part B*, **56**, 2195-2201.

Miller, L.M., Wang, Q., Telivala, T.P., Smith, R.J., Lanzirotti, A. and Miklossy, J. (2006). Synchrotron-based infrared and X-ray imaging shows focalized accumulation of Cu and Zn co-localized with b-amyloid deposits in Alzheimer's disease. *J. Struct. Biol*. **155**, 30-37.

Nascentes, C.C., Arruda, M.A., Nogueira, A.R. and Nóbrega, J.A. (2004). Direct determination of Cu and Zn in fruit juices and bovine milk by thermospray flame furnace atomic absorption spectrometry. *Talanta*., **64**, 912-917.

Nedeltcheva, T., Atanassova, M., Dimitrov, J. and Stanislavova, L. (2005). Determination of mobile form contents of Zn, Cd, Pb and Cu in soil extracts by combined stripping voltammetry. *Analytica Chimica Acta*, **528**, 143-146.

Pohi, P. and Prusisz, B. (2007). Determination of Ca, Mg, Fe and Zn partitioning in UHT cow milks by two-column ion exchange and flame atomic absorption spectrometry detection. *Talanta*., **71**, 715-721.

Santon, A., Irato, P., Medici, V., D'lncà, R., Albergoni, V. and Sturniolo, G.C. (2003). Effect and possible role of Zn treatment in LEC rats, an animal model of Wilson's disease. *Biochim. Biophys. Acta*, **1637**, 91-97.

Wu, Y., Li, N.B. and Luo, H.Q. (2008). Simultaneous measurement of Pb, Cd and Zn using differential pulse anodic stripping voltammetry at a bismuth/poly(p-aminobenzene sulfonic acid) film electrode. *Sensors and Actuators B*, **133**, 677-681.

Zaporozhets, O., Petruniock, N., Bessarabova, O. and Sukhan, V. (1999). Determination of Cu(II) and Zn(II) using silica gel loaded with 1-(2-thiasolylazo)-2-naphthol. *Talanta*., **49**, 899-906.

Zareba, S., Szarwiło, K. and Pomykalski, A. (2005). Determination of Fe(II) and Zn(II) by spectrophotometry, atomic absorption spectrometry and ions chromatography methods in Vitrum. *Farmaco*., **60**, 459-464.

Zheng, F. and Hu, B. (2007). Preparation of a high pH-resistant AAPTS-silica coating and its application to capillary microextraction (CME) of Cu, Zn, Ni, Hg and Cd from biological samples followed by on-line ICP-MS detection. *Anal. Chim. Acta*, **605**, 1-10.