

DETERMINATION OF TRACE ZINC IN WATER, SOIL AND RABBIT BLOOD SAMPLES USING CLOUD POINT EXTRACTION COUPLED WITH ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY

YANG, X. P.^{1*} – JIA, Z. H.¹ – YANG, X. C.¹ – LUO, N.¹ – LIAO, X. J.²

¹*College of Chemistry and Chemical Engineering, Chemical Synthesis and Pollution Control Key Laboratory of Sichuan Province, China West Normal University, Nanchong 637000, China*

²*Exposure and Biomonitoring Division, Health Canada, Canada*

**Corresponding author*

e-mail: xiupeiyang@163.com

(phone: +86-817-2568081; fax: +86-817-2568067)

(Received 22nd Jul 2016; accepted 9th Nov 2016)

Abstract. An improved preconcentration method named as cloud point extraction (CPE) with ultraviolet-visible spectrophotometry (UV-vis) has been developed for zinc preconcentration and determination. Non-ionic surfactant Triton X-100 was used as extractant based on the complexation reaction of Zn (II) ions with 1-(2-pyridylazo)-2-naphthol (PAN). Sodium dodecyl sulfate (SDS) and NaCl electrolyte solution worked as cloud point reagent and synergic reagent, which successfully decreased the cloud point temperature (CPT) of Triton X-100 to 0 °C. The enriched analyte in the surfactant-rich phase was determined by spectrophotometry at 549 nm. The influence factors relevant to CPE were evaluated and optimized, including concentrations of Triton X-100 and SDS, concentration of chelating agent, pH as well as salt effect. Under the optimal conditions, the proposed CPE-UV-vis method showed linear calibration within the range 10-1000 ng/mL of Zn (II) and the limit of detection of the method was 3.3 ng/mL with a preconcentration factor of 38. The relative standard deviation (RSD) was found to be 2.8% ($C_{Zn(II)} = 100$ ng/mL, $n = 11$). The proposed method was applicable to the determination of trace levels of zinc in real water, soil and blood samples with satisfactory results.

Keywords: cloud point extraction; ultraviolet-visible spectrophotometry; 1-(2-pyridylazo)-2-naphthol; sodium dodecyl sulfate; environmental chemistry

Introduction

In recent years, the toxicity and the effect on human health and the environment of trace elements have been receiving growing attention in pollution and nutritional studies. Zinc is an essential trace element for humans, animals and plants that plays a key role in variety of physiological and pathological processes such as enzyme regulation, gene expression, catalytic function of the protein, apoptosis and so on (Falchuk, 1998; Berg and Shi, 1996; O'Halloran, 1993; Hambidge and Krebs, 2007; Vallee and Falchu, 1993). Disorders in zinc metabolism have led to several severe neurological diseases like Alzheimer, diabetes and epilepsy (Fischer-Walker and Black, 2004; Frederickson et al., 2005; Koh et al., 1996; Tsvetkov et al., 2008; Ho, 2004; Wei et al., 2004). Zinc deficiency can result in skin problems, slow healing of wounds, reduce the senses of taste and smell, increase susceptibility to infections, decrease fertility and loss of appetite and hair (Galbeiro et al., 2014). On the other hand, zinc is also a metal pollutant in the environment (Mansur et al., 2008; Wan et al., 2016). Too much zinc may impact the soil microbial activity and cause phytotoxic effects. Therefore, there is an increasing need for simple, sensitive, and inexpensive methods for

the determination of low levels of zinc in various samples collected from environment, biological fluid and water.

Many different techniques have been applied to the measurement of Zn(II) ions in a variety of samples, including atomic absorption-emission spectrophotometry (Sun et al., 2006) and electrochemical (Ge et al., 2003). Although they provide quantitative data, these methods have basic limitations in terms of equipment cost, complexity, sample processing and run times, and they are not appropriate for the direct analysis of Zn(II) ions because of their trace levels and matrix interferences in samples. For these reasons, preliminary preconcentration/separation and matrix-removal steps are frequently required to ensure the accuracy and precision of the analytical results.

Currently, the most widely used preconcentration methods are liquid-liquid microextraction (Liao et al., 2011; Pesavento et al., 2009), solid-phase extraction (SPE) (Panhwar et al., 2015; Liu, 2013), electrochemical deposition (Sun et al., 2000) and CPE (Ohashi, 2004; Ulusoy et al., 2012). Separation and preconcentration based on CPE have attracted considerable attention in the last few years mainly because those procedures can decrease or eliminate the use or generation of toxic substances. It is in agreement with the “green chemistry” principles. Moreover, compared with the classical liquid-liquid extraction method, CPE technique has become increasingly popular due to its high enrichment factor, high recovery, rapid phase separation, low cost, low consumption of organic solvents and the ability of combination with different detection. CPE combined with many modern analytical techniques such as atomic absorption spectrometry (AAS), spectrophotometry have been used to measure the concentration of metal elements in samples from environmental to human (Baig et al., 2011; Song et al., 2016). Spectrophotometric analytical methods and instruments have been widely applied in analytical chemistry for decades and offered many appealing characteristics, including simple instrumentation, rapid response times, and easy operation (Zarei, 2007). Coupled some advanced preconcentration techniques with traditional spectrophotometer, trace or ultra-trace levels of metal ions could be successfully detected (Wen et al., 2011; Wen et al., 2011). Furthermore, 1-(2-pyridylazo)-2-naphthol (PAN) is widely used in all kinds of metal detection as a well-known benign ligand in CPE (Afkhami and Bahram, 2006).

Based on the above consideration, we described the new zinc UV-vis spectrophotometry analysis method based on the CPE by using Zn(II)-PAN complex. The Zn(II)-PAN processed by the technology of CPE show the maximum absorption wavelength at 549 nm. In the light of the absorption in visible region, we determined the concentration of Zn(II) ions by UV-vis spectrophotometry. The proposed method possesses the advantages of high enrichment factor, simplicity, sensitivity, low cost, selectivity and environmental benign. What is lack of the method is far too much manual handling, and the automation should be improved. This method was successfully applied to the determination of Zn(II) ions in water, soil and rabbit blood samples.

Experiment

Reagents

Stock standard solutions of $1000 \text{ mg} \cdot \text{L}^{-1}$ Zn(II) were prepared by dissolving ZnSO_4 in 2% (v/v) HNO_3 . Working solutions were obtained daily by stepwise dilution from standard stock solution with doubly-distilled water (DDW). The chelating reagent at

4×10^{-3} mol/L was prepared by dissolving PAN in ethanol. Both the non-ionic surfactant Triton X-100 and the surfactant SDS were purchased from Aladdin. In this work, 50 g/L of Triton X-100 and 1% (w/w) of SDS were prepared and used in this work. The pH of the solutions was adjusted by using $0.2 \text{ mol} \cdot \text{L}^{-1} \text{NH}_3 \cdot \text{H}_2\text{O}$ and $0.2 \text{ mol/L NH}_4\text{Cl}$. DDW was used throughout the work.

Samples preparation

River water was collected from Xi River and Huatan River (Nanchong, PR China), respectively. Tap water was freshly obtained from our laboratory after allowing the water to flow for 5 min. All water samples above were filtered through a $0.45 \mu\text{m}$ pore size membrane filter to remove suspended particulate matters prior to use.

The soil was sampled from the mountain, gardens and fields located in China West Normal University, respectively. Classification methods through four points were used to classify the soil collected, and these soil samples were dried at 120°C for up to 24 h until constant dry weight was achieved. After removal of chunks of rock and grassroots, the dry soil samples were disaggregated in the ceramic mortar. The sample was sieved through a 100 -mesh sieve. The resulting samples were used for later zinc analysis. Tools used for this procedure such as the mortar and the sieve were washed with anhydrous ethanol before the sample processing.

A 5.0 g dried soil samples was weighted into a plastic tube and 0.1 mol/L HCl solution (1:10, v/v) was added. The resulting mixture was subject to stirring evenly for overnight, followed by keeping in room temperature oscillation for 3 h and then filtering. The filtrate was transferred to a 100 mL volumetric flask and diluted it to the mark for zinc analysis.

Into a 100 mL conical flask was added 5.0 mL of rabbit blood sample and 5.0 mL of concentrated HNO_3 . The resulting solution was left at fumehood for overnight. After adding 2 mL of 30% H_2O_2 , the sample was evaporated to 1 mL on a thermo-regulated hot plate. This 1 mL solution was transferred to a 50 mL-volumetric flask and diluted it to the mark for zinc analysis.

Apparatus

UV2550 ultraviolet and visible spectrophotometer (Shimadzu, Japan) was used for quantitative analysis of zinc. A centrifuge (Model TDZ5-WS) (Shanghai Lu Xiangyi centrifuge Instrument Co., Ltd, Shanghai, China) was employed to facilitate phase separation. The pH values were measured by Ohaus Starter 3C pH meter (Pine Brook, NJ, USA).

Operating procedure

Into a 10 mL centrifuge tube containing an appropriate amount of standard solution of Zn(II), was added 0.14 mL of 4×10^{-3} mol/L PAN solution, 0.4 mL of buffer solution (ammonia and ammonium chloride buffer, pH=8.5), 0.8 mL of 50 g/L Triton X-100 solution, 0.2 mL of 5% (w/w) SDS solution and 1.5 mL saturated NaCl solution. The resulting solution immediately appeared cloudy phenomenon. DDW was used to dilute the solution to the mark of 10 mL centrifuge tube followed by shaking well. The procedure is shown in *Fig. 1*. The solution containing the analyte was stored in the refrigerator for 30 min at around 0°C . Separation of the phases was achieved by centrifugating at 4000 rpm for 10 min. This reaction was not subject to cooling in an ice

bath, because a mixed micelle consisting of SDS and Triton X-100 was used as an extracting agent in which the temperature of cloud point was 0 °C. Anhydrous ethanol was added to the surfactant-rich phase (200 µL) to reduce its viscosity and promote sample treatment prior to the analysis by UV-Vis spectrophotometer. Blank solution was also conducted in the same procedure and measured in parallel to the sample solutions. The extraction rates and recoveries were calculated according to the equations below:

$$\text{Extraction rate (\%)} = (m_{\text{measured}} / m_{\text{initial}}) \times 100\% \quad (\text{Eq.1})$$

$$\text{Recovery (\%)} = ((m_{\text{measured}} - m_{\text{initial}}) / m_{\text{added}}) \times 100\% \quad (\text{Eq.2})$$

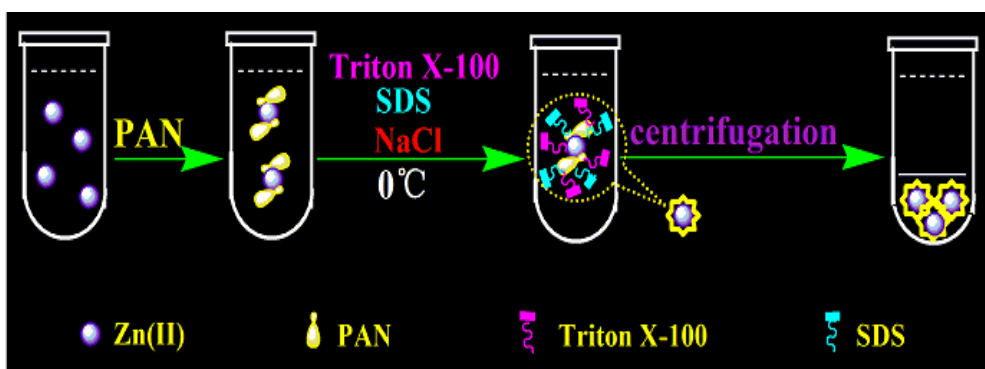


Figure 1. The procedure of cloud point extraction

Results and discussion

Absorption spectra

In this work, UV-vis spectrophotometry was coupled with the advanced CPE preconcentration to accomplish the determination of Zn(II) ions. The maximum wavelength of absorption of the complex of Zn(II)-PAN was the foundation of this quantitative analysis. Absorption spectra of different concentrations of Zn(II)-PAN complex after CPE are shown in Fig. 2, indicating the maximum absorption wavelength was found at 549 nm. The blank absorbance of all reagents utilized was subtracted in the process of the determination of zinc.

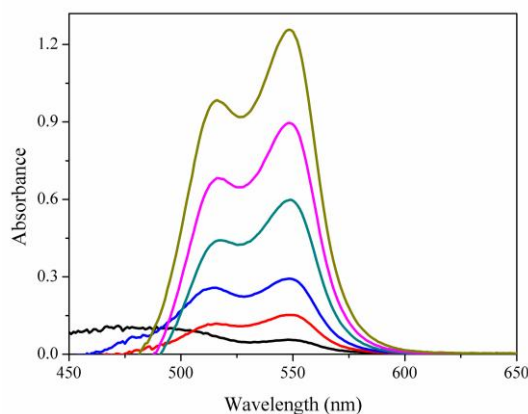


Figure 2. Absorption spectra of different concentrations of Zn(II)-PAN complex after CPE

Volume of the NaCl

According to reported, the CPT of mixed system of Triton X-100 and SDS was lowered to 0 °C after added a certain amount of NaCl solution, and then the phase separation was easier to achieve without heat, because they could lead to the rupture of hydrogen bonds of micelles and force the hydrophobic groups out of the water phase (Zhu et al., 2006). In order to assess the effect of the ionic strength in this work, the experiments were carried out by adding NaCl with different dosage (0.4-2.0 mL) to the solutions before CPE. As can be ascertained from Fig. 3, the extraction rate of Zn(II) ions is greatly increased with the volume of saturated NaCl added from 0.4 to 1.2 mL. After that, the extraction rate of Zn(II) ions is remarkably decreased with the dosage of NaCl from 1.2 to 2.0 mL. As a result, 1.2 mL was chosen as the optimal condition for the proposed method.

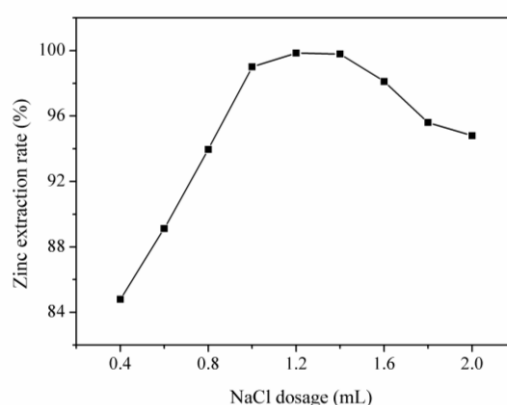


Figure 3. Effect of NaCl dosage on the extraction efficiency of Zn (II) ions. CPE conditions: Zn (II) (100 ng/mL); SDS 0.1% (w/w); Triton X-100, 0.4% (v/v); PAN, 0.14 mL (4×10^{-3} mol/L) and pH=8.5

Concentration of SDS

In those established methods, SDS was used as cloud point reagent and synergic reagent, which combined with Triton X-100 could reach a better result than those attainable with the individual components separately in the process of extraction of metal, which is known as synergism (Li et al., 2007). Furthermore, SDS also was used to decrease the CPT (Zhu et al., 2006). In this work, it was found that the CPT of TX-100 was decreased to 0 °C by adding SDS and the above NaCl. Zn(II) ions can be separated from the resulting mixture when storing the extraction system in the refrigerator for about 30 min. The impacts of the concentration of SDS on the zinc extraction rate were evaluated from 0.025% to 0.250%. As shown in Fig. 4, the extraction rate of Zn(II) ions is increased by increasing the SDS concentration up to 0.1%. When the SDS concentration exceeds 0.1%, there is no any improvement observed in the zinc extraction rate. Such observations may be due to the fact that 0.1% SDS is good enough to completely extract zinc from the mixture. As a consequence, 0.1% SDS was selected in the following experiments.

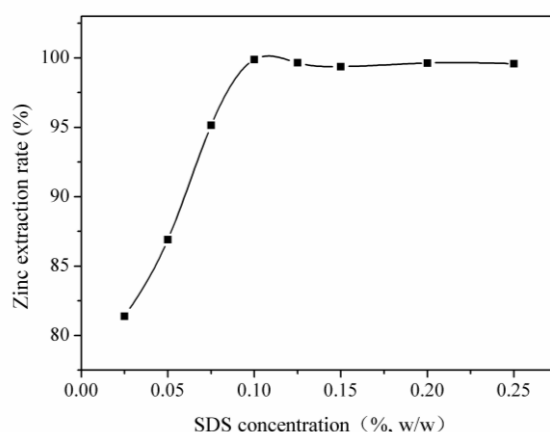


Figure 4. Effect of SDS concentration on the extraction efficiency of Zn (II) ions. CPE conditions: Zn(II), (100 ng/mL); saturated NaCl, 1.2 mL; Triton X-100, 0.4% (v/v); PAN, 0.14 mL (4×10^{-3} mol/L) and pH=8.5

Effect of pH

The precondition of separation of metal ions by the CPE method involves the formation of a complex with sufficiently hydrophobic character being extracted into the small volume of the surfactant-rich phase. The extraction yield of Zn(II) ions may depend on the pH as Zn(II) ions and PAN can form stable compounds only under alkaline conditions. Therefore, the CPE of Zn(II) ions was performed in buffer solutions at a pH range of 7.0-11.0. The results are shown in Fig. 5. It can be seen that the extraction rate of Zn(II) ions is increased dramatically with an increase in pH up to 8.5. In contrast, when the pH is higher than 8.5, the extraction rate of Zn(II) ions is decreased with increasing pH and this observation may be due to the hydrolysis of Zn(II) ions. Hence the optimal buffer pH value was set at 8.5 for CPE.

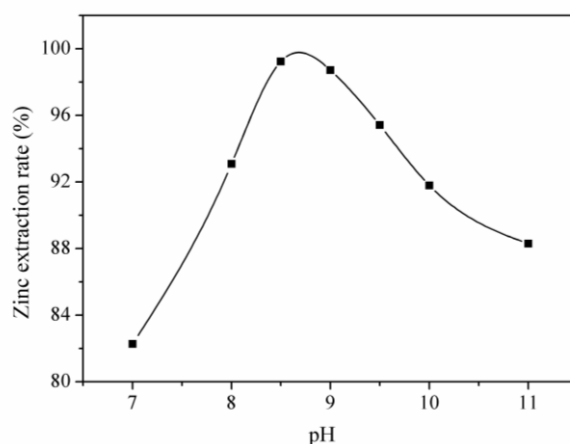


Figure 5. Effect of pH on the extraction efficiency of Zn(II) ions. CPE conditions: Zn(II), (100 ng/mL); saturated NaCl, 1.2 mL; SDS 0.1% (w/w); Triton X-100, 0.4% (v/v) and PAN, 0.14 mL (4×10^{-3} mol/L)

Volume of the PAN

The chelating concentration is very important to the CPE efficiency and subsequent UV-vis spectrophotometer determination. As the principle reagent in this work, PAN was selected to form Zn-PAN complex. The effect of the chelating reagent dosage on the extraction efficiency was investigated in the range of 0.06-0.20 mL. As shown in Fig. 6, the extraction rate of Zn(II) ions is increased with an increase in the dosage of PAN from 0.06 to 0.14 mL. The extraction rate is gradually decreased when the PAN dosage is higher than 0.14 mL. The excessive PAN could be co-extracted into the surfactant rich phase, thus posting negative effects on the extraction efficiency of target analytes. Therefore, 0.14 mL PAN was chosen for the further study.

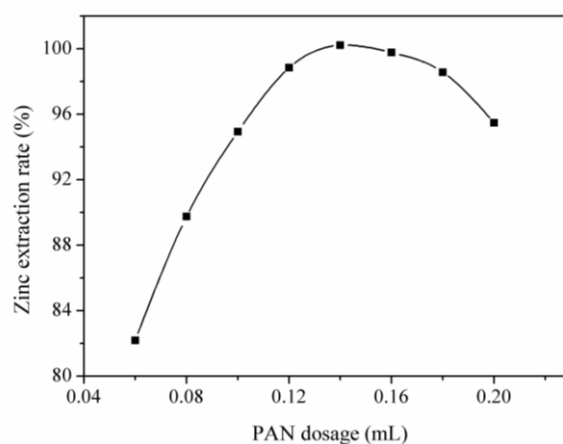


Figure 6. Effect of PAN dosage on the extraction efficiency of Zn (II) ions. CPE conditions: Zn(II), (100 ng/mL); saturated NaCl, 1.2 mL; SDS 0.1% (w/w); Triton X-100, 0.4% (v/v) and pH=8.5

Effect of triton X-100 concentration

A successful CPE would maximize the enrichment factor through minimizing the phase volume ratio. The surfactant concentration is the main parameter affecting the phase ratio. Triton X-100 is one of the non-ionic surfactants extensively used in CPE because of its commercial availability with high purity, low toxicity, low cost and so on. Thus, the influence of different Triton X-100 concentrations on the CPE extraction efficiency of Zn(II) ions was examined, and the results are presented in Fig. 7. As can be seen, the extraction rate of Zn(II) ions is increased with an increase in Triton X-100 concentration up to 0.4%, then reaching a plateau, which is considered as complete extraction. This observation (Fig. 7) is similar to that observed in Fig. 4 in which the influence of the different SDS concentrations on the extraction efficiency of Zn (II) ions is presented. So a concentration of 0.4% (v/v) Triton X-100 was used for subsequent experiments.

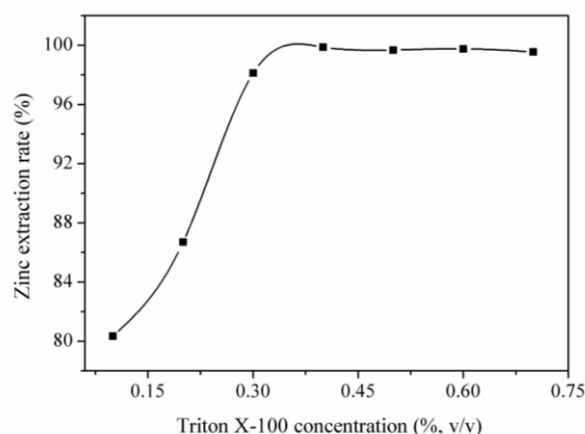


Figure 7. Effect of Triton X-100 concentration on the extraction efficiency of Zn(II) ions. CPE conditions: Zn(II), (100 ng/mL); saturated NaCl, 1.2 mL; SDS 0.1% (w/w); PAN 0.14 mL (4×10^{-3} mol/L) and pH=8.5

Interference study

Because PAN is a universal chelating reagent which can coordinate with other metal elements, and interferences may occur due to the competition of other metal ions for PAN. In this work, several typically potential interfering metal ions were selected for the evaluation of the selectivity of the proposed CPE, including Na(I), K(I), Ca(II), Mg(II), Ni(II), Cu(II), Ba(II), Pb(II), Cd(II), Co(II), Mn(II), Cr(III) as well as Fe(III). A series of solutions of 100 ng/mL Zn(II) containing the metal ions mentioned above at different concentrations were prepared and determined by UV-vis spectrophotometry after CPE, respectively. The results are summarized in *Table 1*, indicating that all interfering ions of study have no obvious interference on the determination of zinc. According to studies in literature, most of the ions of investigation can form a complex with PAN, but the maximum absorption wavelength of all kinds of complex is different. In addition, most of these ions can be precipitated at pH 8.5. Therefore, the selectivity of this method was secured and fairly satisfactory.

Table 1. Investigation of interferences from co-existing metal ions on the determination of 100 ng/mL Zn(II) by using CPE preconcentration followed by UV-Vis spectrophotometer determination

Ions	Interference/metal ratio	Recovery, %
Mg(II)	1000	100.2
Cu(II)	50	99.9
Mn(II)	500	93.6
Pb(II)	500	91.2
Fe(III)	100	94.3
Na(I)	1000	102.8
Ni(II)	300	95.2
Ba(II)	1000	101.1
K(I)	1000	105.2
Ca(II)	1000	100.7
Cd(II)	300	93.5
Cr(III)	30	98.7
Co(II)	50	95.3

Analytical figures of merit

Analytical figures of merit of the developed CPE-UV-vis spectrophotometric method for the determination of zinc were evaluated. Under the optimal experimental conditions, some characteristics of this method such as linear range, correlation coefficients, limit of detection (LOD), repeatability and enrichment factor were investigated, respectively. The calibration curve was linear in the range of 10-1000 ng/mL Zn(II) ($R^2 > 0.997$) and the linear equation was $A=0.00291C+0.001914$ (where A is the absorbance and C is the concentration of Zn(II), ng·mL⁻¹). The detection limit (LOD) of this method calculated as three times the standard deviation of eleven replicate measurements of the blank solution using the CPE method was 3.3 ng/mL. Relative standard deviations (RSD) for eleven replicate determinations 100 ng/mL of Zn(II) was 2.8%. The enrichment factor which was calculated as the ratio of the slopes for the calibration curves subjected to preconcentration procedure and direct sampling (without preconcentration) was 38.

Application

In order to confirm the applicability of the proposed method, it has been applied to the determination of Zn(II) ions in water, soil and biological samples. The reliability of the presented method was proved by spiking experiments and independent analysis. The results of this study shown in Table 2 indicate that the recoveries are in the range of 97.7-101.5 % and confirm the accuracy of the method and independence from the matrix effects.

Table 2. Determination of zinc (II) in water samples, soils and rabbit blood samples by the proposed method

Samples	Added, (ng/mL)	Found, (ng/mL)	R.S.D, %	Recovery, %
Tap water	0	27.5	1.5	-
	100	127.7	1.7	100.2
	400	427.0	1.2	99.8
River water (Huatan river)	0	48.5	1.8	-
	100	148.1	1.3	99.6
	400	440.9	1.6	98.1
River water (Jialing river)	0	49.7	1.4	-
	100	149.6	1.5	99.9
	400	454.4	1.7	101.2
Rabbit blood sample	0	185.3	2.5	-
	100	283.0	3.1	97.7
	400	587.9	3.0	100.7
Mountain soil	0	147.2	2.4	-
	100	245.3	2.9	98.1
	400	548.8	2.3	100.4
Garden soil	0	161.7	3.0	-
	100	262.9	2.7	101.2
	400	562.9	2.1	100.3
Cultivated soil	0	173.0	3.2	-
	100	270.8	2.8	97.8
	400	569.9	2.4	99.2

Conclusions

The method of CPE combined with UV-vis spectrophotometry for the determination of trace zinc was successfully established. The surfactant Triton X-100 was used as the extractant and SDS worked as cloud point reagent and synergic reagent for extraction, which lowered the CPT of Triton X-100 more than 60 °C and assisted the subsequent extraction process. The limit of detection for Zn(II) was 3.3 ng/mL, with sensitivity enhancement factor (EF) of 38. The methodology offers a simple, highly sensitive and selective, cost-effective and environmental benign alternative that can be applied to the determination of zinc in various samples collected from environment, biological fluid and water with satisfactory analytical results.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (no. 21277109), the Program for Young Scientific and Technological Innovative Research Team in Sichuan Province (2014TD0020) and the Sichuan Province Science and Technology Support Plan Project (2015SZ0204).

REFERENCES

- [1] Afkhami, A., Bahram, M., (2006): Cloud point extraction simultaneous spectrophotometric determination of Zn(II), Co(II) and Ni(II) in water and urine samples by 1-(2-pyridylazo) 2-naphthol using partial least squares regression. – *Microchimica Acta* 155: 403-407.
- [2] Baig, J. A., Kazi, T. G., Arain, M. B., Shah, A. Q., Kandhro, G. A., Afridi, H. I., Khan, S., Kolachi, N. F., Wadhwa, S. K. (2011): Inorganic arsenic speciation in groundwater samples using electrothermal atomic spectrometry following selective separation and cloud point extraction. – *Analytical Sciences* 27: 439-445.
- [3] Berg, J. M., Shi, Y. (1996): The galvanization of biology: a growing appreciation for the roles of zinc. – *Science* 271: 1081-1085.
- [4] Falchuk, K. H. (1998): The molecular basis for the role of zinc in developmental biology. – *Molecular and Cellular Biochemistry* 188: 41-48.
- [5] Fischer-Walker, C., Black, R. E. (2004): Zinc and the risk for infectious disease. – *Annual Review of Nutrition* 24: 255-275.
- [6] Frederickson, C. J., Koh, J. Y., Bush, A. I. (2005): The neurobiology of zinc in health and disease. – *Nature Reviews Neuroscience* 6: 449-462.
- [7] Galbeiro, R., Garcia, S., Gaubeur, I. (2014): A green and efficient procedure for the preconcentration and determination of cadmium, nickel and zinc from freshwater, hemodialysis solutions and tuna fish samples by cloud point extraction and flame atomic absorption spectrometry. – *Journal of Trace Elements in Medicine and Biology*, 28(28): 160-165.
- [8] Ge, B., Scheller, F. W., Lisdat, F. (2003): Electrochemistry of immobilized CuZnSOD and FeSOD and their interaction with superoxide. – *Biosensors and Bioelectronics* 18: 295-302.
- [9] Hambidge, K. M., Krebs, N. F. (2007): Zinc deficiency: a special challenge. – *Journal of Nutrition* 137(4): 1101-1105.
- [10] Ho, E. (2004): Zinc deficiency, DNA damage and cancer risk. – *Journal of Nutritional Biochemistry*. 15: 572-578.
- [11] Koh, J. Y., Suh, S. W., Gwag, B. J., He, Y. Y., Hsu, C. Y., Choi, D. W. (1996): The role of zinc in selective neuronal death after transient global cerebral ischemia. – *Science* 272: 1013-1016.

- [12] Li, Y., He, X. J., Cao, X. L., Zhao, G. Q., Tian, X. X., Cui, X. H. (2007): Molecular behavior and synergistic effects between sodium dodecylbenzene sulfonate and Triton X-100 at oil/water interface. – *Journal of Colloid and Interface Science* 307(1): 215-220.
- [13] Liao, X. X., Liang, B., Li, Z. Z., Li, Y. F. (2011): A simple, rapid and sensitive ultraviolet–visible spectrophotometric technique for the determination of ultra–trace copper based on injection–ultrasound–assisted dispersive liquid–liquid microextraction. – *Analyst* 136(21): 4580-4586.
- [14] Liu, Z. L. (2013): Fractal theory and application in city size distribution. – *Information Technology Journals* 12(7): 4158-4162.
- [15] Mansur, M. B., Rocha, S. D. F., Magalhães, F. S., Benedetto, J. S. (2008): Selective extraction of zinc(II) over iron(II) from spent hydrochloric acid pickling effluents by liquid–liquid extraction. – *Journal of Hazardous Materials* 150: 669-678.
- [16] O'Halloran, T. V. (1993): Transition metals in control of gene expression. – *Science* 261: 715-725.
- [17] Ohashi, A., Tsuguchi, A., Imura, H., Ohashi, K. (2004): Synergistic Cloud Point Extraction Behavior of Aluminum(III) with 2-Methyl-8-quinolinol and 3,5-Dichlorophenol. – *Analytical Science* 20: 1091-1093.
- [18] Panhwar, A. H., Kazi, T. G., Afridi, H. I., Arain, S. A., Brahman, N. K. D., Arain, M. S. (2015): A new solid phase microextraction method using organic ligand in micropipette tip syringe system packed with modified carbon cloth for preconcentration of cadmium in drinking water and blood samples of kidney failure patients. – *Spectrochimica Acta Part A* 138: 296-302.
- [19] Pesavento, M., Alberti, G., Biesuz, R. (2009): Analytical methods for determination of free metal ion concentration, labile species fraction and metal complexation capacity of environmental waters: A review. – *Analytica Chimica Acta* 631: 129-141.
- [20] Song, S. G., Li, X. P., Margaritis, D. P. (2016): Electric vehicle electric-hydraulic regenerative braking strategy based on variable current feedback. – *Journal of Mechanical Engineering Research and Developments* 39(2): 403-412.
- [21] Sun, Y. C., Mierzwa, J., Lan, C. R. (2000): Direct determination of molybdenum in seawater by adsorption cathodic stripping square–wave voltammetry. – *Talanta*, 52: 417-424.
- [22] Sun, Z., Liang, P., Ding, Q., Cao, J. (2006) Cloud point extraction preconcentration of manganese(II) from natural water samples using 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone and triton X-100 and determination by flame atomic absorption spectrometry. – *Analytical Science* 22: 911-913.
- [23] Tsvetkov, P. O., Popov, I. A., Nikolaev, E. N., Archakov, A. I., Makarov, A. A., Kozin, S. A. (2008): Isomerization of the Asp7 residue results in zinc-Induced oligomerization of alzheimer's disease amyloid β (1–16) peptide. – *Biochemistry* 9(10): 1564-1567.
- [24] Ulusoy, H. I., Gürkan, R., Ulusoy, S. (2012): Cloud point extraction and spectrophotometric determination of mercury species at trace levels in environmental samples. – *Talanta* 88: 516-523.
- [25] Vallee, B. L., Falchu, K. H. (1993): The biochemical basis of zinc physiology. – *Physiological Reviews* 73: 79-118.
- [26] Wan, R., Kong, D. Q., Liu, J. P. (2016): Study on outdoor high temperature for summer air conditioning by gumbel distribution model. – *Journal of Mechanical Engineering Research and Developments* 39(2): 296-302.
- [27] Wei, G., Hough, C. J., Li, Y., Sarvey, J. M. (2004): Characterization of extracellular accumulation of Zn^{2+} during ischemia and reperfusion of hippocampus slices in rat. – *Neuroscience* 125: 867-877.
- [28] Wen, X. D., Deng, Q. W., Guo, J., Yang, S. C. (2011): Ultra-sensitive determination of cadmium in rice and water by UV–vis spectrophotometry after single drop microextraction. – *Spectrochimica Acta Part A* 79: 508-512.

- [29] Wen, X. D., Yang, Q. L., Yan, Z. D., Deng, Q.W. (2011): Determination of cadmium and copper in water and food samples by dispersive liquid-liquid microextraction combined with UV-vis spectrophotometry. – *Microchemical Journal* 97: 249-254.
- [30] Zarei, A. R. (2007): Cloud point formation based on mixed micelle in the presence of electrolyte for extraction, preconcentration, and spectrophotometric determination of trace amounts of hydrazine in water and biological samples. – *Analytical Biochemistry* 369: 161-167.
- [31] Zhu, X. H., Zhu, X. S., Wang, B. S. (2006): Cloud point extraction for speciation analysis of inorganic tin in water samples by graphite furnace atomic absorption spectrometry. – *Journal of Analytical Atomic Spectrometry* 21: 69-73.