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# Laser-induced thermal lens spectrometry after cloud point extraction for trace analysis of mercury in water and drug samples

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## Abstract

**Background:** We have developed spectrometric determination of mercury in micro volume using laser-induced thermal lens spectrometry (LI-TLS) after cloud point extraction (CPE). TLS as a sensitive method is particularly suited for small volume samples such as that of the remained phase after CPE.

**Results:** Under optimum conditions, the calibration graph was linear in the range of 1 to 50  $\mu\text{g L}^{-1}$  with a limit of detection (LOD) of 0.2  $\mu\text{g L}^{-1}$ . The preconcentration factor of 200 was achieved for 10-mL samples containing the analyte, and relative standard deviations were lower than 4%.

**Conclusion:** Combination of LI-TLS with CPE introduced a powerful method for trace analysis of mercury. The method was successfully applied for the determination of  $\text{Hg}^{+2}$  in water and drug samples.

**Keywords:** Thermal lens spectrometry; Mercury; Cloud point extraction; Triton X-114

## Background

In recent years, the toxicity and effects of trace elements on human health and the environment are receiving increasing attention in pollution and nutritional studies. Mercury is considered as a chemical pollutant and has become widespread in the environment mainly as a result of anthropogenic activities (Eisler 2004; Pacyna and Pacyna 2006). It has been found that mercury accumulates itself in vital organs and tissues such as the kidney and brain. Mercury and its compounds are considered as health hazards, and reports of mercury poisoning as a result of industrial, agricultural, and laboratory exposure as well as its suicidal use are numerous.

Since mercury concentrations in waters are expected to be very low, some powerful techniques are required that a few of them show enough sensitivity. Different analytical techniques have been used for mercury determination at low concentration including cold vapor atomic absorption spectrometry (CV-AAS) (Pourreza and Ghanemi 2009), cold vapor atomic fluorescence spectrometry (CV-AFS) (Geng et al. 2008), flow injection-inductively coupled

plasma-optical emission spectrometry (FI-ICP-OES) (Wuilloud et al. 2002), and high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) (Chen et al. 2009).

Laser-induced thermal lens spectrometry (LI-TLS) is a subgroup of photothermal spectrometry methods, which is an ultrasensitive means to measure optical absorbance. The first photothermal spectroscopic method applied for sensitive chemical analysis is thermal (photothermal) lens spectrometry (TLS) (Gordon et al. 1964), and the first measurement of the thermal lens effect was performed by Gordon et al. (1965) using a simple single beam apparatus (Gordon et al. 1965). Thermal lens effect has been developed as a powerful analytical technique for measurement of low absorbance. The utility of the techniques has increased substantially because it has been recently demonstrated that, in addition to ultrasensitivity, the techniques are particularly suited for small volume samples (Kitamori et al. 2004). In fact, the combined ultrasensitivity and small volume capability make it possible to successfully use them as powerful techniques for analytical devices.

The effect of thermal lens is the result of the temperature rise, which is subsequent to absorption of optical radiation and nonradiative relaxation of the excited

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molecules. Due to the Gaussian profile of the excitation laser, the temperature gradient produces a refractive index gradient which is maximum at the beam center and behaves like a converging or diverging lens depending on whether  $dn/dT$  is positive or negative, respectively (Georges 1994; Snook and Lowe 1995). A steady-state condition is obtained when the rate of laser heating equals the rate of heat loss due to the thermal conductivity of the solvent and the finite temperature rise (Dovich and Harris 1979; Imasaka et al. 1980).

The thermal lens signal is sensitive to the thermo-optical properties of the medium, namely the temperature-dependent refractive index  $dn/dT$ , the thermal conductivity  $k$ , and heat capacity  $Cp$ . In addition, the signal depends on the sample absorbance and heat yield (Piepmeier 1986; Bialkowski 1996).

Mercury in different samples is present at lower levels than the detection limits of sensitive analytical methods, and its determination is spectroscopically and chemically interfered with other major constituents. Much effort has been put to solve these difficulties by the development of various techniques. Preconcentration and separation can solve these problems and lead to a higher confidence level and easy determination of trace mercury. The classical liquid-liquid extraction and separation methods are usually time consuming and labor extensive and require relatively large volumes of high-purity solvents. The additional concern is disposal of the solvent used, which creates a severe environmental problem. Cloud point extraction (CPE) is an attractive technique that reduces the consumption and exposure to a solvent, disposal cost, and extraction time. Moreover, small volume of the surfactant-rich phase obtained through this method permits the design of extraction schemes that are simple, cheap, highly efficient, speedy, and of lower toxicity to the environment than those extractions using organic solvents (Paleologos et al. 2001, 2002; Manzoori and Bavili-Tabrizi 2002; Chappuy et al. 2010; Shah and Kazi 2011).

Cloud point methodology was used for the extraction and preconcentration of many metal ions after the formation of complexes. These metal ions can subsequently be analyzed using analytical systems such as AAS, ETAAS, ICP, GC, HPLC, CE, spectrophotometry (Sanz-Medel et al. 1999; Paleologos et al. 2005; Martinez and Gonzalo 2000; Giokas et al. 2001; Stalikas 2002; Afkhami et al. 2006), and specially LI-TLS.

The aim of our research is to combine laser-induced thermal lens spectrometry, as a sensitive method, with cloud point extraction for determination of mercury. The connection of CPE with TLS could be a powerful analytical technique for mercury because a low volume of sample remains after CPE and the use of organic solvent enhancement in TLS.

## Experimental

### Thermal lens spectrometer setup

A single-laser thermal lens spectrometer was designed in our laboratory (Figure 1). The optical direction was designed in a horizontal-vertical mode. In order to obtain thermal lens signal, the diode laser (532 nm, 50 mW, TEM<sub>00</sub>) was used as the pump/probe source. The laser beam was focused by an 18-cm focal length lens and passed through the micro cell located at the confocal distance. The laser beam was allowed to irradiate the sample or blocked using a chopper. After filtration, the beam intensity change was measured through a 1-mm pinhole by a photo-detector that was located at a 200-cm distance from the sample cell. An operation amplifier amplified the output signal from the photo-detector, and it was digitized by the analog to a digital converter (ADC). The digital signals were processed in a personal computer by laboratory-developed software. For each sample, the signal was derived from the average of three recordings.

### Data treatment

The TLS technique is based on the temperature gradient that is produced in an illuminated sample by nonradiative relaxation of the energy absorbed from a TEM<sub>00</sub> laser beam. Under CW laser excitation and weak absorbing species, the TLS signal depends on the relative changes in the laser beam center intensity. Initial signal  $I_{(0)}$  reflects only the Beer's law response of the sample, and after sufficient time, when a steady-state temperature difference is reached, the intensity at the detector will be  $I_{(\infty)}$ . Then,  $\Delta I/I$

$$\frac{\Delta I}{I} = \frac{I_{(0)} - I_{(\infty)}}{I_{(\infty)}} = \frac{2.303AP(-dn/dT)}{\lambda k} \quad (1)$$

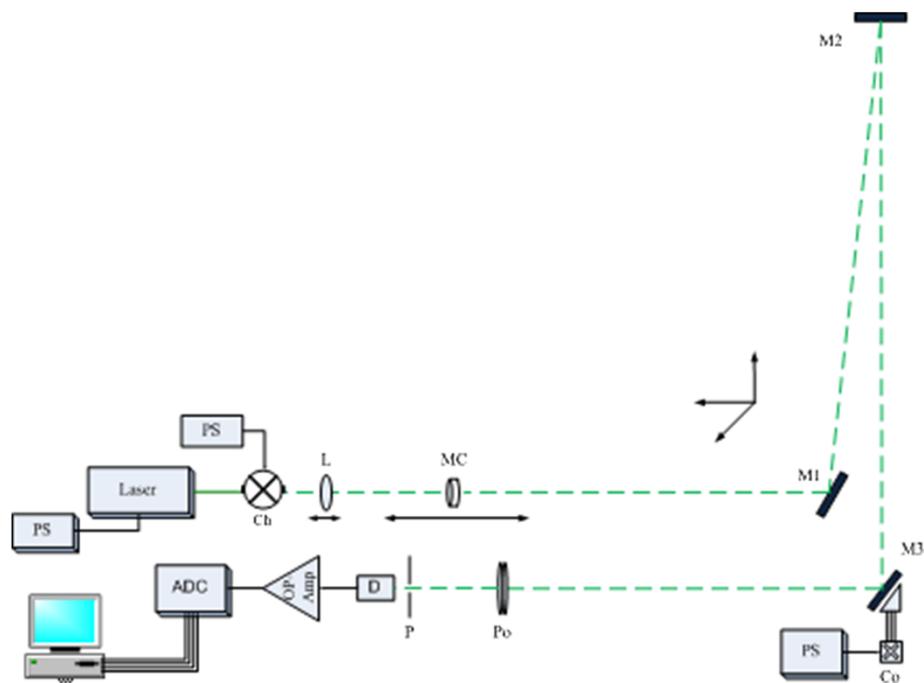
$P$  is the power of the laser,  $\lambda$  is the wavelength,  $dn/dT$  is the temperature coefficient of the refractive index, and  $k$  is the thermal conductivity of the solvent (Bialkowski 1996). This can also be rewritten as:

$$\frac{\Delta I}{I} = 2.303EA \quad (2)$$

where

$$E = \frac{P(-dn/dT)}{\lambda k} \quad (3)$$

$E$  represents the so-called enhancement factor compared to conventional transmission measurements. It is clear that, in addition to the sample absorbance and excitation laser power, the thermal lens signal is directly affected by the thermo-optical properties of the solvent such as the  $dn/dT$  and  $k$  values of the solvent (Dovich and Bialkowoki 1987; Franko and Tran 1996). Nonpolar organic solvents such as benzene, carbon tetrachloride,



**Figure 1** Schematic diagram of single-laser thermal lens spectrometer. PS, power supply; Ch, chopper; L, lens; MC, microcell; M1,M2,M3, mirror; Co, controller; Po, polarizer; P, pinhole; D, detector; Op-Amp, operational amplifier; ADC, analog digital converter; PC, personal computer.

and hexane should provide good media for thermal lens measurements owing to their high  $dn/dT$  and low  $k$  values. Conversely, water, which is the most powerful and widely used solvent in spectrochemical analysis, is considered to be the worst medium for thermo-optical techniques because it has very low  $dn/dT$  and high  $k$  values.

#### Reagents and apparatus

All chemicals used were of analytical reagent grade. The nonionic surfactant, Triton X-114 (Acros, Fair Lawn, NJ, USA), was used without further purification. Stock standard solution of mercury at a concentration of  $1,000 \text{ mg L}^{-1}$  was prepared from  $\text{HgCl}_2$  (Merck, Darmstadt, Germany). Working standard solutions were obtained through appropriate dilution of the stock standard solution. A solution of  $9.9 \times 10^{-4} \text{ mol L}^{-1}$  dithizone (Merck) was prepared in the pure ethanol (Merck). The NaOH solution was prepared by dissolving the appropriate NaOH (Merck) amount in the high-purity water. The materials and vessels used for the trace analysis were kept in 10% nitric acid for at least 24 h and subsequently washed four times with doubly distilled water.

A thermostatic bath (IKA, Königswinter, Germany) maintained at the desired temperature was used for cloud point extraction experiments, and phase separation was performed using a centrifuge (Sigma, Seelze, Germany). A  $100\text{-}\mu\text{L}$  quartz micro cell with 1-mm light path was

used as a determination cell (Hellma GmbH, Müllheim, Germany).

#### Conclusion

The lack of sensitivity associated with the need for detection of micro amounts of samples is probably one of the most frequently encountered problems in trace chemical analysis. As a whole, it was shown that TLS solved these problems, because in addition to the major advantage of thermal lens spectrometry (sensitivity), it gives the possibility to analyze a very low volume of the sample ( $15 \mu\text{L}$ ); on the other hand, the volume of the remained phase after cloud point extraction is in the micro liter range. Therefore, TLS is suitable for analysis of the remained phase after the cloud point extraction, and this combination method (CPE-LI-TLS) exhibits high enhancement factor because of the preconcentration enhancement factor in CPE and the enhancement factor of TLS. In fact, the combined ultrasensitivity and small volume capability make it possible to be successfully used as powerful techniques for analytical devices. Moreover, in this work, by using an alkaline aqueous solution, the selectivity and sensitivity of the TLS method were improved. In alkaline media, the  $\text{Hg(II)}$ -dithizone secondary ( $\text{M}_2\text{L}_2$ ) complex is formed which has higher absorption than the ML complex and enhances the  $\text{M}_2\text{L}_2$  complex to background absorption at 539 nm.

In general, this improved method provides good accuracy, recovery (95.3%), and precision. Also, it presents

a detection limit of  $0.2 \text{ ng mL}^{-1}$  and enhancement factor of 179 compared with other determination methods. However, it seems that any other real samples which are available in low volumes, such as biological and biofluid samples, can be analyzed for their mercury contents by the proposed method.

## Methods

### Extraction and preconcentration procedure

Aliquots of 10.0 mL of the sample containing mercury, Triton X-114 (0.1% (w/v)), dithizone ( $1.96 \times 10^{-6} \text{ mol L}^{-1}$ ), and NaOH (0.5% (w/v)) were kept in a thermostated bath at  $54^\circ\text{C}$  for 6 min. Separation of the aqueous and surfactant-rich phases was accomplished by centrifugation for 5 min at 3,500 rpm. In this condition (alkaline media), no overlapping was observed between blank and complex spectra. The supernatant aqueous phase was separated completely. Then, the surfactant-rich phase was heated to dry in the oven at  $100^\circ\text{C}$  to remove the remaining water. Water is a poor solvent owing to its low  $dn/dT$  and high  $k$  or  $C_p$  values; therefore, for reaching a suitable thermal lens signal, all of the water remaining in the surfactant-rich phase must be eliminated. To decrease the viscosity of the dried surfactant-rich phase and to facilitate sample handling, 50  $\mu\text{L}$  of ethanol was added. The 15- $\mu\text{L}$  homogenized resultant solution was introduced into the micro cell located at the thermal lens spectrometer. The thermal lens spectrometer was set at a wavelength of 532 nm and a chopper frequency of 0.55 Hz. Intensity at the detector over time was obtained during thermal lens effect for each sample, and then the thermal lens signal was calculated by Eq. 1.

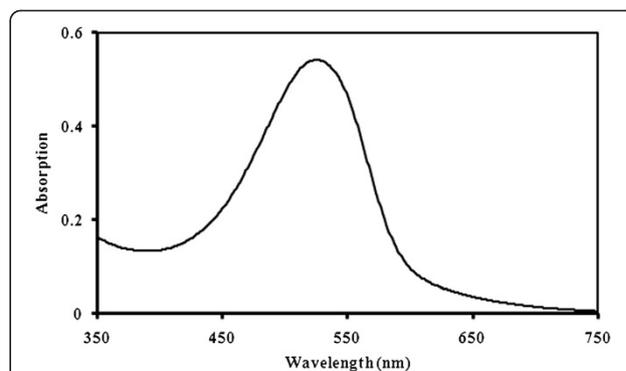
## Results and discussion

### Selection of wavelength

The absorption spectrum of the mercury-dithizone secondary complex in the remaining phase after dissolving using ethanol was studied (Figure 2). This complex shows absorption in  $\lambda_{\text{max}} = 539 \text{ nm}$ . This was well matched to the wavelength of the laser beam used ( $\lambda = 532 \text{ nm}$ ).

### Time of steady-state thermal lens

The steady-state thermal lens effect is due to the formation of the temperature-dependent refractive index gradient. In order to optimize the required time for the steady-state thermal lens effect, the laser beam path to the sample was continuously blocked and opened by a chopper at different frequencies. The intensity of detector output over time was evaluated for  $25 \mu\text{g L}^{-1}$  of mercury-dithizone secondary complex after CPE at a 532-nm laser wavelength. It was found out that the frequency of 0.55 Hz (time of 1.82 s) is suitable for the



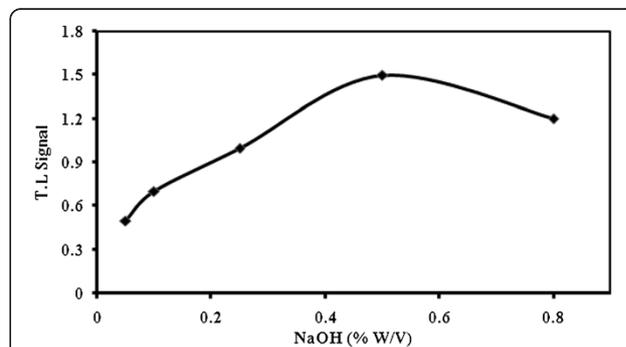
**Figure 2** Absorption spectra of Hg(II)-dithizone complex.

Conditions: 0.1% (w/v) Triton X-114, 0.5% (w/v) NaOH,  $1.96 \times 10^{-6} \text{ M}$  dithizone, and  $100 \mu\text{g L}^{-1} \text{ Hg}^{+2}$ .

steady-state condition which is favorable to build up and decay the thermal lens effect.

### Optimization of experimental parameters

Because of many difficulties such as high background absorption, high CPE temperature, and unpractical complete separation of the surfactant-rich phase from aqueous solution with the use of dithizone in complexation and CPE of the mercury at neutral or acidic media, we carried out our experiments in alkaline media with the addition of NaOH solution (5% w/v) to the sample solutions prior to complexation and CPE. The addition of NaOH by acting as salting-out agent markedly facilitated the phase separation process and decreased the temperature of the cloud point, since it altered the density of the bulk aqueous phase. Moreover, the alkaline solution resulted in the Hg(II)-dithizone secondary ( $M_2L_2$ ) complex that had higher absorption than the Hg(II)-dithizone primary complex formed in neutral solution and enhanced the Hg(II)-dithizone complex to background absorption ratio at 539 nm. This effect was examined with the addition of NaOH, ranging from 0.05 to 0.8% (w/v). As shown in Figure 3, the maximum



**Figure 3** Effect of the concentration of NaOH on thermal lens

signal of  $25 \mu\text{g L}^{-1} \text{ Hg}^{+2}$ . Other conditions: 0.1% (w/v) Triton X-114,  $1.97 \times 10^{-6} \text{ M}$  dithizone, and equilibration temperature  $54^\circ\text{C}$ .

**Table 1 Comparison of the LI-TLS and other methods of mercury determination**

Determination technique	Sample volume (mL)	Solvent volume (mL)	EF <sup>a</sup>	LDR <sup>b</sup> (µg L <sup>-1</sup> )	LOD <sup>c</sup> (µg L <sup>-1</sup> )	Reference
HG-AAS	100	-	20	0.05 to 100	0.039	Song et al. (2006)
CV-AAS	3,000	-	150	-	0.005	Shamsipur et al. (2005)
ICP-OES	10	0.5	18.7	0.25 to 100	0.056	Li and Hu (2007)
Spectrophotometry	50	1	11	5.0 to 80.0	0.83	Niazi et al. (2009)
LI-TLS	10	0.05	179	1.0 to 50.0	0.2	Present work

<sup>a</sup>Enhancement factor. <sup>b</sup>Linear dynamic range. <sup>c</sup>Limit of detection.

TLS signal for Hg<sup>+2</sup> was achieved in the 0.5% (w/v) concentration of NaOH. Moreover, the addition of a salt could markedly facilitate the phase separation process, since it altered the density of the bulk aqueous phase.

Optimal equilibration time and equilibration temperature are necessary to complete reactions and to achieve easy phase separation and preconcentration as efficiently as possible. Therefore, the change of thermal lens signal in equilibration temperature was studied within the range of 35°C to 75°C. It was found that a temperature of 54°C is adequate for extraction of Hg<sup>+2</sup>. The dependence of thermal lens signal upon equilibration time was studied in the range of 3 to 15 min, and the optimum time of 6 min was chosen as a suitable time.

The concentration of the dithizone as a chelating agent was subsequently studied for its effect on the thermal lens signal of Hg<sup>+2</sup>. The variation of the thermal lens signal over the dithizone concentration was evaluated in the range of  $0.49 \times 10^{-6}$  to  $2.97 \times 10^{-6}$  mol L<sup>-1</sup> for 10 mL aliquots of solutions containing 25 µg L<sup>-1</sup> Hg. A concentration of  $1.96 \times 10^{-6}$  mol L<sup>-1</sup> was chosen as the optimum dithizone concentration.

The effect of Triton X-114 concentration was evaluated, and it was found that a quantitative extraction of Hg<sup>+2</sup> can be obtained with the Triton X-114 concentration in the range of 0.025% to 0.3% (w/v). The maximum of thermal lens signal observed for the Triton X-114 concentration is higher than 0.1% (w/v). In order to achieve a good enrichment factor, 0.1% (w/v) was chosen as the optimal Triton X-114 concentration.

#### Figures of merit

From measurements made under the optimum conditions described above, the calibration graph was linear in the range of 1 to 50 µg L<sup>-1</sup>. The calibration equation is  $A = 6.42 \times 10^{-2}C + 11.9 \times 10^{-2}$  with a correlation coefficient of 0.9957, where *A* is the thermal lens signal for Hg<sup>+2</sup> in the surfactant-rich phase and *C* is the concentration of Hg<sup>+2</sup>(µg L<sup>-1</sup>) in the sample solution. The limit of detection, defined as  $C_L = 3S_B/m$ , where *C<sub>L</sub>*, *S<sub>B</sub>*, and *m* are the limit of detection, standard deviation of the

blank, and the slope of the calibration graph, respectively, was 0.2 µg L<sup>-1</sup>.

The reproducibility of the method was studied for five replicate determinations of Hg<sup>+2</sup> in an aqueous sample spiked with 25 µg L<sup>-1</sup> of Hg<sup>+2</sup> after extractions. The relative standard deviation (RSD) was lower than 4%. The enhancement factor (calculated as the ratio of the slopes of the calibration graph with and without preconcentration) and preconcentration factor (calculated as the ratio of sample volume before and after preconcentration) were 179 and 200, respectively.

Table 1 compares the sample volume, solvent volume, enhancement factor, limit of detection, and linear dynamic range of other determination methods with LI-TLS after cloud point extraction of mercury. It shows that in comparison with other sensitive determination methods such as hydride generation atomic absorption spectrometry (HG-AAS) and cold vapor atomic absorption spectrometry (CV-AAS), the LI-TLS has lower sample volume and needed solvent volume, higher enhancement factor, and good limit of detection.

#### Interferences

The effect of interfering ions was studied at different concentrations on the thermal lens signal of a solution containing 25 µg L<sup>-1</sup> of each analyte. An ion was considered to interfere when its presence produced a variation in the thermal lens signal of the sample of more than 5%. Among the interfering ions tested - CO<sub>3</sub><sup>-2</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup>, NH<sub>4</sub><sup>+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup> at the concentration of 500 times higher than that of the Hg<sup>+2</sup> concentration;

**Table 2 Determination of mercury in water samples**

Sample	Added (µg L <sup>-1</sup> )	Found (µg L <sup>-1</sup> ) <sup>a</sup>	Recovery (%)
Soft water	-	ND	-
	10	10.14 ± 0.25	101.4
	25	25.12 ± 0.32	100.5
Physiology serum	-	ND	-
	10	9.53 ± 0.27	95.3
	25	23.55 ± 0.29	94.2

<sup>a</sup>Mean ± S.D. (n = 3).

$\text{Cr}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Al}^{3+}$ , and  $\text{Fe}^{3+}$  at the concentration of 200 times higher than that of the  $\text{Hg}^{+2}$  concentration; and  $\text{F}^-$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Pb}^{2+}$  at the concentration of 80 times higher than that of the  $\text{Hg}^{+2}$  concentration - no interfering was observed.

### Application

To verify the accuracy of the method, the developed method was applied for the determination of  $\text{Hg}^{+2}$  in the soft water and physiology serum. Along with the samples, several known amounts of  $\text{Hg}^{+2}$  were spiked to examine the reliability of the method. For this purpose, 10 mL of each sample was preconcentrated, following the proposed procedure. The results are shown in Table 2. As shown in Table 2, the recovery of the spiked amounts was in the range of 94.2% to 101.4%, which demonstrates the reliability of the proposed method.

### Abbreviations

CMC: critical micelle concentration; CPE: cloud point extraction; CPT: cloud point temperature; CV-AAS: cold vapor atomic absorption spectrometry; CV-AFS: cold vapor atomic fluorescence spectrometry; FI-ICP-OES: flow injection-inductively coupled plasma-optical emission spectrometry; HPLC-ICP-MS: high-performance liquid chromatography-inductively coupled plasma mass spectrometry; LI-TLS: laser-induced thermal lens spectrometry; LOD: limit of detection.

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