Development of a Hyphenated Method Called DLLME/TLM for Trace Analysis of Cd

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ABSTRACT: *Dispersive Liquid-Liquid Microextraction / Thermal Lens Microscopy (DLLME/TLM) was developed as a new combination method for preconcentration and determination of Cd. Thermal Lens Microscopy is suitable for the determination of analyte after DLLME because of the low volume of the remained phase after DLLME and increasing of the enhancement factor for the nonpolar organic solvents. Some effective parameters on the micro extraction, complex formation and combination were selected and optimized. Under optimum conditions, the calibration graphs were linear in the range of 0.05-20 µg/L with the detection limit of 0.008 µg/L. The Relative Standard Deviation (RSD) for 1 and 10 µg/L of cadmium was 3.1 and 2.3, respectively. The enhancement factor of 1200 was obtained from a sample volume of 10.0 mL. DLLME/TLM method was applied to the analysis of real samples. The accuracy of the method was proved by using standard reference materials and micro spectrophotometry.*

KEYWORDS: *Thermal lens microscopy; Dispersive; Real samples; Laser; Cadmium.*

INTRODUCTION

Thermal Lens Spectrometry (TLS) as an analytical spectrometry method was introduced by *Gordon et al.* [1]. In TLS absorption of a pump laser beam, followed by relaxation of the electronic energy gives rise to a thermal gradient. The gradient behaves as a divergent lens in defocusing a probe laser beam. This defocusing process can be performed by the same laser beam, which heats the sample (single laser TLS), or by a second laser beam (dual laser TLS); the change of intensity in the probe beam center at the far field is proportional to the concentration of the absorbing species. This absorptiometric technique has been shown to provide superior sensitivity and improved spatial resolution when compared to conventional spectrometry. Consequently, many procedures for the TLS determination of trace and ultra-traces of highly absorbing species have been described [2]. The following equations show the TLS signal:

$$\frac{\Delta I}{I} = \frac{I(0) - I(\infty)}{I(\infty)} = 0.52\theta + 0.13\theta 2 \qquad (1)$$

Where:

$$\theta = \frac{2.303P(-dn/dt)A}{\lambda k} \qquad (2)$$

$\Delta I/I$ is the relative change of intensity at the pump beam center, P is the pump power, (dn/dT) is the temperature coefficient of the refractive index,

A is the absorbance, λ is the wavelength of the probe laser, and k is the thermal conductivity of the medium.

As understood from Eq. 2, a high sensitivity is obtained with large powers, highly absorbing species and solvents with good thermo optical properties, that is, with large values of the (dn/dT)/k ratio.

Thermal Lens Microscopy (TLM), excitation and probe beams coaxially directed to the objective lens. Duo to the absorption of the excitation beam and its consequent non-radiative relaxation, the thermal gradient around the focused beam is generated. Since temperature dependency of refractive index, the temperature gradient treats as a thermal lens and causes the changes of probe beam intensity. The number of intensity changes is related to the degree of lens and that is proportional to the concentration of sample [3-5].

For the determination of trace amount of elements, it is necessary to use an efficient and highly performance separation and preconcentration method. This is Due to their very low concentration and the matrix effect especially in the environmental samples [6]. Separation and preconcentration techniques, such as liquid-liquid extraction [7], ion exchange [8], Cloud Point Extraction (CPE) [9] and Solid Phase Extraction (SPE) [10,11]; can solve these problems, leading to a higher confidence level and an easy determination of the trace elements.

Solvent extraction due to its simplicity, convenience, wide scope, etc. has been one of the most extensively studied and widely applied methods in separation and preconcentration procedure. Because of the high use of solvent in liquid-liquid extraction methods, several novel micro extraction techniques are being developed in order to reduce the analysis step, increase the sample throughput and to improve the quality and the sensitivity of the analytical methods. The Homogenous Liquid-Liquid Extraction (HLLE) [12, 13], the Liquid Phase Micro Extraction (LPME) [14], are widely used methods of sample preconcentration. They are employed in the separation and preconcentration of environmental contaminants and can solve some of the problems, encountered with conventional pretreatment techniques. A novel microextraction technique as a high performance and powerful preconcentration method termed Dispersive Liquid-Liquid MicroExtraction (DLLME) was demonstrated by Asadi and co-workers [15]. Simplicity of the operation, speed, low coast, high recovery, and

high enrichment factors are some advantages of DLLME. The performance of DLLME was illustrated with determination of polycyclic aromatic hydrocarbons (PAHS) [15], organophosphoros pesticides (OPPS) [16], chlorobenzene [17], Trihalomethanes (THMS) [18], chlorophenoles [19], selenium [20], cadmium [21], and lead [22] in water and environmental samples.

The lack of sensitivity associated with the need for detection of minute amounts of various species in complex samples is probably one of the most frequently encountered problems in chemical analysis of natural samples [23]. It is therefore not surprising that many analytical methods developed to cover the need for environmental pollutants research. This is also the case for Thermal Lens Spectrometry (TLS), which has been recently applied in environmental research, primarily because of its extreme sensitivity [24].

Heavy metal contamination presents a significant threat to the ecosystem due to sever toxicological effects of the living organism. Cadmium is one of the most hazardous elements of human health because it causes adverse effects on metabolic processes. Cadmium has been pointed as the sixth most poisonous substance jeopardizing human health Through the food chain systems. This element is transferred into animals and human beings, causing severe problems. The world health organization has reported tolerable weekly intakes of Cd as 0.007 mg/kg body weight. Therefore, highly sensitive determination methods of trace Cd in biological and drink water samples need to be established. Some analytical methods for trace determination of cadmium are the Flame Atomic Absorption Spectrometry (FAAS) [25], the Electothermal Atomic Absorption Spectrometry (ETAAS) [26], and Inductively Coupled Plasma Emission Spectrometry (ICP/OES) [27].

In the present work, we report on the results obtained for the preconcentration and determination of cadmium by using hyphenated DLLME/LI-TLS. The proposed method was applied to the analysis of water, juices and drug tablets and good results were obtained.

EXPERIMENTAL SECTION
Apparatus
A schematic illustration of TLM is shown in Fig. 2. Briefly, the TLM system consisted of an inverted microscope (IMM-420, SAIRAN) equipped with
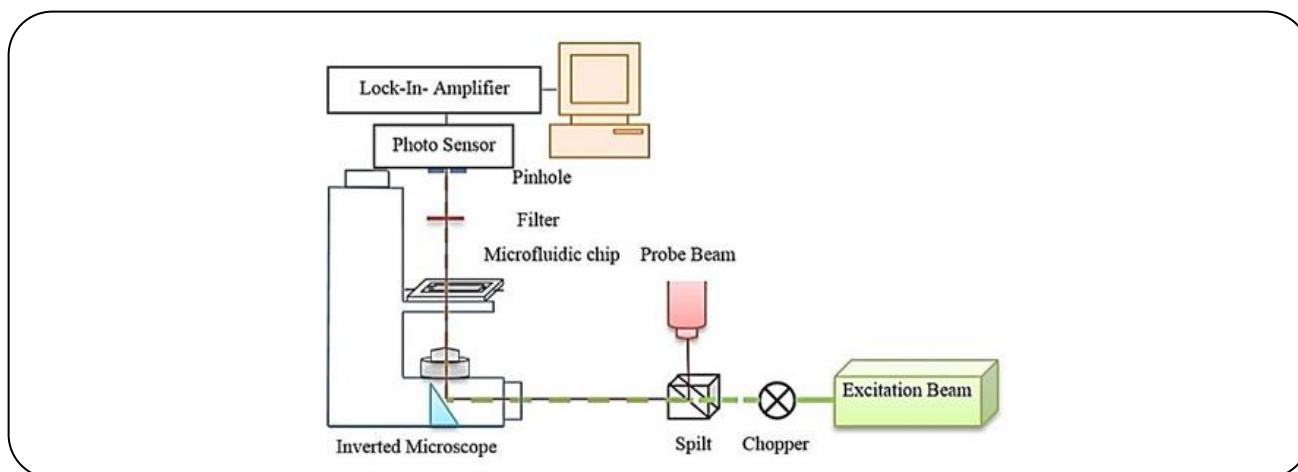
Fig. 1: Schematic diagram of the thermal lens Microscope.

two lasers and other optical devices. The excitation beam was a 50mW diode solid-state laser with an emission line of 532nm which was modulated with a mechanical chopper. The modulation frequency was 500Hz. A 5 mW laser 652 nm was used as probe beam. The two beams were coaxially aligned with a 50% beam splitter and introduced into an inverted microscope. Magnification and numerical aperture of the objective lens were 20X and 0.4, respectively. After passing through the sample in the microchip, the excitation laser was cut by an interference filter. The probe beam diverged and after passing through a pinhole was detected by a photodiode connected to a lock-in-amplifier (SRS-10, Stanford Research Instruments). The time constant of the lock-in amplifier was 10 ms.

Phase separation was performed using a centrifuge (Sigma203, Osterode am harz, Germany). A microchip with 1 mm light pass was used in the experiment. The pH values were measured with a pH-meter (Metrohme691, Herisau, Switzerland), supplied with a glass-combined electrode. A 5 microlitre syringe (Hamilton, Switzerland) was used to transfer organic remained phases to microchip.

Reagents and solutions

All solutions were prepared with high pure water (SG, Barsbuttel, Germany). The stock cadmium solution (1000 mg/L) was prepared from $CdCl_2$ (Merck, Darmstadt, Germany). Working standard solutions were obtained by appropriate dilution of the stock standard solution with high pure water prior to analysis. The chelating agent (dithizone) was supplied from (Merck,

Darmstadt, Germany). A solution of 10^{-3} mol/L dithizone was prepared by dissolving the appropriate amount of this reagent and diluting to 25 mL with ethanol (analytical grade) and was kept in refrigerator (4ºC) for 1 week. The rest of the used chemicals were of analytical grade. A 2 mol/L Na_2SO_4 (Merck) solution was used in order to adjust ionic strength.

The NaOH solution was prepared by dissolving the appropriate NaOH amount (analytical grade, Merck) in high pure water. The pipettes and vessels used for the trace analysis were kept in 10% (v/v) nitric acid at least overnight and subsequently washed three times with high pure water.

The tap, well, river and sea waters, different kinds of juices and drug tablets as real samples were tested for the method development. Standard reference material, SRM 1643d (trace elements in water) (from National Institute of Standard and Technology (NIST), Gaithersburg, MD, USA) and micro spectrophotometry were used for validation of the proposed method.

Analytical Procedure

The extraction of the cadmium trace amount from an aqueous sample into a micro volume of the organic solvent (carbon tetrachloride) was made by complexation with dithizone. Ten milliliters of high pure water were placed in a 10 mL screw cap glass test tube with a conical bottom and were spiked with the cadmium concentration of 10 μg/L and 20 μL of 10^{-3} mol/L dithizone solution. 0.4 mL of acetone (disperser solvent), containing 12 μL carbon tetrachloride (extraction solvent), were injected

rapidly into a sample solution with a 1 mL insulin syringe (Tyco Healthcare, Gasport, UK). A cloudy solution (water, acetone and carbon tetrachloride) was formed in the test tube. In this step, the cadmium-dithizone complex was extracted into the fine droplets of carbon tetrachloride. The mixture was centrifuged for 3 min at 3500 rpm. After this process, the dispersed fine droplets of carbon tetrachloride were sedimented at the bottom of the conical test tube (7 μL ±1); five microliters of this sedimented phase were removed using a 5 microliters Hamilton syringe, manually injected into the microchip and the thermal lens signal was obtained for analyte.

RESULTS AND DISCUSSION

TLM is a powerful analytical technique for the measurement of low absorbance in low volume samples. On the other hand, an organic solvent is obtained after DLLME is an excellent solvent for thermo-optical measurements, because of its low thermal conductivity and high variation in refractive index with temperature. This causes enhancement in signal measurement to conventional spectrophotometry (Eq. (3)).

$$TL_{signal} = 2.303E.A \qquad (3)$$

DLLME/TLM as a powerful hyphenated preconcentration/determination technique can be applied for the analysis of analyte in low volume (microliter) and low concentration.

In order to evaluate and obtain the maximum signal by DLLME/TLM, the effect of different parameters, influencing the complex formation, the extraction and preconcentration conditions, and coupling capability were optimized. These parameters were the type and the volume of extraction solvent, the type and the volume of the disperser solvent, pH, the chelating agent concentration, the extraction time, the ionic strength and the solvent effect. Finally, these optimal conditions were applied to extract and detect cadmium in real samples.

Effect of type and volume of the extraction solvent

Some properties must be considered for the selection of the extraction solvent. The extraction solvent should have a higher density than water, an extraction capability of the interesting compounds and low solubility in water.

Carbon tetrachloride, chloroform, carbon disulfide, and dichloromethane were employed and compared in the cadmium-dithizone complex extraction. A number of sample solutions were tested using 0.4 mL acetone, containing different volumes of the extraction solvents and 20 μL dithizone to achieve a 7 μL volume of the sedimented phase. The solubility of the extraction solvents in water is different. Therefore, to recover a 7 μL of the sedimented phase at the bottom of the test tube, it is necessary to add an excess amount to reach this solubility level. Thereby, 12, 24, 36 and 84μL of carbon tetrachloride, chloroform, dichloromethane, and carbon disulfide were used, respectively.

Among the above solvents, carbon tetrachloride demonstrated the maximum TLM signal. Its sedimented phase can easily be removed by a micro syringe to be introduced into the microchip and has less consumption volume due to its low solubility. Consequently, carbon tetrachloride was the best extraction solvent to be selected.

In order to evaluate the effect of the extraction solvent volume, the solution containing different volumes of carbon tetrachloride were subjected to the same DLLME procedures. The experimental conditions were fixed and included the use of 0.4 mL acetone, containing different carbon tetrachloride volumes (12-48 μL) and 20 μL of dithizone. With the volume increase of carbon tetrachloride from 12 to 48 μL, the volume of the sedimented phase increase from 7 to 28 μL. As a result, at a low volume of the extraction solvent, a high TLM signal was attained. Thereby, in the following studies, the optimum volume of 12 μL was selected for the extraction solvent, consisting of the CCl_4 minimum required volume to reach 7 μL of the sedimented phase.

Effect of type and volume of the disperser solvent

The most important point for the selection of the disperser solvent is its miscibility with both organic (extraction solvent) and aqueous (sample solution) phase. For this purpose different solvents such as methanol, acetonitrile, acetone, and tetrahydrofuran were examined. Several sample solutions were studied using 0.4 mL from each disperser solvent, containing 12 μL carbon tetrachloride (extraction solvent) and 20 μL dithizone (chelating agent). The resulting data illustrated no significant difference among ethanol, acetonitrile and acetone, apart from tetrahydrofuran that presented the lowest TLM signal. Thus, acetone was selected because of, low toxicity and cost.

After choosing acetone as the disperser solvent, it was necessary to optimize its volume. For defining the optimal ethanol volume various experiments were performed with different acetone volumes (0.1, 0.3, 0.4, 0.5, 0.7 and 0.9 mL), containing carbon tetrachloride. It was required to change the CCl$_4$ volume by changing the ethanol volume in order to obtain a constant volume of the sedimented phase (7 μL ± 1) in all experiments. Under these conditions, the volume of the sedimented phase remained constant (7 μL ± 1). The result showed that there was no considerable variation on the TLM signals by using 0.3-0.5 mL of acetone as disperser solvent. Volumes of lower than 0.3 mL of disperser solvent could not form stable cloudy solutions. The TLM signals slightly decrease when the volume of acetone exceeded 0.5 mL. It is clear that by increasing the volume of acetone, the solubility of the complex in water increases. Therefore, the TLM signal decrease. Thus, in order to achieve better and more stable cloudy solution, 0.4 mL of acetone was selected as optimum volume.

Effect of the complexation and extraction time

Complexation and extraction time are important factors in most preconcentration procedures. Since the complex formation of cadmium with dithizone is fast, the time of complex formation is less than 3 min.

In DLLME, extraction time is defined as the time between the injection of the disperser solvent containing the extraction solvent, and the commencement of the centrifugation. The effect of extraction time was Examined in the range of 0-20 min. The results showed that the extraction time has no significant effect on TLS signal. It is revealed that, after the formation of the cloudy solution, the surface area between the extraction solvent and the aqueous phase is infinitely large. The most time consuming step is the centrifuging of sample solution in the extraction procedure, which is about 4 min.

Effect of pH

Separation of metal ions by DLLME involves complex formation with sufficient hydrophobicity to be extracted into the small volume of the non-polar organic phase. Because pH plays a unique role in the metal-chelate formation and the subsequent extraction, the pH of the sample solution was the critical factor evaluated for its effect on the DLLME preconcentration of Cd (II).

The effect of pH on the TLS signal of cadmium-dithizone complex was investigated in the range of 3-13 using hydrochloric acid and sodium hydroxide. The results illustrated that the TLM signal of cadmium-dithizone complex is high up to pH 4 and is nearly constant in the pH 4-13. Therefore, pH 5 was chosen for the subsequent experiments.

Effect of dithizone concentration

The next influential factor that effects on the TLS signal were the dithizone amount. This study was conducted in the dithizone concentration range of 0.1 to 6 μmol/L in samples. In this case, TLM signal increased with the increasing of the dithizone amount up to 1.2 μmol/L, reaching a plateau. A concentration of 2 μmol/L in 10.0 mL of the aqueous solution was chosen as the optimum amount for the cadmium determination. In this procedure, 20 μL of 1x10^{-3} mol/L of dithizone solution was added to the samples.

Effect of ionic strength

For investigating the influence of the ionic strength on the DLLME performance, several experiments were performed by adding varying Na$_2$SO$_4$ amount from 0.01 to 0.5 mol/L. The rest of the experimental conditions were kept constant. The resulting data disclosed that the salt addition from 0.01 to 0.5 mol/L had no significant impact on the TLS signal. These observations offered the possibility of utilizing this method for the cadmium separation from saline solution up to 0.5 mol/L.

Effect of foreign ions

The 1,5 diphenylthiocarbazone (dithizone) chelating agent reacts with several transition metals and semi-metals. The influence of metals that might replace cadmium in the original dithizone chelate was investigated with the purpose of identifying the potential interferents. The tolerance level was defined as the maximum concentration of the foreign ion causing a change in the analytical signal no higher than 5%, when compared with the signal of 10 μg/L cadmium alone. As can be seen in Table 1, most of the cations and anions did not interfere with extraction and determination of cadmium. The relative recovery range for investigated ions was in the range of 95-104.5%. However, Cu^{+2} and Hg^{+2} had adverse effect on the absorbance

Table 1: Effect of interference ion in 10 µg/L Cd (ll) in the water sample using DLLME/TLM.

Foreign ions	Concentration (µg/L)	Interferents/Cd^{+2} ratio
K^+, Li^+, NH_4^+, Mg^{2+}, Cr^{3+}, Al^{3+}	5000	500
Fe^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+}	500	50
Cu^{2+}, Hg^{2+}	50	5
Ag^+, Cr^{3+}	200	20
Pb^{2+}	1000	100
F^-, Cl^-, NO_3^-, CO_3^-, SO_4^{2-}	5000	500

Table 2: Thermo-optical properties and amounts of enhancement parameter for several solvents.

solvent	dn/dT (k^{-1})	K (W/m K)	E^a
Carbon tetrachloride	-6.12×10^{-4}	0.103	558.394
Carbon disulfide	-8.09×10^{-4}	0.150	506.892
Chloroform	-6.03×10^{-4}	0.117	484.384
Dichloromethane	-5.5×10^{-4}	1.22	423.702
Acetone	-5.42×10^{-4}	0.190	268.104
Water	-0.91×10^{-4}	0.598	14.144

^{a} *Calculated at 532nm and 50mW of laser*

at the concentration of 5 times more than that of cadmium. Therefore, interferences effect of these ions for analysis of real samples was eliminated in the presence of 1.0×10^{-3} mol/L thiourea. The corresponding results are listed in Table 1.

Effect of organic solvent

A general trend in Eq. (1) shows the enhancement of the thermal lens signal is obtained for the smaller thermal conductivity (k) and the larger absolute amount of temperature dependent refractive index (dn/dT). The order of magnitude of E predicts that the organic solvent will produce a high enhancement for LI-TLS relative to Beer's law. As it is shown in Table 2, organic solvents have better thermo-optical properties than the water medium at thermal lens spectrometry [28-30]. We have calculated E parameter for organic solvents and water at 532 nm and 50 mW (Table 2). The results show that the E parameters for non-polar organic solvents are higher than those of polar organic solvents and water. Therefore, it must be advantages to use organic solvents to improve the sensitivity of LI-TLS after DLLME method for analysis of elemental and molecular analytes in ultra-trace level. Therefore, Suitable solvents must dissolve related

complexes and they should have good thermo-optical properties.

The effect of organic solvents such as carbon tetrachloride, carbon disulfide, dichloromethane, chloroform, and acetone was investigated on the thermal lens signal after DLLME. The results illustrate that a good correlation exists between the thermal lens signal obtained for Cadmium-dithizone complex in various solvents and the calculated E parameter. Since the thermal lens signal is the highest in the carbon tetrachloride media, it was chosen as a suitable extraction solvent.

The analytical figure of merit

Table 3 summarizes the analytical characteristics of the optimized method, the linear range, the limit of detection, the reproducibility, enhancement factor, and preconcentration factor. The calibration graphs were linear in the Cadmium concentration range of 0.05-20 µg/L. The detection limit (calculated as three times of the standard deviation of 7 blank measurements, divided by the slope of the calibration carve) for the Cadmium cations was found to be 0.008 µg/L.

Concerning the reproducibility of the method, it was evaluated with 10.0 mL from the solutions, containing

Table 3: Analytical characteristics of DLLME/TLM for determination of Cadmium.

Parameter	Analytical feature
Linear range (µg/L)	0.05-20
Limit of detection (µg/L) (36/m, n=7)	0.008
R.S.D.(%)(n=7, 10 µg/L)	2.3
R.S.D.(%)(n=7, 1 µg/L)	3.1
[a]Preconcentration factor	800
[b]Enhancement factor	1200
Sample volume (mL)	10
Sample preparation time (min)	< 3
Sample introduction volume (µL)	20
[c]Determination time (sec)	6

a) Preconcentration factor is calculated as the ratio of the slopes of calibration carve obtained by the conventional LLE-TLM and initial analyte concentration in the aqueous sample

b) The enhancement factor is calculated as the ratio of slope of preconcentration samples to that obtained without preconcentration

c) Averaging of 3 time determination

the analyte ions in the Cd (II) concentration of 1 and 10 µg/L. The relative standard deviation (R.S.D.) for 7 replicate was 3.1 and 2.3% for 1 and 10 µg/L, respectively.

Under the optimized condition, the preconcentration factor of 800 was obtained as the ratio of the analyte concentration in the sedimented phase and the initial analyte concentration in the aqueous sample. The analyte concentration in the sedimented phase was calculated from the calibration graph obtained by the conventional LLE/TLM. The enhancement factor, calculated as the ratio of the slopes of the calibration curve before (using acetone as solvent) and after preconcentration (in carbon tetrachloride) which was 1200. The enhancement and preconcentration factor was attained with only a sample volume of 10.0 mL.

Real samples preparation

Real water samples were collected from different sources. Therefore the situation of each region will influence the amount of organic and inorganic substances in the samples. To reduce the matrix effect of each sample, certain concentration of some reagents such as potassium cyanide, ascorbic acid, EDTA and thiourea were tested as masking agents. Finally, a 1.0×10^{-3} M solution of thiourea was used to eliminate the matrix effect rising from interfering ions. From this point of view the tolerable limit of Cd-dithizone complex increases in the presence of interfering ions.

To reduce the matrix effect in fruit juices they

were centrifuged and the upper solution was separated for further analysis. All aqueous samples were filtered using a 0.45 µm pore size membrane filter to remove suspended particular matter. The colorless solution was diluted to a ratio of 1:10 with high pure water and the proposed method for analyses was performed. To reduce the interfering ions a 1.0×10^{-4} M solution of thiuorea was used.

Prior to the analysis of drugs, a simple and rapid procedure was used to prepare the samples. To reduce the organic matrix in analysis of drugs samples and to prevent their co-precipitation with analyte into the sedimented phase a piece of desired drug was powdered and transferred to a 100 mL volumetric flask; 5 mL of HNO_3 2 mol/L solution was added, followed by the addition of 3 mL of H_2SO_4 solution. Then the flask was manually shaken. To promote oxidation of sample 2 mL of hydrogen peroxide was added and the flask shakes manually again and then takes to volume by addition of high pure water. The solution was then adjusted to desirable pH and the recommended procedure was followed. To reduce the interfering ions a 3.0×10^{-4} M solution of thiuorea was used. The results are given in Table 3.

Analysis of real samples

To test the reliability of the recommended procedure, the method was applied to the determination of cadmium in tap, river, well and sea water samples.

Orange, berry and apple juices were used to analyses

Table 4: Determination of cadmium in tap, well, river water, juices and tablet drug samples and relative recovery of spiked cadmium in tap, well, river water, juices, and tablet drug samples.

Sample	Concentration of Cd^{+2}, mean ± S.D.[a] (µg/L)	Added Cd^{+2} (µg/L)	Found Cd^{+2} mean ± S.D.[a] (µg/L)	Recovery (%)
Tap water[b]	2.40±0.11	5	7.46	99.4
Well water[c]	3.20±0.15	5	8.45	97.0
Rain water[d]	1.10±0.12	5	6.35	100.6
River water[e] 1	n.d[i]	5	4.90	99.0
River water 2	1.3±0.10	5	6.45	102.3
Orange juice[f]	2.8±0.10	5	7.38	99.7
Apple juice[f]	2.5±0.13	5	8.25	101.3
Berry juice[g]	1.5±0.18	5	6.12	98.4
Drug tablet 1	n.d.	5	5.23	103.0
Drug tablet 2	n.d.	5	4.87	99.6
Standard sample	Certified value	Added	Found value	Recovery (%)
NIST SRM 1643d	6.57±0.27		6.36±0.30	98.4

sample	Spiked (µg/L)	TLM		Micro spectrophotometry	
		Found (µg/L)	Recovery (%)	Recovery (%)	Found (µg/L)
Tap water	5	4.88	99.6	5.2	102.4
River water	5	4.73	92.6	4.97	97.5
Standard sample[a]	5	5.3	104.0	4.95	98.6

a) standard deviation (n=3), b) from drinking water system of Tehran, c) from Tehran, d) collected in Tehran, Iran
e) nika river water, f) From Zarnush Co., Urmia, Iran, g) From Aftab Co., Urmia, Iran.

as real samples because of their low matrix effect on DLLME (Table 3).

Also, drug samples were investigated for determination of minute amounts of cadmium. There was not found any cadmium in drug samples or the cadmium amount was less than the limit of detection. The accuracy of the method was verified by the analysis of the samples spiked with known cadmium amount. The relative recoveries of cadmium at the spiking level of 5 µg/L for two drug samples were obtained.

Method validation

The validation of the presented procedure was performed by the analysis of standard reference material (SRM 1643d, water trace analysis) according to the proposed method. It was found that the analytical results are in good agreement with the standard values (Table 4). Also, the data obtained by micro spectrophotometry

coupled with DLLME for some real samples were in good agreement with data obtained by proposed method. It can conclude that the proposed method is accurate and free from systematic error. Statistical analysis of these results showed that there was no significant difference between actual and certified concentrations (t-test, P=0.05).

Comparison with other methods

A comparison of the represented method with the other reported preconcentration methods for the lead preconcentration and determination from water samples are given in Table 5. DLLME/TLM presented a low limit of detection (0.008 µg/L), a high preconcentration factor (800) and a high enhancement factor (1200). These characteristics are of great interest for laboratories in the trace analysis of metal ions. The proposed method were compared with solid phase extraction/ atomic absorption spectrometry (SFE/AAS) [31], flow injection/atomic

Table 5: Characteristics of performance data obtained by using DLLME/TLM and other preconcentration-determination techniques for determination of cadmium in real samples.

method	LOD[a] (µg/L)	R.S.D.(%)[b]	Preconcentration or enhancement factor	Sample volume (mL)	Linear range (µg/L)	Reference
SPE/AAS	0.15	2.8	250	500	Up to 200	31
FI/AAS	0.11	6.3	117	-	1-10	32
SD/GFAAS	0.021	10<	-	25	-	33
LLE/GFAAS	0.18	2.5	-	10	-	34
DLLME/TLM	0.008	3.1-2.3	800[c] 1200[d]	10	20-0.05	Proposed method

a) Limit of detection. b) Relative standard deviation.
c) Preconcentration factor. d) Enhancement factor

Absorption Spectrometry (FI/AAS) [32], Simple Determination/ Grafite Furnace Atomic Absorption Spectrometry (SD/GFAAS) [33] and Liquid-Liquid Extraction/ Grafite Furnace Atomic Absorption spectrometry (LLE/GFAAS) [34].

CONCLUSIONS

Thermal Lens Microscopy (TLM) combined with Dispersive Liquid-Liquid Micro Extraction (DLLME) was developed for the first time. This combination method was evaluated for the preconcentration and the determination of trace amounts of cadmium (at sub-µg/L level). The laser induced-thermal lens spectrometry as an ultra-sensitive spectrometry method is suitable for analysis of the low volume of the remained phase after the DLLME. The dispersive liquid-liquid extraction is an easy, safe, rapid and inexpensive method for the preconcentration and separation of trace metals from aqueous solution to low volume organic phase. The use of a non-polar organic solvent in DLLME causes a large enhancement of thermal lens signal in analyte determination and this causes TLM a robust analytical technique after DLLME for trace and ultra-trace analysis.

This combination method exhibits a high enhancement factor (1200) as a result of the preconcentration and enhancement effect of organic solvent in TLM. Also, the preconcentration factor of 800 was attained with only a 10.0 mL of the sample. In this method, the sample preparation time (less than 3 min) and the sample determination time (less than 6 sec), as well as the consumption of the toxic organic solvents (at microliter level) was minimized without affecting the method sensitivity.

On the other hand, in addition to simplicity and low cost, this method is comparable with the other sensitive methods such as GFAAS for cadmium determination. The proposed method could be readily applied to the determination of other metals with the help of other various chelating agents, extractable by other organic solvents.

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