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Development of a novel flow injection liquid–liquid microextraction method for the on-line separation and preconcentration for determination of zinc(II) using 5-(8-hydroxy-2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophane as a sensitive and selective fluorescent chemosensor

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ABSTRACT

A novel flow injection analysis (FIA) system based on liquid–liquid microextraction and fluorimetric determination was developed for the determination of traces of the Zn^{2+} ion using 5-(8-hydroxy-2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophane (**L**) as a sensitive and selective fluorimetric sensor, with λ_{ex} = 373 nm and λ_{em} = 530 nm, and hexanol as the extracting organic solvent. In the designed FIA system, the phase separation takes place via gravitation forces in the absence of any segmenter. The influence of pH and ionic strength of the solution, amount of ligand, nature of counter ion, volume of organic solvent, extraction time and coil length was investigated. Under optimized experimental conditions, the calibration curve found to be liner over a concentration range of 0.025–4.53 μg mL^{-1} (R^2 = 0.9951) with a limit of detection of 2.3 ng mL^{-1}. The enrichment factor was 45 and relative standard deviation for 7 replicate determinations was 2.43%. The method is very fast and uses low levels of organic solvents. The proposed method was applied successfully to the determination of zinc(II) in human hair, human serum and two inorganic sludge samples.

1. Introduction

Zinc is an essential trace element of great importance for humans, animals and plants, as a +2 cation with a variety of biochemical functions in their metabolism including cell replication, protein synthesis, gene expression and cell division [1,2]. Inadequate zinc(II) absorption, increases zinc(II) losses from the body or increased requirements for zinc(II) and will result in zinc(II) deficiency in human organisms, which leads to several disorders such as growth retardation, the decrease of the immunological defense, eye lesion and some skin diseases [3]. It has also been shown that a number of neurological diseases, such as Parkinson and Alzheimer diseases are closely related to metabolic disorders of zinc(II) [4]. Thus, due to the importance of zinc(II) determination in trace levels in different biological, pharmacological and environmental samples, the development of simple and sensitive analytical methods for the determination of trace levels of zinc is still a challenging subject.

The commonly used analytical methods for the quantitative determination of Zn^{2+} include flame-AAS (FAAS) [5,6], electrothermal atomic absorption spectrometry (ET-AAS) [7], inductively coupled plasma mass spectrometry (ICP-MS) [8], inductively coupled plasma atomic emission spectroscopy (ICP-AES), [9] UV–Vis spectrophotometry [10–12] and electroanalytical techniques [13–15]. Although these methods can exhibit low detection limits, they are time consuming, expensive and could suffer from serious matrix interferences.

Molecular fluorescence spectroscopy is particularly a suitable optical sensing method, which has been widely used for chemical analyses. This is because of its inherent sensitivity, simplicity, ease of automation, cost effectiveness and, to some extent, selectivity, especially for toxic, mutagenic, or carcinogenic substances [16–20]. Thus, in the past two decades, an increasing interest has been focused on the development of fluorescent sensing materials, which offer distinct advantages in terms of sensitivity, selectivity, response time and remote sensing [21–25]. The key point in the development of such fluorescence sensors is the design of a molecular fluorescence sensing element, which usually consists of a fluorophore (signaling moiety) linked to an ionophore (recognition moiety), called as a fluoroionophore. Therefore, the recognition process by the receptor part is converted to a change in the

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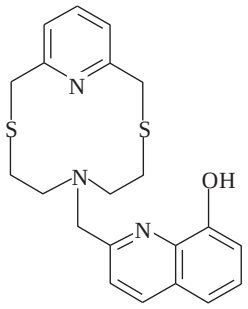
fluorescence signal of the signaling moiety, brought about by the perturbation of such photoinduced processes as energy transfer, charge transfer, electron transfer, formation or disappearance of excimers and exciplexes. Obviously, the topology, size and nature of donating atoms of the recognition moiety play the most important role in determining the selectivity behavior and binding efficiency of the chemosensor.

In recent years, we have introduced novel fluorogenic reagents based on different derivatives of small size mixed aza–thia crown ethers for selective fluorimetric determination of traces of some transition and heavy metal ions [26–31]. In recent works [27,31], we found that 5-(8-hydroxy-2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophaneas (**L**) exhibits selective fluorescence enhancement response towards Zn^{2+}, over Cu^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+} ions upon formation of the corresponding 1:1 complex in MeCN/H_2O (4:1, v/v). The results clearly indicated a synergic cooperation between the receptor and signaling units in determining the Zn^{2+}-selective response by **L**. Thus, in this work, we decided to use this ligand as a very sensitive and selective chemosensor for separation, preconcentration and fluorimetric determination of zinc(II) ion using a flow injection liquid–liquid microextraction method.

Mainly due to its simplicity and versatility, the flow injection analysis (FIA) has been rapidly grown during the past two decades [32]. The FIA methods avoid problems such as tedious procedures, sample contamination and high reagent consumption, and are known as tools for enhancing selectivity and sensitivity of determination processes in complex matrices [33–35]. These advantages are expected to greatly enhance when continuous liquid–liquid microextraction is coupled to FIA. To the best of our knowledge, in this work we describe the first use of liquid–liquid microextraction within a FIA system.



L

2. Experimental

2.1. Reagents

Hexanol as extracting organic solvent was obtained from BDH and used after saturation with doubly distilled water. Analytical grade sodium dodecylsulfate (SDS) and nitrate salts of all cations used (all from Merck) were of the highest purity available and used as received. The fluorescent chemosensor 5-(8-hydroxy-2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophaneas (**L**) was synthesized purified and characterized as reported before [27]. A 5.0×10^{-4} mol L^{-1} ligand solution was prepared with dissolving appropriate amount of **L** in haxanol. After each use, this solution was covered with aluminum foil and stored in refrigerator for further experiments. A stock solution of Zn^{2+} (2.0×10^{-3} mol L^{-1}) was prepared using $Zn(NO_3)_2 \cdot 6H_2O$ and standardized with EDTA. A Borax buffer of pH 8.2 (4.5 mmol L^{-1}) was used. Doubly distilled deionized water was used throughout.

2.2. Apparatus

A JASCO Spectrofluorometer model FP-6200 operated with Et-272 accessory and equipped with a 16-μL flow cell was used for all fluorescence measurements. Time driving conditions of the instrument software were set as: band width, 5 nm; response time, 0.1 s; data patch, 0.1 s. The excitation and emission wavelengths were set as 373 nm and 530 nm, respectively. A ThermoHaak C10 thermostatic bath was used for the adjustment of sample temperature at the desired value. A Hettich EBA20 centrifuge and a Heidolph heater-magnet stirrer NR3001K, were used in batch experiments. The pH measurements were made using a Metrohm 3345 pH meter.

The designed FIA system, operating according to a volume-based mode, is shown in Fig. 1. An Ultra Lab model pp-040p peristaltic pump (Tehran, Iran) with four lines furnished with TygonR tubes was used to propel the solutions. The Rheodyne 5041 model 3-way valves were used for loading/extraction/injection steps. A home-made glass column of 5 mL inner volume, placed horizontally after the extraction coil, was used as a phase separator in the FIA system. The coils used were made of PTFE tubing (i.d. 0.5 mm).

2.3. Flow injection procedure

In the FIA system shown in Fig. 1, the separation coil **C(1)** was kept in a water bath thermostated at 35 °C, and two 3-way valves **V(1)** and **V(2)** were used to provide different paths required in the separation process. After cleaning and conditioning of FIA with distilled water and hexanol, respectively, the extraction and determination of zinc(II) was carried out as follows. In the loading stage **(A)**, the sample solution buffered at pH 8.2 with Borax buffer containing Zn^{2+} ions and 0.03 mmol L^{-1} SDS was loaded into the separation coil **C(1)** (3 m length) at a rate of 1.4 mL min^{-1}, using pump **P(4)**. Simultaneously, and under the same experimental conditions, 150 μL of 5×10^{-4} mol L^{-1} hexanol solution of **L** was loaded into the solvent coil **C(2)**. In this stage, both **V(1)** and **V(2)** valves were in position 0. In mixing stage **(B)**, the valve **V(2)** was switched to position 1 and valve **V(1)** remained in position 0, so that the extraction solvent containing ligand enters into the separation coil with sample back pressure. In extraction stage **(C)**, before solvent exits from separation coil **C(1)**, the valve **V(1)** was switched to position 1 and separation process was started in the isolated coil using pump **P(1)** and the valve **V(2)** was switched to position 0. Finally, after an extraction time of 2.5 min, the valve **V(1)** was switched to position 0, so that the condition was changed to that of stage **(A)**. Now, the isolated separation coil was opened and the entire processed materials entered the phase separator. After a few seconds, the phase separation is occurred under the gravitational force and, consequently, under the pressure of pump **P(3)** and at a flow rate of 10 mL min^{-1}, the contents of the phase separator tube is entered into the flow cell of the spectrofluorometer and the fluorescence intensity of the organic phase is measured as a sharp peak (Fig. 2).

In order to set the spectrofluorometer wavelength parameters at suitable excitation and emission wavelengths of the complex of Zn^{2+} ion with **L**, the fluorescence intensity of a typical solution of complex was recorded in batch experiments, as shown in Fig. 3.

2.4. Sample preparations

The sample preparation procedure for each of real samples, namely, Ni–Cd and Co filter cakes (Angooran Zinc Plant, Zanjan, Iran), human serum, and human hair, was performed as follows.

To prepare the sample solution of cakes, 0.01 g of each cake which was dried at 110 °C, grounded and homogenized, was taken separately and leached completely using aqua regia (1:3 HNO_3/HCl). The resulted solution after cooling was filtered and diluted up to 10 mL with distilled water.

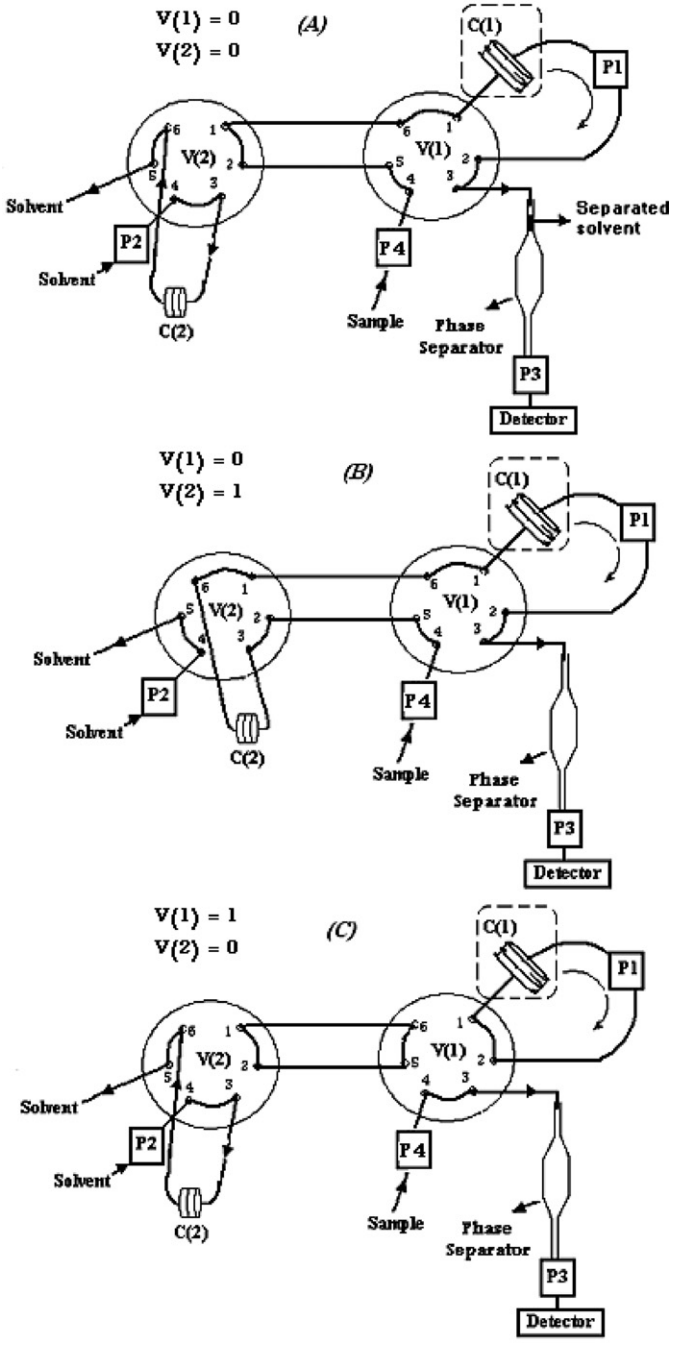


Fig. 1. Manifold of the FIA system: V(1), V(2), 3-way valves; C(1), separation coil; C(2), solvent coil; P(1), P(2), P(3), P(4), peristaltic pump channels. Dotted line show circulator action zone.

Two mL of a human serum sample (Imam Reza Hospital, Kermanshah, Iran) was digested by placing into a crucible and heating at 500 °C for 2 h inside a furnace. The residue was then completely dissolved in a minimum amount of concentrated nitric acid by gentle heating.

The hair sample was first rinsed with acetone and then 0.5 g of the dried sample was accurately weighed and burned in a furnace at 700 °C for 3 h, until a white powder was obtained. The obtained ash was dissolved in a minimum volume of concentrated nitric acid with gentle heating. The solution was first boiled and then cooled and diluted with distilled water to 10.0 mL.

After preparation of the three real samples, aliquots of each solution were diluted with distilled water and buffered at pH 8.2 with

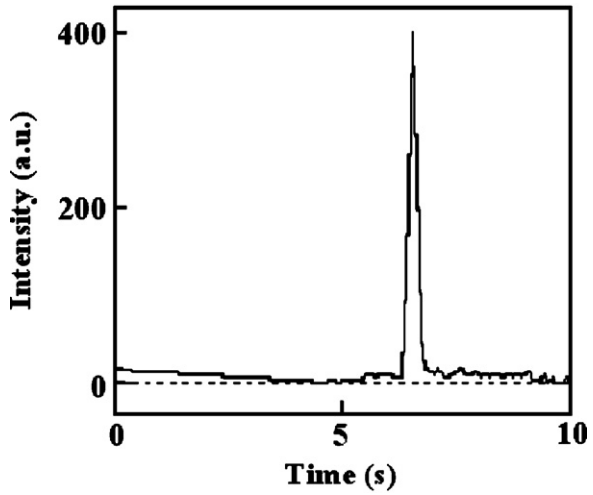


Fig. 2. Sample peak for flow-injection flourimetric detection of 1.5 μg mL^{-1} of Zn^{2+} in time scan mode.

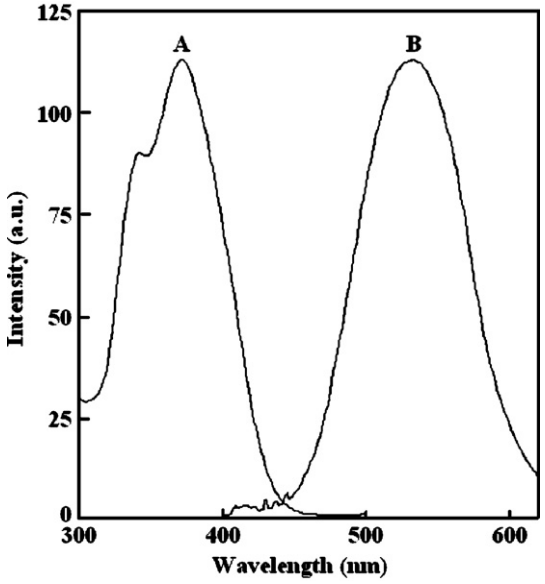


Fig. 3. Absorption (A) and emission (B) spectra for 0.13 mmol L^{-1} of Zn^{2+}–**L** in hexanol.

Borax buffer. SDS was added to a final concentration of 0.03 mm L^{-1} and the concentration of Zn^{2+} was determined following the corresponding recommended procedure.

3. Results and discussion

Since the first report on flow injection–liquid liquid extraction (FI–LLE) by Karlberg and Thelander [36], an extensive amount of research work has been directed towards establishing different types of segmenters, extraction coils, separators, as main components of FI–LLE manifold, as well as its automation using microcontrollers and relative controlling software [37–46]. In 1988 Valcarcel's group reported a novel strategy for liquid–liquid extraction in continues-flow systems without phase separation, in which a single segment of organic solvent interacts with aqueous sample for separation purposes while none of typical components for on-line extraction are incorporated [47,48].

In this work, we employed a more or less similar strategy [49,50] to develop the first flow-injection liquid–liquid microextraction (FI–LLME) method for the selective fluorimetric determination

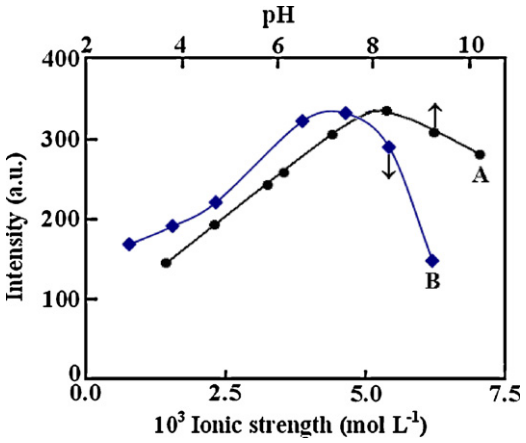


Fig. 4. Effects of pH (A) and ionic strength (B) on extraction of Zn^{2+} ion. Conditions: Zn^{+2}, 1.0 μg mL^{-1}; **L**, 0.15 mmol L^{-1}; SDS, 0.03 mmol L^{-1}; pH, 8.2 (for the case B); ionic strength, 4.5 mmol L^{-1} of borax buffer (for the case A); amount of hexanol, 150 μL; temperature, 25.0 °C; separation flow rate, 1.4 mL min^{-1}; detection flow rate, 10 mL min^{-1}; coil length 3 m; extraction time, 5 min.

of zinc(II) ion in several real samples, using 5-(8-hydroxy-2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophaneas (**L**) as a very sensitive and selective fluorescent chemosensor for Zn^{2+} ion.

In the proposed FI–LLME method, hexanol as the extraction solvent in μL scale is introduced as a single segment into the separation coil of a FIA manifold. Here, the isolated solvent materials start to rotate in an off cycle mode and the segmentation of solvent is carried out with pulsating motions of a peristaltic pump. Then, the zinc content of sample is encountered with the organic solvent and quickly extracted as a selective fluorescent Zn^{2+}–**L** complex (within 1–2 min). After a suitable extraction time, the isolated separation coil is opened and the entire processed material enters into a phase separator. After the gravity phase separation, the content of phase separator is sent to a flow cell and the amount of Zn^{2+} ion present is determined based on the fluorescence intensity of its complex with **L**. A sample fluorescence signal for a 1.5 μg mL^{-1} Zn^{2+} solution obtained after application of the proposed method is shown in Fig. 2.

3.1. Effect of chemical variables on FI–LLME

In order to evaluate the optimum values of chemical variables on the proposed extraction method, the influences of pH of test solution, the amounts of borax buffer and the ligand, and SDS concentrations on the extraction process were studied, as follows.

The fluorescence intensity measurements of the organic phase were made in the presence of a 1 μg mL^{-1} solution of Zn^{2+} at different pH values. The pH of solutions was adjusted by either HCl or NaOH. As it is seen from Fig. 4, curve A, the fluorescence intensity of the extracted solution increased sharply with increasing pH of test solution from 3.0 to 7.8, passing through a more or less plateau between pH 7.8 and 8.3, and further decreasing gradually at pH >8.3. The possible reason for the diminished response at pH <7 could be considered as follows. The ligand **L** used is a hyroxyquinoline-based reagent and it is likely to act as an acidic extractant similar to Kelex 100, so that at low pH values Zn^{2+} ion is extracted into the organic phase via an ion-exchange process. Thus, at higher H^+ concentrations, the extraction of Zn^{2+} will be suppressed considerably. On the other hand, the reduced fluorescence intensity of the organic phase at pH >8.3 could be due to the formation of some hydroxyl complexes of Zn^{2+} ion in solution [51]. Thus, in subsequent experiments, a solution at pH of 8.2, adjusted with a borax buffer, was used.

Meanwhile, the influence of varying concentration of borax buffer of pH 8.2 (for varying the ionic strength of solution) on the extraction of zinc(II) ion with **L** was also investigated in the range of 0.8–6.2 mmol L^{-1}, and the results are shown in Fig. 4, curve B. As is obvious from this figure, the fluorescence intensity of the organic phase increases with increasing borax buffer concentration until a concentration range of about 4–5 mmol L^{-1} is reached. Such initial increase in sensitivity with increasing the buffer concentration could be explained with the higher buffer capacity which will prevent a decrease in pH as a result of the extraction process. However, a further increase in buffer concentration resulted in a sharp decrease in fluorescence intensity, most probably due to competition of borate ion with the ligand for Zn^{2+} ion, and consequent decrease in amount and florescence intensity of the Zn^{2+}–**L** complex in solution. Thus a 4.5 mmol L^{-1} concentration of borax buffer of pH 8.2 was selected for the adjustment of the ionic strength of sample solution.

In the liquid–liquid extraction (LLF) processes, the nature of chelating agent and its concentration are among the most important factors affecting the extent of metal ion extraction. LLE can be used for preconcentration of metal ions after the formation of water-insoluble complexes between the ions and the ligand. In fact, the LLE efficiency depends on the hydrophobicity of the ligand and its complex, the kinetics of complex formation and its transfer between the two phases.

Since the Zn^{2+}–**L** complex formed under optimal experimental conditions is a positively charged species, the presence of a proper lipophilic anion is necessary to accompany the charged complex, in the form of an ion-pair, in order to facilitate the microextraction of the zinc(II) ion in the system. For this purpose, in this work, we found out that SDS, as an anionic surfactant, can act as a lipophilic counter-anion conveniently. Thus, in the next step, the effect of the surfactant concentration on the SSE of 1 μg mL^{-1} of zinc(II) ion in the presence of 1.5 mmol L^{-1} of **L**, as a counter ion was investigated and the results are shown in Fig. 5, curve A. As seen, with increasing concentration of the surfactant, up to about 0.03 mmol L^{-1}, the intensity was increased significantly, emphasizing the predominance of an ion pairing mechanism in the extraction

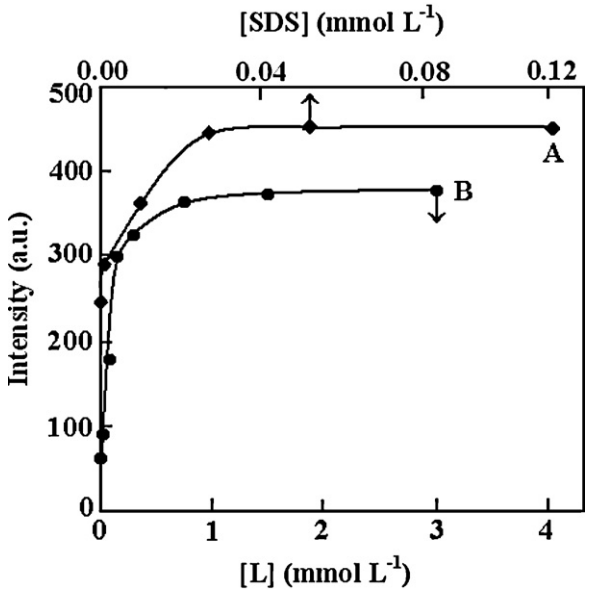


Fig. 5. Effects of SDS (A) and ligand (B) concentrations on extraction of Zn^{2+} ion. Conditions: Zn^{+2}, 1.0 μg mL^{-1}; **L**, 0.15 mmol L^{-1} (for the case A); SDS, 0.03 mmol L^{-1} (for the case B); pH, 8.2; ionic strength, 4.5 mmol L^{-1} of borax buffer; amount of hexanol, 150 μL; temperature, 25.0 °C; separation flow rate, 1.4 mL min^{-1}; detection flow rate, 10 mL min^{-1}; coil length 3 m; extraction time, 5 min.

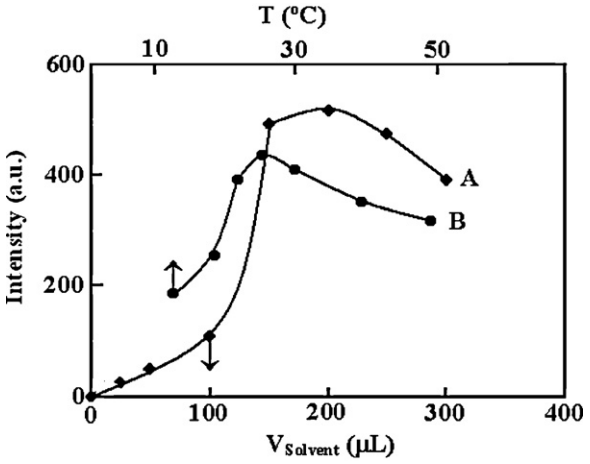


Fig. 6. Effects of amount of extraction solvent (A) and temperature (B) on extraction of Zn^{2+} ion. Conditions: Zn^{+2}, 1.0 μg mL^{-1}; **L**, 0.15 mmol L^{-1} (for the case A); SDS, 0.03 mmol L^{-1} (for the case B); pH, 8.2; ionic strength, 4.5 mmol L^{-1} of borax buffer; amount of hexanol, 150 μL; temperature, 25.0 °C; separation flow rate, 1.4 mL min^{-1}; detection flow rate, 10 mL min^{-1}; coil length 3 m; extraction time, 5 min.

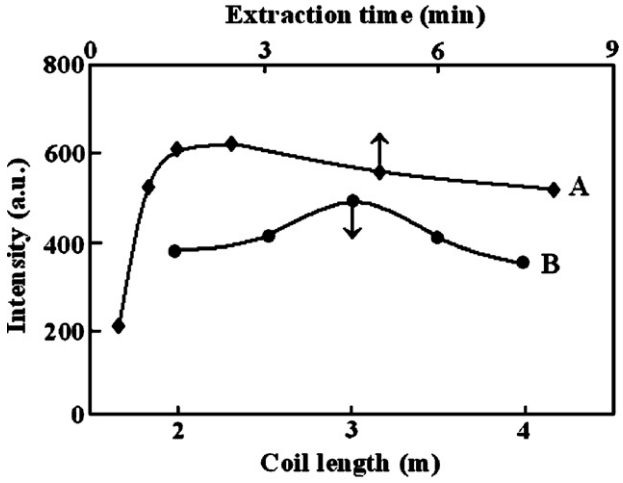


Fig. 7. Effects of extraction time (A) and separation coil length (B) on extraction of Zn^{2+} ion. Conditions: Zn^{+2}, 1.0 μg mL^{-1}; **L**, 0.15 mmol L^{-1}; SDS, 0.03 mmol L^{-1}; pH, 8.2; ionic strength, 4.5 mmol L^{-} of borax buffer; amount of hexanol, 150 μL; temperature, 25.0 °C; separation flow rate, 1.4 mL min^{-1}; detection flow rate, 10 mL min^{-1}; coil length 3 m (for the case A); extraction time, 5 min (for the case B).

process. Further increase in surfactant concentration resulted in no significant change in fluorescence intensity. Thus, a 0.03 mmol L^{-1} concentration of SDS was selected as optimum amount for further experiments.

Fig. 5, curve B shows the effect of varying concentration of **L**, in the range of 0.02–3 mmol L^{-1}, as a suitable selective ligand [27,31] on the liquid–liquid microextraction of 1 μg mL^{-1} of Zn^{2+} ion. As is obvious, maximum extraction efficiency was obtained at concentrations greater than 1.5 mmol L^{-1} of the ligand. Thus, concentration of 1.5 mmol L^{-1} was selected for further extractions. It is worth mentioning that the presence of excess amounts of **L** caused no adverse effect on the extraction process, which is an advantageous point as the procedure could be applied to the analysis of Zn^{2+} in real samples.

3.2. Effect of physical variables on FI–LLME

After setting up the flow injection manifold (Fig. 1), different continuous flow system variables affecting the performance of the proposed FIA system for the liquid–liquid microextraction–fluorimetric determination of traces of Zn^{2+} were optimized as follows.

Based on preliminary experiments on the effect of flow rates on the intensity of the fluorescent signal, a flow rate of 1.4 mL min^{-1} was set for load and separation steps, and the signal recording was carried out at an optimal flow rate of 10 mL min^{-1}.

Influence of amount of hexanol as organic phase, in the range of 50–300 μL, was investigated as a function of solvent coil length (i.e., C(2) in Fig. 1), and the results are shown in Fig. 6, curve A. As seen, the signal intensity increases with increasing volume of the organic phase up 150–200 μL, and then decreases with further increase in the amount of organic phase. The decreased signal intensity at organic volumes larger than 300 μL would be due to the dilution of the extracted Zn^{2+} with **L**, which leads to the diminished fluorescent signals. Thus a hexanol volume of 150 μL was selected for further studies.

In order to verify the effect of temperature on FI–LLME of complex Zn^{2+}–**L** system, a temperature controlled water-bath was integrated into the manifold. The reaction coil C1 was inserted in the water-bath (doted line Fig. 1). The temperature was then varied in the range of 10–50 °C and the results are shown in Fig. 6, curve B. As seen, the signal intensity increases with temperature up to 25 °C and slowly decreases at higher temperatures, most probably due to the increased dynamic quenching of the system at elevated temperatures. Thus, a temperature of 25 °C was selected in order to achieve optimum efficiency.

In the next step, the influence of varying sample volume, via changing of the separation coil length between 2 and 5 m, was tested. From the results shown in Fig. 7, curve A, it is obvious that a reaction coil length of 3 m can do the job perfectly, which was selected as the optimum reaction coil length in further studies.

In order to optimize the extraction process, the extraction time needs to be set to an optimal value. The results of such optimization process are shown in Fig. 7, curve B. As can be seen, the extraction rate is sharply increased within the range of the extraction time from 30 to 150 s, but then decreased slowly. As a result, an extraction time of 150 s was adopted to obtain the highest signal response.

It is worth mentioning that, based on the previously published reports on the one segment mode and segmenter systems [49,50], when a solvent like hexanol is used as the extraction solvent, the differential flow velocities, brought about upon pulsating action of the peristaltic pump, can yield a separation between the sample zone and the extracted analyte zone, in the absence of any segmentation. The predominance of the suggested mechanism of segmentation will result in a wide interaction surface which can lead to lower separation times.

3.3. Figures of merit of proposed method

The calibration graph was obtained by the preconcentration of certain amounts of a Zn^{2+} standard sample solution in the presence of 1.5 mmol L^{-1} of **L** and 0.03 mmol L^{-1} of SDS at pH 8.2 and 25.0 °C. The resulting calibration graph was linear over the range of 0.025–4.53 μg mL^{-1} with a regression equation $F = (185.99 \pm 5.31)$ (Zn^{2+}, μg mL^{-1}) + (45.472 ± 1.45) and $R^2 = 0.9951$, where F is the fluorescence emission intensity of the Zn^{2+}–**L** complex. The precision for 7 replicate measurements of a 1.2 μg mL^{-1} solution of Zn^{2+} ion (Fig. 8) was 2.43% relative standard deviation. The limit of detection, defined as $C_{LOD} = 3S_b/m$ and where S_b is standard deviation of six replicate blank signals and m is slope of the calibration curve after preconcentration [48], was found to be 2.3 ng mL^{-1}. An enhancement factor of 45 was obtained by aspirating a sample into a 3 m separation coil [32]. The proposed FIA system revealed a sam-

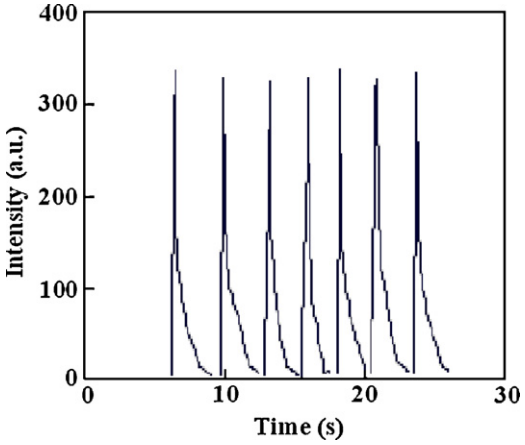


Fig. 8. FIA peaks for 7 replicated determinations of a 1.2 μg mL^{-1} of Zn^{2+} ion under optimized experimental conditions.

pling rate of higher 15 injections per hour. In comparison with the previously published online liquid–liquid extraction methods [35,42–45], the obtained sampling rate of 15 h^{-1} is higher than some reports, i.e., 8 h^{-1} [35,45], and lower than others, i.e., 25 h^{-1} [43], 33 h^{-1} [42] and 60 h^{-1} [44].

3.4. Interference study

The effect of excess amounts of several interfering ions on the determination of a 2.5 μg mL^{-1} Zn^{2+} was studied. The potential interfering ions tested included the common ions at the usual concentrations found in real samples as well as those ions which reduce the extraction of Zn^{2+} ion. The results presented in Table 1 revealed that the recovery of Zn^{2+} in the presence of excesses of all diverse ions tested was almost quantitative. In fact, most of the metal ions listed in Table 1 seem to precipitate at a pH 8.2.

3.5. Analytical applications

In order to evaluate the applicability of the proposed method, it was applied to the determination of Zn^{2+} in Ni–Cd and Co filter cakes (Angooran Zinc Plant, Zanjan, Iran), human serum, and human hair. The procedures for sample preparation and determination of their Zn^{2+} ion content are given in Section 2.4.

Table 1
Effect of interfering ions on determination of Zn^{2+} at different interfering Ion/zinc(II) molar ratius.[a]

Interfering ion	[Interfering ion]/[Zn^{2+}] ratio	Recovery (%)
Na^+	500	99.5
K^+	500	100.0
Ca^{2+}	100	98.5
Mg^{2+}	100	101.0
Cu^{2+}	50	98.0
Pb^{2+}	50	101.0
Co^{2+}	50	97.5
Ni^{2+}	50	98.0
Al^{3+}	50	98.0
Cd^{2+}	20	99.0
Fe^{3+}	20	98.5
Ag^+	5	101.8
Cl^-	500	105.0
NO_3^-	500	103.0
SO_4^{2-}	500	102.0

[a] Experimental conditions: Zn^{+2}, 1.2 μg mL^{-1}; **L**, 0.20 mmol L^{-1}; SDS, 0.03 mmol L^{-1}; pH, 8.2; ionic strength, 4.5 mmol L^{-1} of borax buffer; amount of hexanol, 150 μL; temperature, 25.0 °C; separation flow rate, 1.4 mL min^{-1}; detection flow rate, 10 mL min^{-1}; coil length 3 m; extraction time, 5 min.

Table 2
Determination of zinc(II) in real samples value.

Sample	Amount of zinc(II)	
	Proposed method	ET-AAS
Cobalt cake	172 ± 6 (μg mL^{-1})	168 ± 3 (μg mL^{-1})
Nickel–cadmium cake	154 ± 4 (μg mL^{-1})	151 ± 1 (μg mL^{-1})
Human hair	167 ± 5 (μg g^{-1})	174 ± 3 (μg g^{-1})
Human serum	0.69 ± 0.02 (μg mL^{-1})	0.71 ± 0.07 (μg mL^{-1})

The results of zinc determination in different real samples, obtained by the proposed method and those by electrothermal atomic absorption spectrometry (ET-AAS), are summarized in Table 2. As is obvious, in all cases, there are satisfactory agreements between the results obtained by the two different methods used.

4. Conclusions

Present study reports the first liquid–liquid microextraction–flow injection system (FI–LLME) designed for the selective and sensitive fluorimetric determination of zinc(II) ion using 5-(2-quinolinylmethyl)-2,8-dithia-5-aza-2,6-pyridinophane (**L**) as a novel sensitive and selective fluorescent chemosensor for Zn^{2+} ion. The method is not only selective towards zinc(II) ion but also possesses a relatively wide linear response range with a low limit of detection. Meanwhile, the proposed flow injection system is simple and its overall analysis time is very short. As a micro extraction system, the designed flow method is capable to work with low amount of hexanol, as an organic extraction solvent, so that it results to have low toxicity and to cause much less environmental pollution, as compared to previously reported solvent extraction methods.

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