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A New Dispersive Liquid-Liquid Microextraction Method Followed by Direct GF-AAS Determination Optimized with Experimental Design and Response Surface Methodology for Determination of Ag(I) in Water Samples

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In this research, a rapid, reliable and selective dispersive liquid-liquid microextraction (DLLME) followed by direct injection of microdroplet to graphite furnace atomic absorption spectrometry (GF-AAS) method for the determination of ultra-trace amounts of Ag(I) was developed. Effect of the important experimental parameters on the extraction efficiency of Ag(I) was investigated using response surface methodology (RSM) by performing a central composite design (CCD). A newly synthesized Calixarene (mesotetraspirocyclohexylcalix[4]pyrrole, TSCC4P) was utilized as the chelating agent. The optimal experimental condition was obtained as sample volume: 5 ml, dispersive solvent type: methanol, dispersive solvent volume: 715 μl , extracting solvent: 1,2-dichlorobenzene, volume of extracting solvent: 25 μl , amount of TSCC4P: 127.1 μg , and pH of sample solution: 6.5. Under the optimum conditions Ag(I) ions were extracted into a fine sedimented microdroplet, which 10 μl of it was directly injected into GF-AAS system. The calibration graph was linear over the range of 0.1-10.0 ng ml^{-1} with a detection limit ($S/N = 3$) of 0.02 ng ml^{-1} . The relative standard deviation (RSD%) for ten replicated determinations of 10 ng ml^{-1} Ag(I) was 3.1%. The enrichment factor and extraction recovery were found to be 292 and 96%, respectively. The proposed DLLME-GF-AAS method was successfully applied to the extraction and determination of Ag(I) ions in different real water samples.

Keywords: Silver, Dispersive liquid-liquid microextraction, GF-AAS, Experimental design, Response surface methodology

INTRODUCTION

The name Calixarenes introduced in 1989 by Gutsche for the macrocycles or cyclic oligomers produced from hydroxyalkylation of phenols and aldehydes. These macrocycles have hydrophobic cavities which make useful module for encapsulation of smaller molecule or target ions and may serve as cavitands known in Host-Guest theory [1]. Calixarene derivatives with different functional groups have been reported to use in enzyme mimetic [2], selective liquid membranes [3], electrochemical sensors [4], solid-phase extraction (SPE) [5], and in high-performance liquid chromatography (HPLC) stationary phases [6]. It was

established that the presence of Calixarenes containing nitrogen or sulfur atoms (based on Hard-Soft Acid-Base Theory N is a medium base and S is soft base compared to O) is indicative of more selectivity towards soft metal ions, such as Ag(I), Pb(II) and Hg(II), over alkali metal ions [5,7]. Pyrrole-based tetra-azacrown ethers or calix[4]pyrroles are the originally fully meso-alkyl substituted porphyrinogens which have been extensively carried out as host molecules for anions [8] and neutral substrates [9]. They are deprotonated tetra-anionic N_4 ligands which form σ and/or π -bonds with high-valent transition-metal cations [10].

Silver is an element of great economic importance and its compounds and alloys are widely used in the jewellery industry, preparation of photographic films, mirrors and

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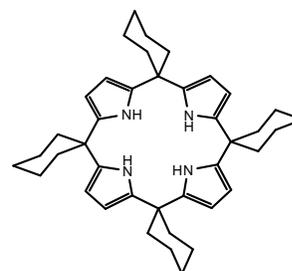
dental fillings production [11]. Moreover, due to its antibacterial properties, its compounds are added to a great variety of products, such as air conditioner [12] and drinking water filters [13]. The rapid determination of this metal in geochemical exploration studies is also of obvious relevance [14]. Recent information about the interaction of silver with essential nutrients, especially Se, Cu and vitamins E and B12, has focused attention on its potential toxicity [15]. However, it is also a significant environmental pollutant and its monitoring and determination in a variety of environmental and biological samples has received today a growing attention [16]. Consequently, a quantitative chemical analysis is needed for accurate, selective and sensitive determination of trace and ultra-trace amounts of silver.

Due to the extremely low concentration of elements and matrix effects in the biological and environmental samples, a separation-preconcentration step is usually required before the analysis of the trace amounts of elements [17]. Of a great variety of separation techniques reported for this purpose we can mention liquid-liquid extraction (LLE) [18], solid-phase extraction (SPE) [19], supercritical fluid extraction (SFE) [20,21], bulk-liquid membrane (BLM) [21,22], emulsion-liquid membrane (ELM) [23] and supported-liquid membrane (SLM) [24]. LLE, known as the most general and the oldest preconcentration and separation classical extraction method in analytical chemistry, is tedious, time-consuming and usually requires large volumes of expensive and potentially toxic organic solvents [25].

Single-drop microextraction (SDME), using microliter-range organic solvents, has been newly developed as a popular, inexpensive, fast and proper alternative for classical extraction methods [26]. However, this technique suffers from instability of the drop and needs along equilibrium times for complete extraction of analyte. Fast stirring breaks up the organic drop and results in air bubble formation. Some newer liquid microextraction strategies such homogenous liquid-liquid microextraction (HLLME) and dispersive liquid-liquid microextraction (DLLME) was proposed to raise the analytical performances and also to resolve the problems of SDME. Regard to other microextraction methods, the main advantages of DLLME are speed, simplicity of operation, low sample volume and

low cost, and usually shows high recoveries and enrichment factors (EFs) [27,28].

Graphite furnace atomic absorption spectrometry (GF-AAS) is a micro-amount sample analysis method with high sensitivity. Similarly, DLLME is a miniaturized sample pretreatment technique. So, the aim of this work is to develop a simple, reliable and sensitive method by coupling DLLME and GF-AAS to develop an efficient and powerful analytical strategy for the selective pre-concentration of ultra-trace amounts of silver in water samples by using a newly synthesised porphyrin intermediate (meso-tetraspirocyclohexylcalix[4]pyrrole), which is briefly named TSCC4P.



meso-tetraspirocyclohexylcalix[4]pyrrole (TSCC4P)

EXPERIMENTAL

Reagents and Standards

All used organic solvents including methanol, ethanol, acetonitrile, acetone, chloroform, carbon tetrachloride, dichloromethane, tetrachloroethylene, chlorobenzene and 1,2-dichlorobenzene were of the analytical-grade and purchased from Merck chemical company (Darmstadt, Germany). Silver nitrate and other salts were of the highest purity available from Merck. All acids were of the analytical-grade purity from Merck or Fluka (Buchs, Switzerland). A 1000 $\mu\text{g ml}^{-1}$ stock standard solution of Ag(I) was prepared by dissolving appropriate amount of silver nitrate in nitric acid solution (2% v/v). Working standard solutions were prepared by appropriate dilution of the stock solution with doubly distilled water. Synthesis, characterization and purification of meso-tetraspirocyclohexylcalix[4]pyrrole (TSCC4P) was carried out as described previously [5].

Instrumentation

A Shimadzu AA6650 atomic absorption spectrometer (AAS) equipped with a deuterium (D2) lamp for background correction, a GFA-EX7 graphite furnace with high-density pyrolytic coated graphite cuvettes, and a ASC-6100 autosampler was applied for the determination of silver under the factory's recommended schedule. A silver hollow cathode lamp (Hamamatsu Photonic Co. Ltd., L233-series) was used as radiation source. Sample injection volume was 10 μl in all experiments. The temperature program for the graphite atomizer was optimized and its steps are listed in Table 1. Pure Ar (99.99%) supplied by Roham Gas Co. (Tehran, Iran) was used as purge gas for the GF-AAS analyses.

The pH values were measured with a Jenway 4030 digital pH-meter, containing a combined glass-calomel electrode. An Eppendorf 5810 centrifuge (Hamburg, Germany) was used to centrifuge and accelerate the phase separation in DLLME process. All of the 14 ml screw cap glass test tubes with conical bottom, which were used as extraction vessel, were maintained into 0.1 M HNO_3 overnight and then washed with doubly distilled water and acetone for proper cleaning and avoiding memory effect. Different micro-syringes with 10, 25, 50 and 500 μl volumes were purchased from Hamilton (Hamilton, USA).

Statistical Software

The Statgraphics Centurion XV, 2005 statistical software (Statgraphic, StatPoint Technologies, VA, USA) was employed to design experiments and also for modelling

and analysing the obtained data [29].

DLLME Procedure

A 5.0 ml portion of sample solution containing Ag(I) ions, adjusted to pH = 6.5 by sodium dihydrogen phosphate/disodium hydrogen phosphate buffer, was placed in a 14 ml glass test tube. Then, 715 μl of ethanol (as dispersing solvent) containing 25 μl of 1,2-dichlorobenzene (as extracting solvent) and 127 μg of TSCC4P (as complexing reagent) was injected rapidly into the sample solution using a 1.0 ml syringe. The rapid injection disperses the organic solvent into the aqueous sample solution and results in a cloudy system. So, the complexation of Ag ions and TSCC4P ligand and consequently extraction of the resulted adduct occurs during a short period of time. Afterward, the cloudy solution was centrifuged (4000 rpm, 4 min), to separate organic microdroplet and aqueous phases. This process caused the dispersed organic microdroplet to be sedimented at bottom of the conical test tube. Finally, 10 μl of the sedimented phase was withdrawn using a proper microsyringe and directly injected into the GF-AAS system for analysis.

RESULTS AND DISCUSSION

To obtain a high recovery and enrichment factor, the effect of various affecting experimental parameters such as type and volume of dispersing and extracting solvents, pH of sample solution, amount of the chelating agent, extraction

Table 1. Instrumental Parameters for GF-AAS Determination of Silver^a

Stage	Temperature ($^{\circ}\text{C}$)	Heat mode	Time (s)	Ar flow (ml min^{-1})
Drying	150	ramp	10	100
Drying	250	ramp	10	100
Pyrolysis	500	ramp	10	1000
Pyrolysis	500	step	10	1000
Pyrolysis	500	step	3	0
Atomization	1800	step	3	0
Cleaning	2500	step	1	1000

^aSpectrometer parameters: wavelength, 328.1 nm; slit width, 0.5 mm; Ag lamp current, 8 mA.

time, and salt addition effect were optimized using response surface methodology. In order to obtain the best values for the above-mentioned factors, the extraction recovery and enrichment factor were considered in almost. The equations used to calculate the extraction recovery and enrichment factor were considered as reported previously [30].

To ensure the accurate quantization of silver ions and also to identify and control the matrix effect, related to direct injection of organic solvent into GF, some preliminary liquid-liquid extraction experiments followed by GF-AAS determination were conducted. For this purpose, various concentrations of Ag (0.05-20.0 ng ml⁻¹) were spiked in 5.0 ml portions of sample solution (pH = 6.5) and were extracted using 5.0 ml of 1,2-dichlorobenzene, containing 5.0 mg of TSCC4P. Determination of the extracted and remained amounts of Ag(I) ions showed that the extraction was exhaustive in all examined concentrations. Thus, different amounts of silver (over the above-mentioned range) were extracted using the described LLE method. Finally, a standard calibration curve was demonstrated by direct injection of 10 µl of the separated organic phase into GF-AAS, for subsequent analysis of silver in conducting DLLME experiments. To identify the important factors for response surface methodology (RSM), different affecting parameters on the recovery and enrichment factor of the proposed DLLME procedure were studied using one-at-a-time experiments, at first. The first step in the optimization process was selection of extracting solvent. Therefore, carbon tetrachloride, chloroform, dichloromethane, tetrachloroethylene, chlorobenzene and 1,2-dichlorobenzene were used as extracting solvents. For this purpose, 5 ml portions of silver sample solutions (10 ng ml⁻¹) at pH = 6.0 were studied by DLLME using 500 µl of methanol containing 100 µg TSCC4P and 35 µl of each mentioned solvents. Using chloroform and dichloromethane, the formed cloudy solutions were unstable and unsuitable for subsequent centrifugation and appropriate phase separation. Chlorobenzene and 1,2-dichlorobenzene resulted in quantitative extraction whereas, the percentage of extraction for carbon tetrachloride and tetrachloroethylene were 56.5 ± 2.5% and 29.4 ± 3.0%, respectively. However, 1,2-dichlorobenzene was found to form more stable and homogeneous cloudy system

compared to that with chlorobenzene. Therefore, 1,2-dichlorobenzene was selected as the best choice of extracting solvent for subsequent experiments.

Miscibility of disperser with the extracting solvent and aqueous sample solution is the most important provision in DLLME method. Different solvents such as methanol, ethanol, acetonitrile, and acetone were examined as disperser for this purpose. Thus, 5 ml aliquots of silver sample solution (10 ng ml⁻¹) at pH = 6.0 were studied using 500 µl of different dispersers containing 35 µl of 1,2-dichlorobenzene and 100 µg TSCC4P. The obtained enrichment factors for methanol, ethanol, acetonitrile, and acetone were 200.7 ± 4.4, 173.9 ± 4.6, 158.9 ± 3.3 and 179.9 ± 5.2, respectively. According to the results, methanol was selected as the disperser solvent, due to the highest enrichment factor in comparison with other examined solvents. Further experiments indicated that extraction time and salt addition have no significant effect on the extraction efficiency.

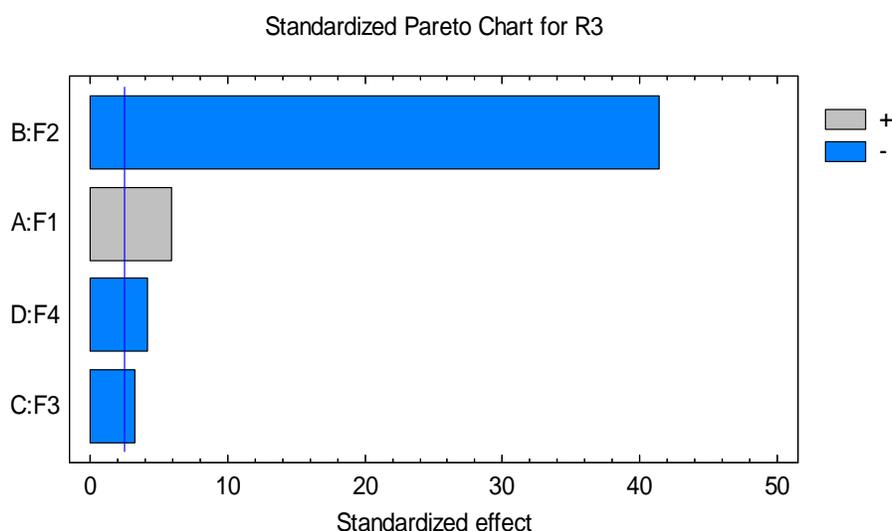
Response Surface Methodology

Experimental design. Optimization of the independent variables (volume of disperser, F1; volume of extractant, F2; TSCC4P amount, F3; and pH, F4) was carried out using response surface methodology (RSM) based on the central composite design (CCD). Those were the factors chosen to be optimized simultaneously based on their importance and their possible interactions. The variables and the high and low levels for each of them, which have been used in the RSM design, are listed in Table 2. The preset values were defined according to the results of one-at-a-time experiments, within practical restrictions for some of the factors.

To characterize significance of the selected factors, analysis of variance (ANOVA) as a classical statistical tool, was performed and EF was selected as the response objective. The results are summarized as Pareto Chart in Fig. 1. Every bar length in Fig. 1 is proportional to the significance of a variable. According to the results, extractant's volume was the most statistically significant factor, which affects the dependent variables, while disperser's volume, pH and amount of the chelating agent

Table 2. The Variables and the Low and High Values Used for Central Composite Design (CCD)

Symbol	Variable name	Low	High	Units	Continuous
F ₁	volume of disperser	350.0	650.0	μl	Yes
F ₂	volume of extractant	30.0	40.0	μl	Yes
F ₃	TSCC4P amount	60.0	140.0	μg	Yes
F ₄	pH	5.5	7.5	-	Yes

**Fig. 1.** Pareto Chart of the main effect of variables obtained from an orthogonal array design.

had very low significances (at $p < 0.05$ level). Therefore, all four variables were considered in the following optimization steps. Since the sample volume and salt addition effect were not significant variables, they were kept constant as 5 ml and 0.0% (w/v), respectively.

Results of central composite design. CCD is one of the most frequently used response surface designs in statistics, which is constructed from several factor combinations. The design consists of three distinct sets of experimental runs: (1) a factorial design in the factors with two levels for each factor, (2) a set of centre points whose values of each factor are the medians of the values used in the factorial portion, which is often implied to improve the precision of the experiment, (3) a set of axial points, experimental runs identical to the centre points except for one factor, which

will take on values both below and above the median of the two factorial levels, and typically both outside their range. Therefore, CCD was concerned with the optimization of the significant variables in order to obtain the best response, in the proposed DLLME-GF-AAS procedure.

A second-order mathematical model correlating the response function with the independent factors could be established using the data provided by CCD. The experiments were designed according to CCD using a 24 factorial and star or 56 runs design with four central points and two responses including recovery (R1) and enrichment factor (R2) using the Statgraphics as shown in Table 3. Individual experiments were carried out randomly. The main effects, interaction effects and quadratic effects were optimized and evaluated in this design.

Table 3. List of Experiments in the CCD for Model Optimization and Their Responses

Run	Block	F ₁ (μl)	F ₂ (μl)	F ₃ (μg)	F ₄ (pH)	R ₁ (%)	R ₂
1	1	500.0	35.0	100.0	6.5	103.52	206.2
2	1	350.0	40.0	140.0	5.5	97.52	151.4
3	1	500.0	25.0	100.0	6.5	92.03	273.9
4	1	650.0	40.0	140.0	7.5	98.43	161.9
5	1	650.0	30.0	140.0	5.5	97.24	239.5
6	1	500.0	35.0	180.0	6.5	102.3	192.3
7	1	350.0	30.0	140.0	7.5	97.35	216.3
8	1	350.0	30.0	60.0	7.5	94.61	213.1
9	1	500.0	35.0	100.0	6.5	102.72	203.0
10	1	350.0	40.0	60.0	7.5	98.44	151.9
11	1	650.0	30.0	140.0	7.5	95.94	231.7
12	1	650.0	30.0	60.0	7.5	97.05	236.7
13	1	350.0	30.0	140.0	5.5	96.57	227.8
14	1	500.0	35.0	100.0	6.5	101.54	197.5
15	1	800.0	35.0	100.0	6.5	95.21	195.1
16	1	500.0	45.0	100.0	6.5	103.42	145.7
17	1	650.0	30.0	60.0	5.5	96.24	243.0
18	1	350.0	30.0	60.0	5.5	93.22	239.0
19	1	350.0	40.0	60.0	5.5	96.07	189.1
20	1	500.0	35.0	20.0	6.5	94.49	194.4
21	1	350.0	40.0	140.0	7.5	99.01	154.2
22	1	200.0	35.0	100.0	6.5	92.03	167.9
23	1	500.0	35.0	100.0	8.5	93.47	177.7
24	1	500.0	35.0	100.0	4.5	83.67	170.8
25	1	650.0	40.0	60.0	7.5	97.23	159.9
26	1	650.0	40.0	140.0	5.5	95.04	160.5
27	1	650.0	40.0	60.0	5.5	94.81	168.1
28	1	500.0	35.0	100.0	6.5	103.03	204.4
29	2	500.0	35.0	100.0	6.5	101.23	201.7
30	2	350.0	40.0	140.0	5.5	96.04	150.5
31	2	500.0	25.0	100.0	6.5	93.14	280.5
32	2	650.0	40.0	140.0	7.5	100.03	164.5
33	2	650.0	30.0	140.0	5.5	96.48	236.5
34	2	500.0	35.0	180.0	6.5	100.23	194.2
35	2	350.0	30.0	140.0	7.5	95.81	214.8
36	2	350.0	30.0	60.0	7.5	96.52	216.4
37	2	500.0	35.0	100.0	6.5	100.12	197.9
38	2	350.0	40.0	60.0	7.5	99.13	155.9
39	2	650.0	30.0	140.0	7.5	97.23	240.7

Table 3. Continued

40	2	650.0	30.0	60.0	7.5	95.16	233.2
41	2	350.0	30.0	140.0	5.5	97.81	231.8
42	2	500.0	35.0	100.0	6.5	95.08	187.9
43	2	800.0	35.0	100.0	6.5	93.85	196.3
44	2	500.0	45.0	100.0	6.5	100.02	144.1
45	2	650.0	30.0	60.0	5.5	97.04	246.3
46	2	350.0	30.0	60.0	5.5	96.22	246.7
47	2	350.0	40.0	60.0	5.5	94.21	184.0
48	2	500.0	35.0	20.0	6.5	96.52	202.8
49	2	350.0	40.0	140.0	7.5	98.13	152.4
50	2	200.0	35.0	100.0	6.5	91.04	169.2
51	2	500.0	35.0	100.0	8.5	94.03	178.1
52	2	500.0	35.0	100.0	4.5	85.21	175.3
53	2	650.0	40.0	60.0	7.5	97.88	162.1
54	2	650.0	40.0	140.0	5.5	97.04	170.2
55	2	650.0	40.0	60.0	5.5	93.01	165.5
56	2	500.0	35.0	100.0	6.5	100.45	204.2

The second-order equation can quantitatively describe the relationship between the responses and independent variables. So, the EF can be predicted at any point within the factor domain, even though that point has not been included in the design, after fitting EF equation by a least-squares regression. EF equation is $EF = C_{\text{drop}}/C_0$; which C_{drop} and C_0 are concentration of the analyte in the sedimented droplet and the initial concentration of the analyte in the aqueous sample, respectively. The mathematical model obtained for the EF, was as:

$$R^2 = 499.634 + 0.113505F_1 - 16.3058F_2 - 0.704609F_3 + 36.5906F_4 + 0.0221667F_1F_4 + 0.148458F_2^2 + 0.195F_2F_4 + 0.094375F_3F_4 - 5.18229F_4^2$$

The experimental data shows a good accordance with the second-order polynomial equations. The coefficients of determination (R^2) were more than 0.9 for EF, which were statistically acceptable at $p < 0.05$ levels. The backward regression method by Statgraphics verified the results, also. The liminal value for R^2 within acceptable limits was 0.90, which indicated good correlating ability for the model and

suitable accordance with the experimental data. Thus, the procedure demonstrates the ability of the model to work as a predictive tool.

The three-dimensional (3D) response surfaces plots relating independent variables with EF were produced also by Statgraphics. The response plots are constructed with two variables kept at their central levels, and varying the others over the experimental ranges. Four samples of the plots are shown in Fig. 2.

Based on the results presented in Fig. 2, EF increases with increasing the disperser volume and TSCC4P amount similar to the result obtained by the one-at-a-time strategy. Reversely, EF decreases by increasing the extractant volume. However, extractant volume cannot be too low, due to the decrease the volume of the microdroplet and its handling difficulties in very low volumes. On the other hand, it is necessary to obtain a constant volume of the sedimented phase in all experiments by keeping the disperser volume constant. Finally, Statgraphics established the following experimental conditions (according to the overall results of optimization): disperser (methanol) volume: 714.2 μl ; extractant (1,2-dichlorobenzene) volume:

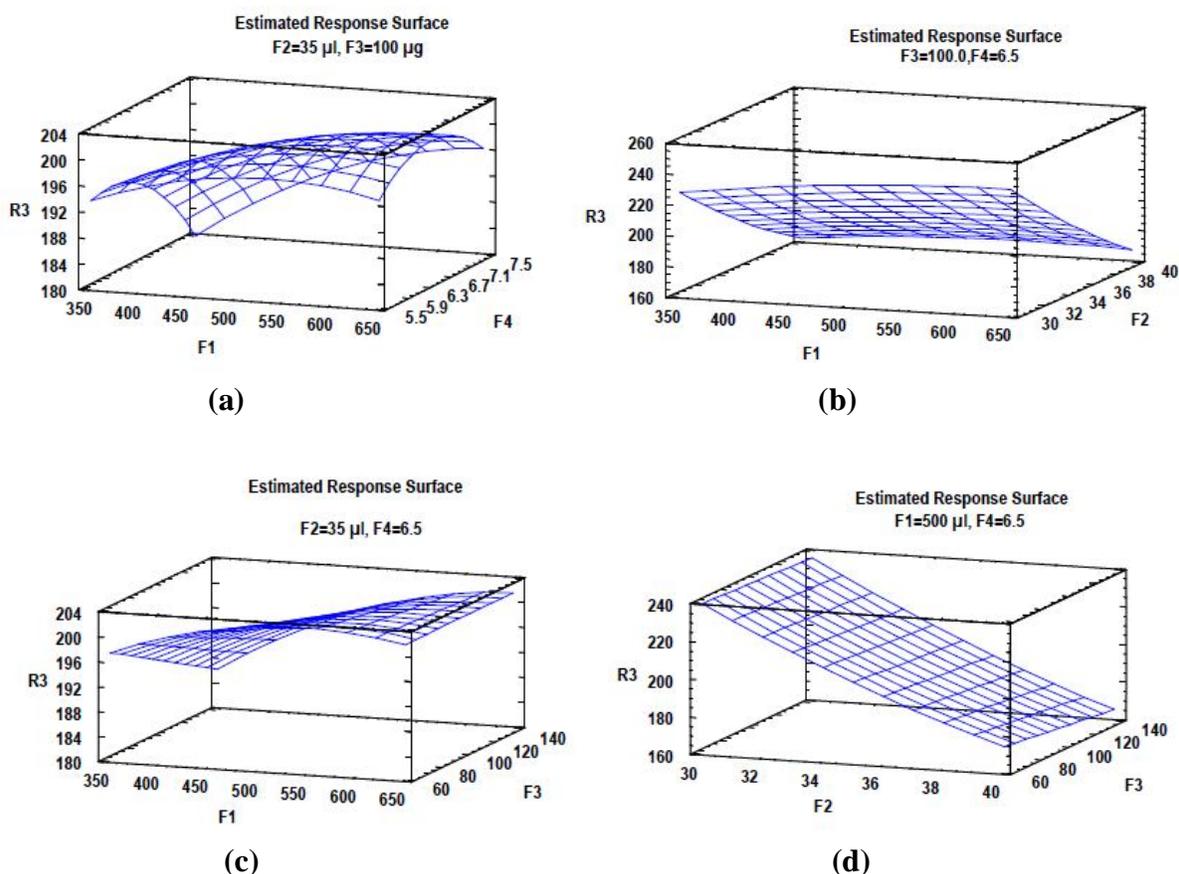


Fig. 2. Plots of response surfaces with the central composite design obtained for: a) volume of disperser vs. pH (volume of extractant, 35 µl; TSCC4P amount, 100 µg), b) volume of disperser vs. volume of extractant (TSCC4P amount, 100 µg; pH, 6.5), c) volume of disperser vs. TSCC4P amount (volume of extractant, 35 µl; pH, 6.5), and d) volume of extractant vs. TSCC4P amount (volume of disperser, 500 µl; pH, 6.5).

25 µl; amount of TSCC4P: 127.1 µg; and pH: 6.47. Under the proposed conditions, the observed experimental EF and extraction recovery were found to be 292 and 96%, respectively.

Analytical Performances

The effect of some important coexisting cations, which may accompany silver ions in real samples, was investigated. For this purpose, 5.00 ml portions of sample solution, containing 10 ng ml⁻¹ of silver and various amounts of interfering ions were examined by the proposed DLLME-GF-AAS method. Coexisting species with ±5% variation in GF-AAS signal were considered as interfere.

The results obtained are summarized in Table 4.

For quantitative analysis, a calibration graph with concentrations ranging over four orders was obtained for the proposed DLLME-GF-AAS method. Under the optimized conditions, linearity was observed over the range of 0.1-10 ng ml⁻¹ with equation of "A = 0.0257C_{Ag(I)} + 0.075" and determination coefficient (R²) of 0.9982. The limit of detection, calculated from LOD = 3S_B/m (where S_B and m are standard deviation of the blank response and slope of the calibration graph, respectively) was found to be 0.020 ng ml⁻¹. Relative standard deviation (RSD%) for ten replicate measurements of 10 ng ml⁻¹ of Ag(I) was 3.1%. The extraction recovery was found to be 96% and total time

Table 4. Effect of Coexisting Ions on the Recovery Percent of Ag⁺ (10 ng ml⁻¹) in Water Samples Using the Proposed DLLME-GF-AAS Method

Ion	Concentration (ng ml ⁻¹)	Ion/Ag(I)	Recovery (%)
Na ⁺	10,000	1000	98.2
K ⁺	4,000	400	99.4
Ca ²⁺	4,000	400	98.7
Mg ²⁺	4,000	400	100.1
Cr ³⁺	3,000	300	101.2
Zn ²⁺	3,000	300	96.5
Fe ²⁺	2,000	200	97.2
Pd ²⁺	2,000	200	98.1
Al ³⁺	2,000	200	95.5
Co ²⁺	1,000	100	100.7
Ce ³⁺	1,000	100	98.3
Ba ²⁺	1,000	100	97.4
Cd ²⁺	1,000	100	95.4
Sr ²⁺	1,000	100	99.5
UO ₂ ²⁺	1,000	100	96.5
Ni ²⁺	1,000	100	101.4
As ⁵⁺	1,000	100	98.8
La ³⁺	1,000	100	102.5
Bi ³⁺	1,000	100	103.2
Mn ²⁺	1,000	100	102.5
Cu ²⁺	1,000	100	99.5
Tl ⁺	1,000	100	100.3
Ce ⁴⁺	1,000	100	97.4
As ³⁺	1,000	100	100.3
Th ⁴⁺	1,000	100	97.9
Ni ²⁺	1,000	100	100.9
Fe ³⁺	1,000	100	97.8
Pb ²⁺	1,000	100	100.6
Hg ²⁺	500	50	96.7
Cl ⁻	10,000	1000	97.3
H ₂ PO ₄ ⁻	10,000	1000	96.5
CH ₃ COO ⁻	10,000	1000	95.6
NO ₃ ⁻	10,000	1000	96.6
SO ₄ ²⁻	5,000	500	98.0
Br ⁻	3,000	300	99.5
Cr ₂ O ₇ ²⁻	3,000	300	99.2
VO ₃ ⁻	3,000	300	98.4
F ⁻	3,000	300	98.2
SCN ⁻	2,000	200	100.6

Table 5. Determination of Ag(I) in Synthetic and Natural Water Samples Using the Proposed DLLME-GF-AAS Method

Sample	Added (ng ml ⁻¹)	Found (ng ml ⁻¹)	Recovery (%)
Synthetic A (containing: 4000 ng ml ⁻¹ of Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺ , and 2000 ng ml ⁻¹ of Fe ²⁺ , Zn ²⁺ , Cr ³⁺)	5	4.79 ± 0.20 ^a	95.80
	7	6.77 ± 0.22	96.71
	10	9.62 ± 0.40	96.20
Synthetic B (containing: 1000 ng ml ⁻¹ of Pb ²⁺ , Fe ³⁺ , Cu ²⁺ , Mn ²⁺ , Cd ²⁺ , Co ²⁺ and 500 ng ml ⁻¹ of Hg ²⁺)	5	4.82 ± 0.20	96.40
	7	6.82 ± 0.30	97.43
	10	9.55 ± 0.53	95.50
Well water (Ghaleh-Sangi Village, near Khoramabad)	-	1.05 ± 0.06	-
	1	2.07 ± 0.12	100.98
	2	3.02 ± 0.20	99.02
	3	3.98 ± 0.21	98.27
Spring water (Sarcheshmch, Mottahare St., Khoramabad)	-	0.72 ± 0.04	-
	1	1.71 ± 0.08	99.42
	2	2.66 ± 0.10	97.79
	3	3.67 ± 0.20	98.66
River water (Khorram-Rood, Papikhaldar Village, Khoramabad)	-	0.93 ± 0.04	-
	1	1.91 ± 0.09	98.96
	2	2.91 ± 0.10	99.32
	3	3.87 ± 0.21	98.47
Tap water (Lorestan University, Khoramabad)	-	0.58 ± 0.03	-
	1	1.56 ± 0.08	98.73
	2	2.52 ± 0.24	97.67
	3	3.57 ± 0.20	99.72

^aStandard deviation for three replicate experiments.

taken for the sample preparation step in the proposed DLLME method was less than 3 min. However, the most time-consuming step was the centrifuging of cloudy solution, about 2 min.

Analysis of Natural Water Samples

To evaluate the applicability of the proposed DLLME-GF-AAS procedure to real samples, with actual complex matrices, it was applied to concentrate and recovery of silver ions from two different synthetic samples. The obtained results (Table 5) showed that the recovery of Ag(I) ions was quantitative in both samples. The proposed method was also carried out to determine silver in four natural waters (well water, spring water, river water and tap water).

The accuracy of the determinations was verified by analysis of the four examined samples, spiked with the known amounts of Ag(I). The relative recoveries were satisfying in all cases (Table 5). These results demonstrated that the presence of different ions in synthetic samples and also in matrices of the well, spring, river and tap water samples had no significant effect on the DLLME extraction and GF-AAS determination of silver ions.

Comparison of the Proposed Method with Similar Published Procedures

A comparison of the characteristic data of the proposed DLLME-GF-AAS method and some of the reported methods for pre-concentration and determination of silver

Table 6. Comparison of the Proposed DLLME-GF-AAS Method to those Reported for Determination of Ag(I)

Preconcentration method	Analysis method	Chelating agent or reagent	Sample volume	Enrichment factor	LOD (ng ml ⁻¹)	RSD (%)	Linear range (ng ml ⁻¹)	Ref.
Proposed method	GF-AAS	<i>meso</i> -tetraspirocyclohexylcalix[4]pyrrole (TSCC4P)	5	292	0.020	3.1	0.1-10	This work
DLLME	GFAAS	Diethyldithiocarbamate (DDTC)	5	132	0.012	3.5	0.1-10	[30]
DLLME	GFAAS	Diethyldithiocarbamate (DDTC)	5	72	0.020	2.7	0.1-5	[31]
Ligandless DLLME	FAAS	-	8	16	1.2	1.5	5-2000	[32]
SI-DLLME	FAAS	Diethyldithiocarbamate (DDTC)	16.2	186	0.15	2.9	0.4-20	[33]
DLLME-SFO	GFAAS	5-(4'-dimethylamino benzylidene)-rhodanine	10	250	0.056	4.3	0.1-10	[34]
DLLME	UV-Vis	Bisindocarbocyanine chloride (BDIC) reagent of ion associate formation	5	-	30	-	70-2100	[35]
LLE	FAAS	Dicyclohexano-18-crown-6 (DC18C6) reagent of ion associate formation	5	30	13	2.5	-	[36]
Coprecipitation	FAAS	Diethyldithiocarbamate (DDTC)	-	26	0.6	2	-	[37]
CPE	ETAAS	-	15	60	0.0012	4.2	0.005-0.1	[38]
CPE	FAAS	Bis((1H-benzo [d] imidazol-2yl)ethyl) sulfane (BIES)	15	42	1.4	2.1	-	[39]
CPE	FAAS	Diethyldithiocarbamate (DDTC)	10	24	1	2.6	5-450	[40]
SPE	FAAS	Diethyldithiocarbamate (DDTC)	-	110	0.2	3.1	-	[41]
SPE	FAAS	-	50	25	1.05	-	-	[42]

are summarized in Table 6. The obtained EF by the proposed DLLME-GF-AAS method is better than those obtained by the previously reported methods [31-42]. Additionally, it was found to be superior to the reported methods by considering its sensitivity, precision, and linear range. The proposed method requires much less amounts of the sample and is quite appropriate for trace analysis applications. In this study for the first time a newly synthesized Calixarene (TSCC4P) was used for the selective separation and preconcentration of ultra-trace amounts of silver in water samples using a novel DLLME-GF-AAS method. Accordingly, the main advantages of our proposed method are high enrichment factor, good sensitivity and selectivity, rapidity, low consumption of organic solvents, and simplicity of operation.

CONCLUSIONS

A new DLLME procedure, coupled with GF-AAS, was developed and optimized with the aid of experimental design. It is a fast, sensitive and selective option for the analysis of ultra-trace amounts of silver in water samples. Response surface methodology (RSM) using central composite design (CCD) was applied as optimization strategy to obtain the best values for the different affecting experimental parameters. It offers significant analytical performance, very good sensitivity and reasonable precision. The developed method has been successfully applied for quantitative analysis of ultra-trace amounts of Ag(I) ions in natural water samples. Other important features of this method, over other methods, are low cost,

low organic solvent consumption, simplicity of operation, rapidity, selectivity and satisfactory enrichment factor. The results also demonstrated that the response surface design is a suitable method, with the least number of experimental runs, for such similar microextraction methods.

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