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Selective Homogeneous Liquid-Liquid Extraction and Preconcentration of Thallium Using a New Calix[4]Pyrrole

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Meso-tetraspirocyclohexylcalix[4]pyrrole (TSCC4P) was synthesized and investigated as a suitable selective complexing ligand for homogeneous liquid-liquid extraction and preconcentration of Tl^+ ions. Zonyl FSA (FSA) was used as a phase-separator agent under slightly acidic pH conditions. Under the optimal experimental conditions ([FSA] = 2.5% w/v, [THF] = 20.6% v/v, [TSCC4P] = 3.4×10^{-4} M and pH = 5.3), 5 μg of Tl^+ in 7 mL aqueous phase could be extracted quantitatively into 30 μL of the sedimented phase. The influence of different experimental parameters on the extraction and determination of Tl^+ were investigated. The maximum concentration factor was 233-fold. The limit of detection of the proposed method was 0.004 ng/mL. The reproducibility of the proposed method was at most 3.4%. The proposed method was applied to the extraction and determination of Tl^+ in different synthetic and human hair samples.

Key Words: **Thallium, Hair, Homogeneous Liquid-Liquid Extraction, Calix[4]pyrrole.**

INTRODUCTION

The past several years have seen a rapidly developing interest in the use of hair analysis as a screening tool in medical practice and assessing the nutritional and toxicological status of the body[1]. Human hair is an attractive biological material because of the simplicity of sampling, storing, transport and handling, as well as for the fact that it can store the information about contamination over a long period. Another advantage of this material is that some trace elements are considerably more concentrated in hair than the other biological materials, which makes analysis easier. Many investigators for monitoring environmental or occupational exposure and evaluating heavy metal poisoning have used the concentrations of toxic elements in human hair[2].

The widely used techniques for the determination of elements in hair are inductively coupled plasma atomic emission spectrometry (ICP-AES), X-ray fluorescence (XRF), neutron activation analysis (NAA), anodic stripping voltam-

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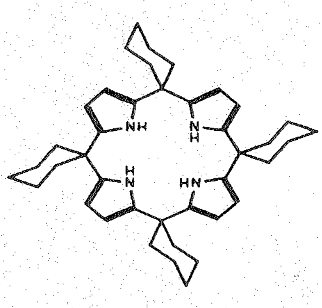
metry (ASV), flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ET-AAS)[1-3]. However, in commonly used analytical methods, separation and/or preconcentration of some trace elements is necessary. On the other hand, due to the demand for ultra-trace analysis, the need for powerful separation and preconcentration methods has increased, particularly for environmental analysis. Therefore, simple, rapid, cleaner and efficient techniques that can be performed easily are required[4-6].

Homogeneous liquid-liquid extraction (HLLE) is a simple and powerful preconcentration method that reduces the extraction time, disposal costs, consumption and exposure to organic solvents. This method extracts the solute in a homogeneous solution into a very small sedimented phase formed from the solution by the phase-separation phenomenon. In HLLE, the initial condition is a homogeneous solution, namely, the surface area of the interface is infinitely large initially. Accordingly, no vigorous mechanical shaking is necessary. The procedure is simple and requires only the addition of a reagent[7]. Recently, HLLE was successfully utilized for the extraction of some organic and inorganic analytes[8-11].

Some of calixarene derivatives containing various functional groups have been incorporated as neutral carriers into ion-selective electrodes (ISEs) sensitive to lithium[12], sodium[13], potassium[14], calcium[15] and cesium[16] ions. However, only calixarene derivatives with functional groups containing nitrogen or sulfur atoms show superior selectivity towards soft heavy metal ions, such as Ag^+, Tl^+, Pb^{2+} and Hg^{2+}, over alkali metal ions[17, 18]. Calix[4]pyrroles are the originally fully *meso*-alkyl substituted porphyrinogens that have been extensively studied as host molecules for anions[19, 20] and neutral substrates[21, 22], and as a deprotonated tetraanionic N_4 ligand that forms σ and/or π-bonds with high-valent transition-metal cations[23].

In this paper, the synthesis and application of a porphyrin intermediate, namely *meso*-tetraspirocyclohexylcalix[4]pyrrole (TSCC4P), as a new complexing ligand for the development of a simple and efficient method for the selective homogeneous liquid-liquid extraction and concentration of Tl^+ ions are reported.



Meso-tetraspirocyclohexylcalix[4]pyrrole (TSCC4P)

EXPERIMENTAL

A Shimadzu AA650 atomic absorption spectrometer equiped with a GFAEX7 graphite furnace (with high-density graphite tube cuvettes), an ASC-6100 autosampler and a D_2 continuous source for background correction was utilized for the determination of Tl^+ under the recommended condition. A jenway 4030 digital pH-meter equipped with a combined glass-calomel electrode was used for pH adjustments. An Eppendorf 5810 centrifuge was used for centrifugation. Ethanol, methanol, acetone, THF and DMSO were of analytical reagent-grade from Merck. The materials for synthesis (pyrrole, diethyl ether, chloroform and cyclohexane) were bought from Aldrich and were used as received. Zonyl FSA was used as received from Aldrich and was a mixture that contained components with carbon numbers in the alkyl group from 6–10. In this study, FSA diluted to 25% (w/v) with water was used.

$$CF_3—(CF_2)_n—CH_2—CH_2—S—CH_2—CH_2—COOH \qquad n = 6–8$$

Zonyl FSA

The pH buffer aqueous solution was prepared at pH = 5.3 by mixing appropriate volumes of 0.2 M acetic acid and 0.2 M sodium acetate solution. The analytical grade thallium(I) nitrate and other salts were of the highest purity available and were dried in vacuum over P_2O_5. Doubly distilled deionized water was used throughout. The standard stock solution of Tl^+ (1000 μg mL^{-1}) was prepared by dissolving an appropriate amount of thallium(I) nitrate in 5 mL of concentrated nitric acid and diluted to 1000 mL with water. Working solutions were prepared by appropriate dilution of the stock solution.

Synthesis of *meso*-tetraspirocyclohexylcalix[4]pyrrole

A solution of 16.2 g (0.242 mol) of pyrrole and 20 mL of absolute ethanol was placed in a three-necked 500 mL flask. The flask was fitted with a stirrer and a reflux condenser and was then placed in an ice-bath. Aqueous hydrochloric acid (1 mL, 37%) was added dropwise to the stirred solution, followed by 23.7 g (0.242 mol) of cyclohexanone over a period of 20 min. The solid formed in the flask stopped the stirring when the addition of cyclohexanone was completed. After standing at room temperature for 1 h, the product was washed with 50 mL of cold water. The solid was removed from the flask by partial dissolution in diethyl ether. Subsequent purification of the residue by column chromatography (silica gel, $CHCl_3$), afforded a novel *meso*-tetraspirocyclohexylcalix[4]pyrrole (TSCC4P) as a white powder with a crystal m.p. of 272–273°C. The identity and purity of TSCC4P was confirmed by elemental analysis, IR, ^1H NMR and ^{13}C NMR spectrometry. IR (KBr, cm^{-1}): 3420 and 3390 ν(NH, pyrrole), 3030 ν(CH, aromatic), 2950 ν(CH, aliphatic), 1580, 1480, 1440, 1380; ^1H NMR ($CDCl_3$, 250 MHz : $\delta = 7.13$ (H_β, pyrrole), 5.98 (NH, pyrrole), 1.99 and 1.55 (H, cyclohexyl); ^{13}C NMR ($CDCl_3$, 62.9 MHz): $\delta = 137$ (C_α, pyrrole), 104 (C_β, pyrrole), 40, 37, 26, 23 (C, cyclohexyl)[24].

Homogeneous liquid-liquid extraction and quantification of Tl^+

A sample solution (7 mL) containing 5 μg of Tl^+, 2 mL of THF containing 2.0 mg of TSCC4P and 1 mL of 25% (w/v) FSA aqueous solution were placed in a 50 mL cylindrical glass vial fitted with a plastic cap. An acetic acid-sodium acetate buffer solution (1 mL) was then added in order to adjust the pH value of the mixture to 5.3. Thus, the final concentrations were [TSCC4P] = 3.4×10^{-4} M, [THF] = 20.6% v/v, [FSA] = 2.5% (w/v) and pH = 5.3. The mixture was allowed to stand for 10 min at room temperature and then centrifuged at 2500 rpm for 15 min. The volume of the sedimented phase was determined using a 100 μL microsyringe and transferred directly into the cuvette (graphite tube) of ET-AAS instrument. The Tl^+ concentration was then determined against a reagent blank using a suitable external linear calibration curve.

Determination of Tl^+ in human hair samples

The sample of hair were cut from the same zone of scalp for all categories (6 samples), namely from the occipital region at the distance of *ca.* 1 cm from the scalp. 1–2 g of hair samples was collected in polyethylene bags, which were thoroughly closed and labelled. Cleaning of hair is considered to be a critical step and of major importance with respect to the analytical procedure. Hair samples were washed by a standard procedure proposed by the International Atomic Energy Agency[1]. 0.5 g of the sample was agitated with 25 mL of acetone, then three times with double distilled water and finally with 25 mL of acetone. The contact time of the cleaning medium with the sample was 10 min. The samples were finally dried for 16 h at 110°C.

Different methods were used for digestion of hair. Among the tested methods, digestion with concentrated HNO_3 and H_2O_2 was most effective with minor interferences for the drop formation. Thus 0.5 g of the sample was treated with 5 mL of concentrated HNO_3 and 5 mL of H_2O_2 for 1.5 h at 100°C. The remaining solution was then heated on an electrical heater until just before drying for complete evaporation of HNO_3 and H_2O_2. Finally, the residual was washed with double distilled water and diluted to 50 mL in a volumetric flask.

The thallium content of a 7 mL portion of the thus-treated aliquot was extracted using the proposed method into a drop (sedimented phase) and then injected directly into the cuvette of ET-AAS spectrometer and quantified using an appropriate linear calibration graph.

RESULTS AND DISCUSSION

Previous studies showed that *meso*-alkyl substituted calix[4]pyrroles can act as cation sensor materials and some calix[4]pyrroles-based thallium and silver ion-selective membrane electrodes[25] have exhibited excellent electrochemical response characteristics and selectivity for Tl^+ and Ag^+. Preliminary experiments showed that Tl^+ ions were sufficiently extracted from a solution with slightly acidic medium in a system containing THF and FSA as a phase-separator agent. The proposed method was examined as a selective separation and preconcentration HLLE method for the ET-AAS determination of Tl^+ ions.

Effect of the pH

A fluorosurfactant, such as HPFOA ($pK_a = 1.01$, 20°C, $I = 0.1$) or Zonyl FSA ($pK_a = 6.5$, 50% v/v THF), dissolves in water at a pH value higher than its acid dissociation constant of the carboxylic acid group. If the pH of this aqueous solution is lower than its acid dissociation constant, the fluoro-surfactant precipitates as a needle-like crystalline solid due to the charge neutralization of the carboxyl ion. However, if a small amount of water-miscible organic solvent, such as THF, DMSO, DMF, dioxane, acetone or acetonitrile, coexists in this system, the fluoro-surfactant precipitates in the water-miscible liquid phase with a μL volume scale. The solute is then extracted into the sedimented phase[7].

In order to investigate the influence of the pH on the HLLE of Tl^+ ions, the pH of aqueous samples was varied over the range of 3.0–6.0 (Fig. 1), by using 1.0 M solutions of HNO_3 or NaOH, and the recommended procedure was followed. The results showed that the highest extraction percentages were obtained in the pH range of 4.7–5.5. Due to the dissolution of FSA, no precipitated phase was formed at pH values greater than 6.5. Thus, a pH value of 5.3 was used for further studies. An acetic acid-sodium acetate buffer solution was used in order to adjust the pH value to 5.3.

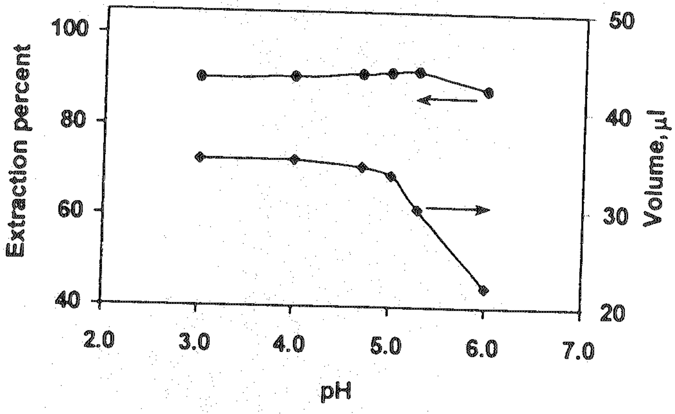


Fig. 1. Effect of the pH on the per cent extraction and the volume of the sedimented phase (5 μg of Tl^+ ions in 7 mL of sample solution)

Effect of nature and volume of the water-miscible organic solvent

Different organic solvents, such as THF, DMSO, acetone, ethanol and methanol, were tested for the HLLE of Tl^+ ions using TSCC4P. TSCC4P has limited solubility in DMSO, ethanol and methanol. On the other hand, using of acetone results in a solid sedimented phase. The application of THF as a water-miscible organic solvent causes complete phase-separation with the least amount of solvent, resulting in a viscose spherical drop suitable for handling with a microsyringe. Thus, THF was selected for subsequent experiments. Furthermore, the volume of THF was optimized. Hence, the volume per cent of THF was varied over the range of 10–35% v/v (Fig. 2). It was found that the extraction per cent of Tl^+ was relatively independent of the volume per cent of THF in the range studied. However, the volume of the sedimented phase increased with increasing the volume per cent of THF. The high volume percentages resulted in a high-volume sedimented phase and a low concentration factor and a low-volume percentage caused a solid sedimented phase, instead of an oily drop. Hence, a 20.6 volume per cent of THF was used as the optimal volume for the HLLE of Tl^+ ions using TSCC4P.

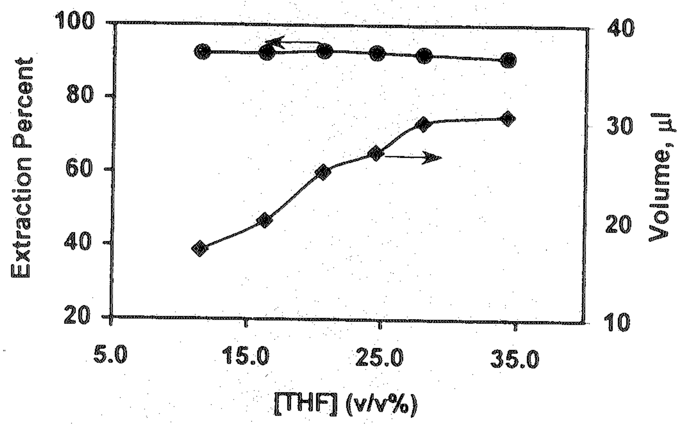


Fig. 2. Effect of THF concentration on the per cent extraction and the volume of the sedimented phase (5 µg of Tl^+ ions in 7 mL of sample solution, pH = 5.3)

Effect of the FSA concentration

In order to investigate the optimum amount of FSA on the quantitative HLLE of Tl^+ ions using TSCC4P, the extraction of 5 µg of Tl^+ from 7 mL of the sample solutions was conducted by varying the concentration of FSA (Fig. 3). As can be seen, the extraction of Tl^+ ions is relatively quantitative in the range of 2.0–5.0% (w/v) of FSA. Hence, subsequent HLLE experiments were carried out with 2.5% (w/v) of FSA.

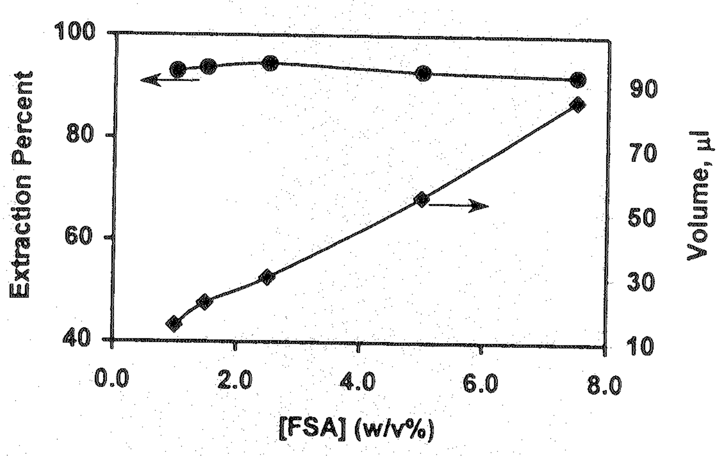


Fig. 3. Effect of FSA concentration on the per cent extraction and the volume of the sedimented phase (5 µg of Tl^+ ions in 7 mL of sample solution, [THF] = 20.6% v/v and pH = 5.3)

Effect of the TSCC4P concentration

The effect of the TSCC4P concentrations on the extraction percentage of Tl^+ is shown in Fig. 4. The results show that the extraction per cent of Tl^+ ions was quantitative for TSCC4P concentrations higher than 2.6×10^{-4} M. In very high concentrations of TSCC4P, phase-separation occurred precociously before the extraction was completed and some solid sediment around the drops remained. Thus, a concentration of 3.4×10^{-4} M of TSCC4P was used for further studies.

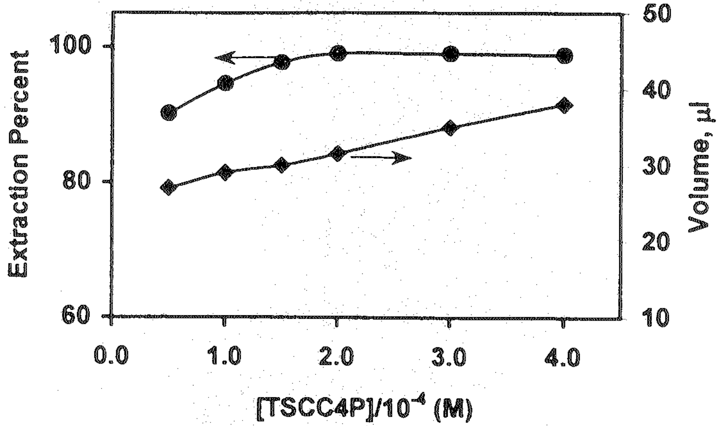


Fig. 4. Effect of TSCC4P concentration on the per cent extraction and the volume of the sedimented phase (5 µg of Tl^+ ions in 7 mL of sample solution, [THF] = 20.6% v/v, [FSA] = 2.5% w/v and pH = 5.3)

Analytical performance

When 5 µg of Tl^+ ions in 7–50 mL of solutions were examined under the optimal experimental condition ([FSA] = 2.5% w/v, [THF] = 20.6% v/v, [TSCC4P] = 3.4×10^{-4} M and pH = 5.3) using the proposed method, the extraction was quantitative in all cases. The maximum concentration factor of the proposed method, under the optimal experimental conditions, was 233-fold (*i.e.*, 30 µL of the sedimented phase was produced from 7 mL of the sample solution).

In order to investigate the selective extraction and determination of Tl^+ ions using TSCC4P from binary mixtures with various metal ions, and aliquot of a solution (7 mL) containing 5 µg of Tl^+ ions and different amounts of other cations were taken and the recommended procedure was followed. The results (Table-1) show that the Tl^+ ions in the binary mixtures were extracted almost completely, even in the presence of up to 10 mg of some cations.

TABLE-1
SEPARATION OF Tl^+ IONS FROM BINARY MIXTURES USING TSCC4P[a]

Diverse ion	Amount taken (µg)	Recovery of Tl^+ (%)	Diverse ion	Amount taken (µg)	Recovery of Tl^+ (%)
Na^+	10000	96.9	Ni^{2+}	2000	98.0
K^+	5000	98.8	Zn^{2+}	2000	98.5
Ca^{2+}	2000	97.0	Cr^{3+}	2000	97.4
Mg^{2+}	2000	99.1	Cu^{2+}	1000	95.8
Sr^{2+}	2000	98.8	Pb^{2+}	1000	95.8
Mn^{2+}	2000	97.3	Al^{3+}	1000	95.7
Fe^{3+}	2000	97.5	UO_2^{2+}	200	96.6
Co^{2+}	2000	96.6			

[a] 5 µg of Tl^+ ion in 7 mL of sample solution ([FSA] = 2.5% (w/v), [THF] = 20.6% (v/v), [TSCC4P] = 3.4×10^{-4} M and pH = 5.3).

The limit of detection (LOD) of the proposed method for the determination of Tl^+ ion was studied under the optimal experimental conditions. The LOD obtained from 3σ of the blank and dividing the resulting value to the concentration factor (*i.e.*, 233) was 0.004 ng/mL. The reproducibility of the proposed method for the HLLE and determination of 5 μg of Tl^+ ion from 7 mL of the sample solution was also studied. The results obtained in 10 replicate measurements revealed an R.S.D. of 3.4%.

To evaluate the applicability of the proposed method to samples with different matrices, it was applied to the separation and recovery of Tl^+ ions from three different synthetic samples (Table-2). The results show that in all samples, the Tl^+ ion recovery was quantitative. The proposed method was also applied for the determination of Tl^+ ion in human hair samples. A satisfactory agreement exists between the results obtained by the proposed method and those reported by ICP-AES (Table-3).

TABLE-2

RECOVERY OF 5 μg Tl^+ ION FROM THE THREE SYNTHETIC SAMPLES[a]

Synthetic sample	Recovery (%)
A: Na^+, K^+, Ca^{2+} and Mg^{2+}, 1 mg of each cation	98.2 (1.7)[b]
B: Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+} and Mn^{2+}, 0.5 mg of each cation	97.8 (2.1)
C: Na^+, K^+, Ca^{2+} and Mg^{2+}, 0.5 mg of each cation and Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+} and Mn^{2+}, 0.25 mg of each cation	98.0 (2.8)

[a] 7 mL of each sample solution ([FSA] = 2.5% (w/v), [THF] = 20.6% (v/v), [TSCC4P] = 3.4×10^{-4} M and pH = 5.3) was taken.

[b] Values in parentheses are %R.S.Ds. based on three replicate analyses.

TABLE-3

DETERMINATION OF THALLIUM IN DIFFERENT HUMAN HAIR SAMPLES (ARSHIA BARBERS SHOP, KHORRAM-ABAD, SEPTEMBER 2004)[a]

Hair sample	Thallium/Hair sample (μg/g)	
	Proposed method	ICP-AES
A: Age: 30, Gender: Male, Smoking: No, Fish consumption: Medium	0.99 (3.5)[b]	0.84 (1.0)
B: Age: 18, Gender: Male, Smoking: No, Fish consumption: Medium	0.74 (2.8)	0.59 (2.1)
C: Age: 15, Gender: Male, Smoking: No, Fish consumption: High	0.43 (3.2)	0.64 (3.4)
D: Age: 33, Gender: Male, Smoking: High, Fish consumption: Low	0.68 (2.2)	0.92 (1.8)
E: Age: 50, Gender: Male, Smoking: High, Fish consumption: Low	0.84 (2.1)	0.69 (1.0)
F: Age: 28, Gender: Male, Smoking: Medium, Fish consumption: Medium	0.45 (3.1)	0.48 (2.8)

[a] 7 mL of each sample solution ([FSA] = 2.5% (w/v), [THF] = 20.6% (v/v), [TSCC4P] = 3.4×10^{-4} M and pH = 5.3) was taken.

[b] Values in parentheses are % R.S.Ds. based on three replicate analyses.

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