

Cloud Point Extraction Coupled to Flame Atomic Absorbance Spectroscopy for Cobalt (II) determination with Azo-Azomethine Dye

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Abstract— The azo-azomethine dye 6,6'-((1E,1'E)-((4-methyl-1,2-phenylene)bis(azanylylidene))bismethanylylidene))bis(2-methoxy-3-(o-tolyldiazenyl) phenol) (L₃) were synthesized and characterized by UV-visible, FT-IR, ¹H-NMR and Mass spectroscopy. The effect of the several factors on the CPE pre-concentration of Cobalt (II) with L₃ was optimized. Extensive thermodynamic study has been presented to understand the mechanism of extraction and solubilization of studied complex in Triton X-100 micelles. Under the optimized conditions, enrichment factor of 54 is achieved leading to limit of detection and limit of quantitation of 11.5 and 38.4 ngmL⁻¹ respectively. Under the optimal conditions the calibration plot is linear in the range of 0.025-3 µg mL⁻¹, the precision (RSD%; n=8) of the proposed method is of 0.823% at 0.05 µg mL⁻¹ of Co (II). This method is applied to the determination of Co (II) in various environmental samples.

Keywords— Cobalt (II), azo-azomethine dye, complex, cloud-point extraction, FAAS, real samples analysis.

I. INTRODUCTION

The potential of micellar systems as an alternative to organic solvent have extensively introduced in the extraction and pre-concentration of metal ions and organic compounds and became one of the most preferred pre-concentration steps for enhancing the sensitivity in inorganic and organic analysis and an important practical application in the use of surfactants in analytical chemistry [1]. In principle, cloud point extraction (CPE) depends on the separation of hydrophobic analyte between two phases generated from non-ionic surfactant solution when heated to its critical cloud point temperature (CPT). One phase is called a surfactant-rich phase (SRP) with a small volume containing the analyte trapped by organized micelles, and other phase is called surfactant-poor phase (SPP) or bulk aqueous phase containing a little surfactant molecules at critical micelle concentration (CMC) [2].

The importance of the determination of trace metal concentration in natural water samples is increasing in contamination monitoring studies. The determination of metal ions traces in biological samples is particularly

difficult because of the complex matrix and the usually low concentration of these elements in such samples, which requires sensitive instrumental techniques and frequently a pre-concentration step [3]. Cobalt is an essential trace element which is present in most body tissues, with the highest concentrations typically found in the liver. It is a component of vitamin B12 (cyanocobalamin), which is involved in the production of the blood red cells and the prevention of pernicious anemia. Cobalt toxicity is quite low compared to many other metals. However, high exposure to this element can cause several health problems such as asthma and skin irritation. Therefore, quantitative determination of cobalt at low concentrations plays an important role in different fields such as environmental analysis, process control and medicine [4]. Some of heavy metals like cobalt is necessary for human life [5]. Most of spectrophotometric methods developed for the determination of cobalt are, unfortunately, not practically usable due to poor sensitivity and partly poor selectivity. Routine spectrophotometric methods are not often sensitive enough to determine low concentrations of cobalt ions in environmental samples only at the µg L⁻¹ level. Consequently, a pre-concentration step is usually required. In spite of recent advances in instrumental analysis, a direct determination of trace elements in complex matrices, such as environmental, biological, mineral, ore and high purity materials, seems still to be difficult because of insufficient sensitivity and selectivity of the methods. Thus, enrichment and separation of the analytes are important for the determination of Co (II), because of its extremely low concentration in natural water. Cobalt determination at trace levels in real samples has been frequently difficult because of low concentration of the metal and matrix interferences [6]. Flame atomic absorption spectrometry (FAAS) is one of the techniques most extensively used for determining various elements with a significant precision and accuracy. This analytical technique is remarkable for its selectivity, speed and fairly low operation cost [7].

In the present study, an attempt has been performed to establish a new procedure for the separation



and extraction of Co (II) by CPE methodology and its detection by FAAS.

II. MATERIALS AND METHODS

A. Apparatus

The UV-Vis spectrophotometer model T 60 (PG Instruments Ltd, Germany) was used for absorbance measurements. The IR spectra were recorded by using FTIR-8400S (Shimadzu, Japan). Mass spectra are recorded using an Agilent Technology (HP) / MS Model 5973 Network Mass Selective Detector. The $^1\text{H-NMR}$ in deuterated DMSO as solvent was recorded on Bruker DMX-500 Spectrophotometer-300 MHz with TMS. Flame Atomic Absorption Spectrophotometric AA-500 AFG (PG Instruments, Japan) was used for Cobalt (II) detection in aqueous solution and in surfactant rich phase.

B. Reagent and materials

All analytical grade reagents were used without further purification. Deionized water was used for diluting the standard, reagents and samples. The nonionic surfactant (Triton X-100) was purchased from Sigma (Sigma Ultra, >99.6% (UK)). Cobalt chloride (II) hexahydrate (97%), *O*-toluidine (99%), NaOH (99%), HCl (37%), NaNO_2 (99%) were purchased from BDH and 3,4-diaminotoluene (97%) from BDH. The $1000 \mu\text{g mL}^{-1}$ stock solution of Co (II) at was prepared by dissolving of 0.4038 g of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in a minimum amount of deionized water in 100 mL volumetric flask and completed to mark with deionized water and mixed thoroughly. A stock solution of $6,6'-((1E,1'E)-((4\text{-methyl-1,2-phenylene})\text{bis}(\text{azanylylidene}))\text{bis}(\text{methanylylmidene}))\text{bis}(2\text{-methoxy-3-(o-tolyldiazanyl)phenol})\text{(L}_3\text{)}$ reagent solution at concentration of $1 \times 10^{-2} \text{ M}$ was prepared by dissolving 0.6267 g in minimum amount of DMSO in 100 mL volumetric flask and diluted to mark with DMSO. Other standard solutions were prepared by dilution with the same solvent.

C. Synthesis of Azo-azomethine and Characterization of Reagents

A2-hydroxy-3-methoxy-4-(o-tolyldiazenyl) benzaldehyde (AZ_3) reagent was prepared by dissolving (2.143 g, 0.02 mol) of *O*-toluidine in mixture containing 30 mL distilled water and 5mL of concentrated hydrochloric acid and cooled to (0-5)°C. The solution was diazotized at (0-5) °C with (1.38 g, 0.02 mol) sodium nitrite which was dissolved in 20 mL of distilled water. Sodium nitrite solution was added dropwise to amine solution and stirring continuously at (0-5) °C and left to stand 30 min. This diazonium solution was added to *O*-vanillin (3.043 g, 0.02 mol) which was dissolved in mixture of 25 mL ethanol and 30 mL of 6% sodium hydroxide. The mixture was stirred continuously for 2h at (0-5) °C in ice-bath. After that the solution was acidified with dilute hydrochloric acid to pH 6 and allowed to stand in the refrigerator overnight. The precipitate was filtered and washed with distilled water and ethanol to remove the excess of unreacted substances. The obtained AZ_3 was recrystallized from ethanol and dried in oven at 50°C for two hours [8]. The purity was confirmed by TLC technique. Yield 80.37%, mp 142-144°C.

The azo-azomethine ligand was synthesized by condensation reaction (Fig. 1) of 3,4-diaminotoluene with AZ_3 by dissolving (2.703 g, 0.01 mol) of AZ_3 in 20 mL absolute methanol then mixed with a solution (0.611 g, 0.005 mole) of 3,4-diaminotoluene dissolved in 10 mL of the same solvent with the addition of four drops of glacial acetic acid as a catalyst followed by reflux for 7 hours. The reaction was followed using TLC [9]. The light brown precipitate was formed. It was filtered, dried and recrystallized from absolute ethanol. Yield 78.13%, mp 253-254°C.

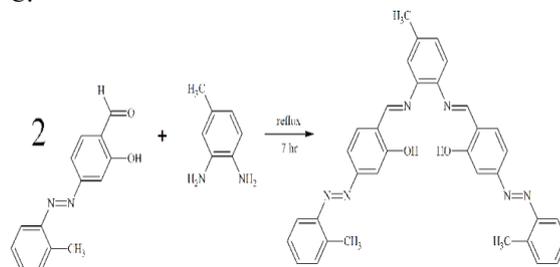


Fig. 1. Reagent synthesis

D. General Procedure for CPE [10]

A typical CPE experiment required the following steps: an aliquot of 10 mL of a solution Co (II) containing solution, 1 mL of $1 \times 10^{-3} \text{ M}$ of L_3 reagent solution, 0.9 mL of 1% Triton X-100 were mixed. The mixture was shaken for 1 min then left to stand for 20 min controlled by thermostated bath at 50°C until formation the cloud solution. Separation of the two phases was achieved immediately when the surfactant-rich phase became very viscous without centrifugation or cooling and settled at the bottom of the tube, allowing the aqueous phase to be easily decanted. After then, the surfactant-rich phase in the tube was dissolved in 1mL of 1 M methanolic HNO_3 solution and the Co atomic absorbance was measured at 270.72 nm using double-beam Atomic Absorption Spectrophotometer AA-500AFG equipped with air-cooled 10 cm slot titanium burner unit premixed for air/acetylene flame. For the determine remainder quantity of Co(II) in aqueous solution after extraction by subtraction quantity of Co(II) measured in the surfactant-rich phase from original quantity, afterward calculate Distribution ratio (D) from the following equation:

$$D = \frac{[\text{M}^{n+}]_{\text{cpL}}}{[\text{M}^{n+}]_{\text{aq}}} \dots \dots \dots (1)$$

Where

$[\text{M}^{n+}]_{\text{cpL}}$: metal ion concentration that transferred to micelle phase

$[\text{M}^{n+}]_{\text{aq}}$: metal ion concentration that remained (residual) in aqueous phase.

E. Application of real samples

The present CPE method was applied for determination of Co (II) ion in river water, tap waters, liquid milk, black tea, tobacco and soil samples. River water was collected from Al-Refaie / Thi-Qar, tap water was taken from Laboratory, liquid milk, black tea, tobacco samples were taken from submarket.

a) Water samples

To remove suspended particulate matter, water sample were filtered through Whatman No. 42 and stored in glass bottles at 4 °C before analysis. 15 mL of it was treated according to the general procedure for CPE. The levels of analyte ion in the samples were determined by FAAS.

b) Raw cow's milk sample

The concentrations in sample were analyzed according to the procedure outlined by Peters [11]. The milk samples need to be brought in to clear solution for analysis by FAAS. For this reason the sample first digested to dissolve the sample and to remove the fat. 1.00 g of raw cow's milk is treated with 5mL (65% nitric acid) and 2mL (30% hydrogen peroxide) and then digested on electric hot plate at 90 °C and the temperature of this mixture was gradually increased to 120 °C. Until brown fumes appeared, indicating completion of oxidation of organic matter. The organic matrix of milk was destroyed and left the elements in to clear solution, after cooling the clear solution was filtered in to 25mL volumetric flask and completed to the mark with deionized water [12]. 15mL of it was treated according to the general procedure for CPE.

c) Tea sample

Accurately weighed 1g of tea sample was transferred in to a crucible and kept in a muffle furnace for ashing at 450 °C for 3 hours and then 5 mL of 6M HCl was added to the crucible. Further, the crucible containing acid solution was kept on a hot plate and digested to obtain a clean solution. The final residue was dissolved in 0.1M HNO₃ solution and made up to 25 mL [13]. 15mL of it was treated according to the general procedure for CPE.

d) Tobacco sample

The cigarette tobacco was prepared by removing the papers and filters from 60 cigarettes of 3 packs. The samples were dried in an oven at a temperature of 80 °C for 6 hours and allowed to cool in a desiccator. The dried tobacco was ground in a mortar with a pestle until powdered finely as much as possible for homogenization, to simplify weighing and to facilitate organic matter destruction (digestion).

The digestion procedure of Campbell and Plank was used. 1g of dried, ground, and homogenized cigarette tobacco was transferred to crucible. 5mL of concentrated HNO₃ acid was added and the crucible was covered with watch glass and allowed to stand overnight. The covered crucible was placed in a muffle furnace with temperature controller and heated at 200°C for 30 min. The crucible was removed and allowed to cool and 2 mL of 30% H₂O₂ was added and digested at the same temperature and time in the same way. This step was repeated to ensure complete digestion. The digest was allowed to dry to 1 to 2 mL at 150 °C. Then 5mL of 1% HNO₃ was added to digest residue and filtered quantitatively through Whatman filter paper No.40 into a 25mL volumetric flask and made up to volume with deionized water [14]. 15 mL of it was treated according to the general procedure for CPE.

e) Soil sample

1g of the soil sample was weighed into a 50 mL beaker and 9 mL of 65% (v/v) HNO₃, 4 mL of 50% (v/v) HF, 1 mL of 37% v/v HCl and 2 mL of 75% v/v HClO₄

were then added and digested at 135 °C for 2 h. The digest was slowly evaporated until the white fumes of HClO₄ appeared. The digest was filtered and made up to 25 mL with deionized water [15]. 15mL of it was treated according to the general procedure for CPE.

F. Slope Analysis for Stoichiometric Determination of Complex

The method involved the extraction of Co (II) ion as from 10 mL aqueous solution containing constant amount of 3 µg mL⁻¹ of Co (II) ion, 1mL of 0.1M of HCl, 0.5mL of Triton X-100 and increasing concentrations of L₃ ranging from (5×10⁻⁴ to 1×10⁻² M). The experiments were subjected to the optimum conditions established by general CPE procedure. After separation, the surfactant-rich phase which contains complex at each concentration ratio was diluted with 1mL of 1M methanolic solution of HNO₃ and the absorbance of each solution was measured at 270.72 nm by FAAS.

III. RESULTS AND DISCUSSION

A. The L₃ reagent characterization

The UV-Vis spectra for the synthesized reagent L₃ presented on Fig. 2, show two distinct absorption peak, the first at wavelength 288nm due to π-π* in aromatic ring [14] and the second peak at 402 nm due to interior intra molecular charge transfer n- π* [15,16] which is belong to the -N=N- and C= N groups.

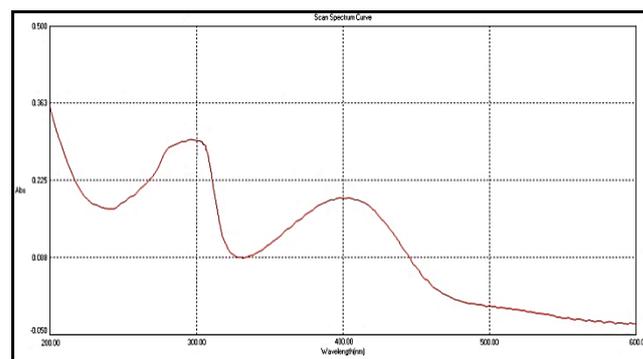


Fig. 2. UV-VIS spectrum for dye (L₃), (C = 1×10⁻³ M in DMSO, pH 6, l = 1 cm)

The IR spectrum was obtained for L₃ ligand in KBr pellets. The spectra show absorption band in 3208 cm⁻¹ belongs to OH [17]. The absorption band at 1639 cm⁻¹ belong to stretching vibration of C=N of imine [15] and a strong band in the range at 1465 Cm⁻¹ due to the stretching vibration of the (N=N) [18,17]. The C=C appear stretching vibration at 1582 Cm⁻¹ [18]. But stretching vibration for the C-H aromatic appear weak band at 3059 cm⁻¹ [18], absorption band at 2970 Cm⁻¹ due to C-H aliphatic [15,19].

The ¹H-NMR spectrum of L₃ was recorded in DMSO [16,20]. ¹H NMR: δ 1.90-2.01 (6H, 1.95 (s), 1.96 (s)), 2.19 (3H, s), 3.76-3.89 (6H, 3.81 (s), 3.84 (s)), 6.93-7.55 (15H, 6.99 (d, J = 8.5 Hz), 7.00 (d, J = 8.4 Hz), 7.09 (dd, J = 8.4, 1.9 Hz), 7.09 (d, J = 8.5 Hz), 7.09 (d, J = 8.4 Hz), 7.19 (dd, J = 8.4, 0.5 Hz), 7.27 (dd, J = 1.9, 0.5 Hz), 7.34 (ddd, J = 6.7, 1.3, 0.4 Hz), 7.37 (ddd, J = 8.0, 1.5, 0.4 Hz), 7.42 (ddd, J = 6.7, 6.5, 1.6 Hz), 7.44 (ddd, J = 6.5, 6.2, 1.3 Hz), 7.45 (ddd, J = 6.5, 6.2, 1.5 Hz), 7.45 (ddd, J = 6.2, 1.5,

0.4 Hz), 7.48 (ddd, $J = 8.0, 6.5, 1.5$ Hz), 7.47 (ddd, $J = 6.2, 1.6, 0.4$ Hz), 8.88-9.07 (2H, 8.93 (s), 9.02 (s)).

The mass spectrum of the L_3 ligand was recorded and the obtained molecular ion (m/z) peaks confirm its proposed formula and geometry. The ligand shows a peak at $M^+ = 626$ corresponding to the molecular ion peak ($C_{37}H_{34}N_6O_4$). It also showed a series of peaks at $m/z = 535, 254, 236, 146, 133, 119, 105, 91$ and 77 corresponding to $[C_{30}H_{27}N_6O_4]^+$, $[C_{15}H_{14}N_2O_2]^+$, $[C_{15}H_{13}N_2O]^+$, $[C_8H_6N_2O]^+$, $[C_7H_5N_2O]^+$, $[C_7H_7N_2]^+$, $[C_7H_7N]^+$, $[C_7H_7]^+$ and $[C_6H_5]^+$ respectively.

B. Optimization of CPE procedure

The effects of several experimental parameters which impact the CPE efficiency were carried out by classical optimization (one-variable-at-a-time). In this approach, we observe the effect of one factor at a time (OVAT) on an experimental response. While only one factor is changed, others are kept at a constant level. Although, the "optimization" performed by OVAT [21] does not ensure at all that the real optimum will be conformed, but it would be valid only if the variables to be optimized would be totally independent from each other (i.e. no interactive effects among the variables). Nevertheless, the classical optimization certainly leads at least to an improvement of the analytical method. Inasmuch as the extraction efficiency of the CPE depends on dual factors, some of regarding the prior formation of a complex with sufficient hydrophobicity and the other for the formation of micelles to obtain the desired separation and pre-concentration. Consequently, the effects of ligand concentration, HCl concentration, incubation time, equilibration temperature, Triton X-100 volume were selected in this study.

a) Effect of reagent Concentration

The variation of absorption signal as a function of reagent concentration was investigated for 10 ml solution containing $3 \mu\text{g mL}^{-1}$ Co (II), 0.5 mL of 1% Triton X-100, 0.1M HCl and varying concentrations of ($1 \times 10^{-4}, 5 \times 10^{-4}, 1 \times 10^{-3}, 5 \times 10^{-3}, 1 \times 10^{-2}, 5 \times 10^{-2}$ M) of L_3 reagent followed CPE procedure. The results are presented on the Fig. 3. Using data on the Co content in the aqueous and micellar phases, the distribution ratio (D) and recoveries (%) were calculated. The results have shown that the analytical response increased with increasing in the concentration of L_3 reagent and reached maximum up to 1×10^{-3} M (L_3), indicating sufficient for complex formation. Thus, a concentration of 1×10^{-3} M of L_3 was selected as optimum. The results also revealed that the concentration of 1×10^{-3} M of L_3 provides a high distribution ratio ($\log D = 0.906$) with highly stable complex formation extractable into surfactant. Since CPE extraction procedure is in thermodynamic behaviour of extracted species [22,23]. It might be divided into two steps: the first step is the formation of complex between L_3 and Co (II) and the second step is the extraction of this complex into the cloud point phase. Thus, L_3 concentration plays effective role for the formation of ion pair complex according to the thermodynamic equilibria. When the concentration of L_3 less than 1×10^{-3} M, it is not

sufficient to reach optimum thermodynamic equilibria for complex formation because the rate of dissociation became more than rate of association if the concentration of L_3 is more than 1×10^{-3} M.

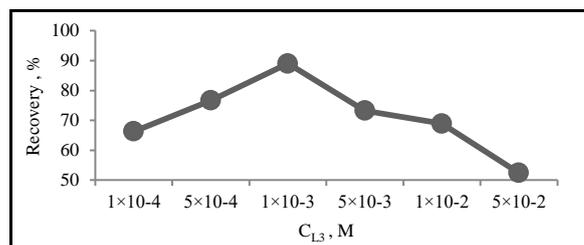


Fig. 3. Effect L_3 concentration on the absorbance values for complex formation by CPE ($3 \mu\text{g mL}^{-1}$ Co (II), 0.1M HCl)

b) Effect of HCl Concentration

The separation of Co (II) by CPE method involves the formation of complex between Co (II) and the protonated reagent in the presence of HCl, with sufficient hydrophobicity and extracted into micelle medium thus obtaining desired enrichment. In this study, 10 mL solution containing $3 \mu\text{g mL}^{-1}$ Co (II), reagent (L_3) 1×10^{-3} M and 0.9 mL of 1% Triton X-100 and varying concentration range of (0.05-2) M HCl was subjected to general CPE procedure. The results in Fig. 4 show the effect of HCl concentration on the extractability of complex into the surfactant-rich phase. The results illustrate that the concentration at 0.8M HCl was found to be optimum for extraction ability according to CPE method. The recovery increases with increase HCl concentration and reaching maximum at 0.8 M HCl. At less than 0.8M HCl, no thermodynamic equilibria can be reached for extraction of complex in micelle cloud point, thus the probability of complex formation might not be achieved of which a decrease in absorbance and distribution ratio D values, while at higher than 0.8 M HCl, no suitable extraction may be occurred due to the deviation of thermodynamic equilibria which leads to dissociate the ion-pair complex according to mass action law and Le Chatelier principle and hence the absorbance suddenly decreases giving lower extraction efficiency [22].

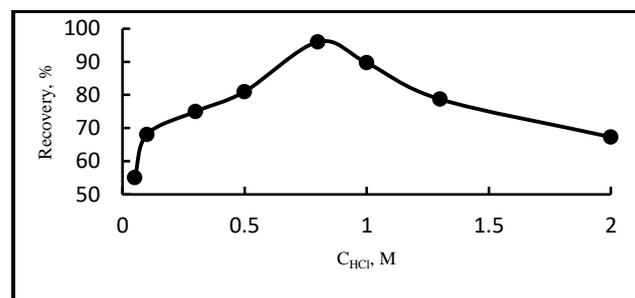


Fig. 4. Effect of HCl concentration on absorbance values for complex formation by CPE ($3 \mu\text{g mL}^{-1}$ Co (II), 1×10^{-3} M reagent (L_3))

c) Effect of Incubation Time

The effect incubation time on extraction is an important parameter in CPE process for a complete reaction to achieve easy phase separation and the pre-concentration to be as efficient as possible. The effect of time on the extraction of Co(II) in the range of (10-45) min was investigated by taking 10ml aqueous solution containing 3

$\mu\text{g mL}^{-1}$ Co(II), 0.8 M HCl, 1×10^{-3} M of L_3 , 0.9 mL of 1% (w/v) Triton X-100, followed CPE procedure. It was also observed that the incubation time of 20min was sufficient for the maximum recovery of cobalt complex. This optimum time is most valuable time to formation CMC with high density and reaches a suitable kinetic energy which gives a good aggregation for surfactant in obtaining a smaller volume and the highest of a cloud point layer. Whereas time of less than 20 min cannot allow to reach maximum kinetic equilibrium for aggregation micelles to form CMC and decline extraction efficiency. But at time of heating of more than 20 min, effect of increasing the diffusion of micelles in aqueous solution by increasing the kinetic energy is more than needed. Thus a decrease in density and increase in volume of cloud point layer occur resulting in minimizing extraction efficiency also [23,16].

d) Effect of Equilibrium Temperature

To ensure phase separation and pre-concentration of an analyte efficiently, optimal equilibration temperature is very crucial parameter for complete reaction which reflect certainly the magnitude of pre-concentration factor of an analyte. The effect of the equilibrium temperature was examined by taking 10 mL aqueous phase containing of $3 \mu\text{g mL}^{-1}$ Co (II) and keeping other factors at optimum conditions. The temperature was varied from 30 - 90 °C at 20 min in a search of optimum value. It was shown that the highest recovery was obtained when the temperature at 60 °C achieving quantitative extraction. Certainly, high temperatures are not suitable for the CPE procedure because these could cause problems of the stability of complex due to thermal decomposition of complex. In this study, 60°C was chosen as the optimized incubation temperature for all experiments.

e) Effect of TX-100 Volume

A successful cloud point extraction procedure should maximize the extraction efficiency by minimizing the phase volume ratio, which improves its enrichment ability. The effect of the surfactant amount on CPE of 10 mL containing $3 \mu\text{g mL}^{-1}$ Co(II) with 1×10^{-3} M L_3 at 0.8 M HCl in the presence of 1% Triton X-100 varying from (0.1-1.5) mL followed the general CPE procedure has been optimized in order to obtain maximum extraction efficiency via minimizing the phase volume ratio. The results are shown in Fig.5.

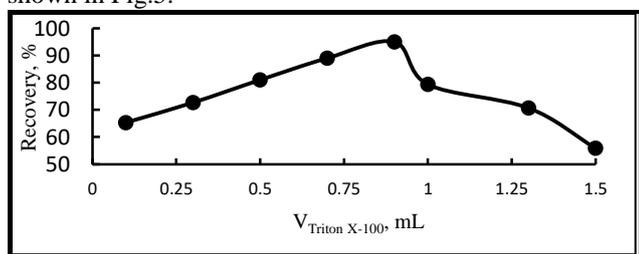


Fig. 5. Effect of Triton X-100 amount on Co(II) CPE: $3 \mu\text{g mL}^{-1}$ Co (II), 0.8 M HCl, 1×10^{-3} M L_3 reagent

Thermodynamic equilibrium for extraction and pre-concentration for any analyte needs a significant amount of surfactant to form micellar phase layer with higher viscous and smaller volume available for the extraction of a major quantity of ion pair complex by giving

higher recovery. Figure (5) reflects the impact of Triton X-100 amount on the pre-concentration of Co (II). It was shown that the recovery increases dramatically and reaches the maximum at 0.9 mL of 1% Triton X-100 and decreases suddenly. At a low amount of surfactant, the recovery is low, perhaps due to the insufficiency of assemblies' formation entrapping the complex quantitatively. Whilst at a higher amount of surfactant, the extraction efficiency is low, probably due to the increase in surfactant-rich phase volume at which the analyte become more diluted (i.e. not good aggregation by effect of diffusion in aqueous phase) or according to thermodynamic behaviour of Triton X-100 which cannot allow to form smaller volume and higher viscous layer resulting in lowest extraction. Consequently, optimum volume of 1% (w/v) Triton X-100 at 0.9 mL was gave a high sensitivity and the highest extraction efficiency for the complex.

C. Stoichiometric Determination of Complex

The Co(II) with L_3 reagent complexation stoichiometry was studied according to the procedure mentioned in Slope Analysis for Stoichiometric Determination of Complex by the dependence of $\log D = f(\log C_{L_3})$. The graph is linear ($R=0.9268$) and the slope is equal to 0.566, indicating the complex ratio of 1:1.

D. Thermodynamic Study

The equilibrium extraction constants (K_{ex}) and thermodynamic parameters for the extraction of complex during the cloud point extraction using Triton X-100 as a mediated extracting agent at temperature were determined 60°C. The equilibrium constants (K_{ex}) at the selected temperature were calculated from the equation.

$$K_{ex} = \frac{D}{[M^{n+}]_{aq} [R]_s} \dots \dots \dots (2)$$

Where D is distribution ratio, $[M^{n+}]_{aq}$ metal ion concentration that remained in aqueous phase and $[R]_s$ concentration of reagent in surfactant phase.

Thermodynamic parameters were also calculated from the relationships:

$$\frac{\Delta \log K_{ex}}{\Delta 1/T} = \frac{-\Delta H_{ex}}{2.303 R} \dots \dots \dots (3)$$

$$\text{Slope} = \frac{-\Delta H_{ex}}{2.303 R} \dots \dots \dots (4)$$

$$\Delta G_{ex} = -RT \ln K_{ex} \dots \dots \dots (5)$$

$$\Delta G_{ex} = \Delta H_{ex} - T \Delta S_{ex} \dots \dots \dots (6)$$

The $\log K_{ex} = f(1/T)$ graph is linear ($R=0.9974$) described by the equation $y=-5.174x+25.37$. The calculated thermodynamic parameters are presented in Table 1.

Table1. Thermodynamic parameters and equilibrium constants for the CPE of Co (II) complex

T°K	ΔH_{ex} (KJ mol ⁻¹)	ΔG_{ex} (KJmol ⁻¹)	ΔS_{ex} (J mol ⁻¹)	D	logK _{ex}
303	0.099	-48.4	159.9	2.9	8.3
313		-52.6	168.3	5.0	8.8
323		-57.4	178.1	9.4	9.3
333		-63.1	189.7	19.4	9.9

It can be seen from table (1) that the enthalpy change (ΔH_{ex}) is equal to $0.099 \text{ KJ mol}^{-1}$ indicating a high efficiency of the extraction process of complex was achieved thermodynamically into the surfactant-rich phase. This is because (1) a strong electrostatic association exists between CoCl_4^{2-} anion with the reagent cation L_3^+ and (2) contribution of the complex in driving water molecules out of surfactant phase in which more of micelles are aggregated enabling the precise extraction of complex especially in extracting of trace amounts. As for ΔG_{ex} is negative in all cases, indicating the extraction process is a spontaneous phenomenon because the complex transference and surfactant phase formation are synchronized processes that occur at the same time. Thus, the more negative value of ΔG_{ex} , the large spontaneous process. The positive values of ΔS_{ex} prove that the solubilized complex molecules are organized in more random fashion during extraction process (i.e. entropic in region). Therefore, the extraction process is endothermic in nature, which is also verified from the positive value of ΔH_{ex} [22,16].

E. Analytical characteristics of the developed method

Calibration curve was obtained under the optimum conditions is linear ($R^2=0.9966$) in the range $0.025\text{-}3 \mu\text{g mL}^{-1}$ Co (II) and described by the equation $\text{Abs} = 2.0712 \times C_{\text{Co}} + 0.1443$. The limit of detection (LOD) of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the standard deviation of the blank ($n=8$). The limit of quantitation (LOQ) is the lowest level of analyte that can be accurately and precisely measured. The limit of quantitation, defined as 10 times the standard deviation of the blank ($n=8$). The limit of detection and limit of quantitation (LOQ) were 11.5 and 38.4 ng mL^{-1} respectively, depending on the standard deviation of the response and the slope of the calibration curve using the following equation: ($\text{LOD}=3\sigma\text{B/s}$), ($\text{LOQ}=10\sigma\text{B/s}$), where σB is the standard deviation of the blank calibration plot and (s) is its slope. Furthermore, for pre-concentration of 15 mL of the working standard solutions based on the slope ratio of calibration curves with and without pre-concentration [24], an enrichment factor of 54 was obtained. To test the reproducibility of the proposed extraction method, the suggested procedure was repeated eight times under optimum conditions. The relative standard deviation (RSD) was measured for $0.05 \mu\text{g mL}^{-1}$ was found to be 0.823% . The consumptive index is 0.277 was defined as the sample volume in milliliters consumed to reach a unit of enrichment factor (EF) [25].

F. Determination of Cobalt (II) in real samples

To evaluate the effectiveness of the developed procedure for the determination of Co^{2+} ion in real samples including milk, tobacco, tea and two different water samples including river and tap water and the results are shown in Table 2. Good recoveries ($101.108 - 104.918\%$) were achieved for all analyzed samples by adding Co (II) standard solution into the sample solution.

Table 2. Recovery percentage of the cobalt ion real samples ($P=0.95$; $n=3$)

Sample	Co Add	Co Found	%Recovery	%Error
River water ^a	0	0.842 ± 0.023	-	-
	0.5	1.408 ± 0.015	104.918	4.918
Tap Water ^a	0	0.340 ± 0.013	-	-
	0.5	0.861 ± 0.021	102.500	2.500
Milk ^b	0	0.147 ± 0.15	-	-
	1	1.163 ± 0.18	101.395	1.395
Tea ^b	0	1.332 ± 0.11	-	-
	1	2.411 ± 0.12	103.388	3.388
Tobacco ^b	0	1.235 ± 0.20	-	-
	1	2.328 ± 0.11	101.145	1.145
Soil ^b	0	0.715 ± 0.16	-	-
	1	1.734 ± 0.23	101.108	1.108

a: All values are $\mu\text{g mL}^{-1}$, b: All values are $\mu\text{g g}^{-1}$

The results have shown that the concentration of Co (II) in river and tap water was above the limit permitted concentration by WHO ($0.01 \mu\text{g mL}^{-1}$) in drinking water [26]. There are limited reports on the concentration of cobalt in milk sample. According to a report of milk composition and synthesis resource library, the concentration of cobalt is 0.6 ppm [27]. The concentration obtained in the present study is very less compared to the reported value. The cobalt concentration in tea was found $1.332 \mu\text{g g}^{-1}$, the determined level of Co (II) in the tea sample was below the limit (1.5 mg kg^{-1}) [28]. Levels of cobalt in tobacco range from <0.3 to $2.3 \mu\text{g/g}$ dry weight, and approximately 0.5% of this cobalt is present in mainstream smoke cobalt. In the present study was ($1.235 \mu\text{g g}^{-1}$). The concentration of cobalt in soil may be several hundred milligrams per kilogram [29] in the present study was below the WHO.

IV. CONCLUSION

The reagent 6,6'-((1E,1'E)-((4-methyl-1,2-phenylene)bis(azanylylidene))bis(methanylylidene))bis(2-methoxy-3-(o-tolyldiazenyl)phenol) was successfully applied in CPE for Co(II) pre-concentration prior to FAAS detection. This work made it possible to provide a quick, simple, safe, affordable, and environmentally responsible approach for pre-concentrating and separating minute amounts of cobalt. The suggested pre-concentration technique enables the measurement of cobalt in real samples.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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