Use of a Hydrophobic Azo Dye for the Centrifuge-Less Cloud Point Extraction–Spectrophotometric Determination of Cobalt

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Abstract: The hydrophobic azo dye 6-hexyl-4-(2-thiazolylazo)resorcinol (HTAR, H) was studied as part of a system for the centrifuge-less cloud point extraction (CL-CPE) and spectrophotometric determination of traces of cobalt. The extracted 1:2 (Co:HTAR) complex, [CoIII(HL−)(L2−)]n, shows an absorption maximum at 553 nm and contains HTAR in two different acid–base forms. Optimum conditions for its formation and CL-CPE were found as follows: 1 × 10−5 mol L−1 of HTAR, 1.64% of Triton X-114, pH of 7.8, incubation time of 20 min at ca. 50 °C, and cooling time of 30 min at ca. −20 °C. The linear range, limit of detection, and apparent molar absorptivity coefficient were 5.4–189 ng mL−1, 1.64 ng mL−1, and 2.63 × 105 L mol−1 cm−1, respectively. The developed procedure does not use any organic solvents and can be described as simple, cheap, sensitive, convenient, and environmentally friendly. It was successfully applied to the analysis of artificial mixtures and real samples, such as steel, dental alloy, rainwater, ampoules of vitamin B12, and saline solution for intravenous infusion.

Keywords: cobalt; cloud point extraction; green analytical chemistry; azo dye; spectrophotometric determination

1. Introduction

Cobalt is a group 9 first-row transition metal with atomic number 27. It is a hard, lustrous, silvery-gray, corrosion-resistant ferromagnetic material, recognized as a new element (1735) by the Swedish chemist Georg Brandt. Pure metal is not found in nature, but its compounds are part of most rocks and soils [1,2]. It is classified as a dispersed element with an average content in the upper crust of 17.3 ppm [3,4]. Cobalt is the essential ingredient of 72 approved minerals in the International Mineralogical Association’s database [5], the most important of which are cobaltite (CoIIIAsS), glaucodot (CoIIIFeAsS), erythrite (CoII3(AsO4)2·8H2O), and skutterudite (CoIIIAs3). However, its economically viable deposits are few and cobalt is usually produced as a by-product of other metals (e.g., Ni, Cu, and Ag) [6,7].

The main applications of cobalt are in rechargeable batteries, superalloys, steels, magnets, pigments, chemicals, ceramics, catalysts, oil drying agents (siccatives), and nutrients [7–9]. As the need for rechargeable batteries continues to grow, some economists expect a significant increase in demand for this element in the coming years [8,10].

Cobalt is an essential trace element for many organisms, including humans. It is utilized by animals only in the form of vitamin B12 (cobalamin), synthesized by specific microorganisms in the presence of sufficient cobalt. This vitamin is important for the development, myelination and function of the central nervous system, DNA synthesis, and red blood cell formation [11]. Cobalt deficiency in humans can lead to health problems,
Numerous spectroscopy techniques have been used for the determination of cobalt, including flame atomic absorption spectrometry (FAAS) [16–19], graphite furnace atomic absorption spectrometry (GFAAS) [20], inductively coupled plasma mass spectrometry (ICP-MS) [21], inductively coupled plasma optical emission spectrometry (ICP-OES) [22], and thermal lens spectrometry [23].

UV/Vis spectrophotometry is considered a good alternative for the determination of inorganics, thanks to its simplicity, low cost, versatility, energy efficiency, sensitivity, and availability [24–26]. It can be easily combined with various sample preparation techniques to improve analytical characteristics and extend the scope of application. The list of such techniques comprises liquid–liquid extraction [27], dispersive liquid–liquid microextraction [28], continuous sample drop flow-based microextraction [29], deep eutectic solvent microextraction [30], and cloud point extraction (CPE) [31–44].

CPE is a modern variant of the classical liquid–liquid extraction, which minimizes (or completely ignores) the use of organic solvents. It complies with the Green Analytical Chemistry principles [45] and is often defined as an “eco-friendly tool” [46]. The application of CPE to trace element analysis usually requires the conversion of the analyte into a hydrophobic electroneutral complex [47–49] that enters the surfactant-rich phase (SRP). This phase is typically separated by centrifugation [49,50], but sometimes a simpler option is possible: separation based on gravitational forces. Given the proper choice of reagent(s) and experimental conditions, gravitational (centrifuge-less, CL) separation is convenient and advantageous, and the time required for the process to complete is not very long.

The aim of this work was to develop a CL-CPE–spectrophotometric procedure for the determination of traces of cobalt, using 6-hexyl-4-(2-thiazolylazo)-resorcinol (HTAR). This novel hydrophobic reagent (Figure 1) has recently been applied in our laboratory for the CL-CPE of vanadium(IV,V) [51] and copper(II) [52].

![Chemical formulae of the HTAR forms: neutral, H2L (I); monoanionic, HL− (II); and dianionic, L2− (III).](image-url)
2. Results and Discussion
2.1. Optimum Conditions

A single factor optimization was carried out in this study. The influence of the following experimental parameters was examined and optimized at room temperature: wavelength of spectrophotometric measurement, pH, HTAR concentration, and TX-114 mass fraction. The effect of incubation time at elevated temperature (50 °C) was also investigated.

Spectra of the extracted species are shown in Figure 2. The Co–HTAR coordination compound obtained in neutral or basic media shows an absorption maximum ($\lambda_{\text{max}}$) at 553 nm (1). Spectral characteristics do not change when a strong oxidizing agent (e.g., ammonium persulfate, APS) is added to the system (the resulting spectral line is identical to spectrum 1). This suggests that the central atom is Co(III), a statement consistent with the literature describing a spontaneous Co(II) → Co(III) oxidation reaction with the dissolved oxygen in systems containing azo dyes [53–58].

![Absorption spectra of the complex (1, 2, 2’’) and the blank (1’, 2’) at two different pH values (ammonium acetate buffer): 7.8 (1, 1’) and 4.7 (2, 2’, 2’’). 2.25 × 10^{-5} \text{ mol L}^{-1} \text{ of Co(II)}, 1 \times 10^{-5} \text{ mol L}^{-1} \text{ of HTAR, 1.64\% of TX-114, } t = 20 \text{ min at 50 °C. The concentration of APS (2’’) was 1.6 \times 10^{-4} \text{ mol L}^{-1}.}](image)

In acidic solutions, the recorded spectrum of the Co–HTAR complex (2) changes to $\lambda_{\text{max}} = 524$ nm. This hypsochromic shift can be attributed to the lower absorbance of the blank (2’), which is determined by the existence of the HTAR reagent mainly in its neutral form (H$_2$L) at pH < 6.7 [52]. The addition of APS does not affect the position of $\lambda_{\text{max}}$, but the absorbance becomes higher (compare spectra 2 (Co-HTAR) and 2’’ (Co-HTAR-APS)). This is consistent with the fact noted by many authors that the oxidation of Co(II) is not as fast and easy at lower pH values.

The effect of pH on the absorbance at 553 nm is shown in Figure 3. An ammonium acetate buffer (3 mL) was used to adjust the pH. The absorbance is maximal and constant in a wide pH range (from 6.0 to 8.3). Further studies were performed at pH 7.8. This pH value was chosen based on the following considerations: (a) it is far from the limit values (6.0 and 8.3); (b) the absorbance of the blank (at $\lambda = 553$ nm) is weakly sensitive to accidental pH deviations.

The effect of HTAR concentration is displayed in Figure 4. The chosen optimal concentration was $1.0 \times 10^{-5}$ mol L$^{-1}$. At concentrations above (1.5–2.0) × $10^{-5}$ mol L$^{-1}$, a slight decrease in absorption is observed.
The reported heating temperatures for CPE systems based on the same surfactant, TX-114, are commonly between 40 °C and 65 °C [23,31,32,35,36,38,39,59–61]. The results of our experiments at 50 °C are represented in Figure 6. As can be seen, the minimum incubation time required is ca. 15 min. To avoid accidental errors caused by insufficient heating, further studies were performed at an incubation time of 20 min.

The effect of the Triton X-114 (TX-114) mass fraction is demonstrated in Figure 5. Further studies were performed in the presence of 8.2 mL (≈8.2 g) of the surfactant solution, which corresponds to an approximate mass fraction of 1.64%.

Figure 3. Effect of pH on the absorbance: 8 × 10^{-6} mol L^{-1} of Co, 2.8 × 10^{-5} mol L^{-1} of HTAR, 2.0% of TX-114, t = 20 min at 50 °C, λ = 553 nm.

Figure 4. Effect of HTAR concentration: 1.6% of TX-114, 3.2 × 10^{-6} mol L^{-1} of Co, pH 7.8, t = 20 min at 50 °C, λ = 553 nm.

Figure 5. Effect of TX-114 mass fraction: 3.2 × 10^{-6} mol L^{-1} of Co, 1 × 10^{-5} mol L^{-1} of HTAR, pH 7.7, t = 20 min at 50 °C, λ = 553 nm.
Figure 6. Effect of incubation time (t) at 50 °C: 1.6% of TX-114, 3.2 × 10^{-6} mol L^{-1} of Co, pH 7.7, 1 × 10^{-5} mol L^{-1} of HTAR, λ = 553 nm.

The last step of the developed CL-CPE procedure is cooling. The samples were kept in a refrigerator (at ca. −20 °C) for 30 min, as in our previous work on Cu(II) extraction with the same surfactant (TX-114) and reagent (HTAR) [52].

The selected optimal CL-CPE–spectrophotometric optimization.

Table 1. The CL-CPE–spectrophotometric optimization.

| Parameter                  | Optimization Range | Optimal Value | Figure |
|----------------------------|--------------------|---------------|--------|
| Wavelength, nm             | Visible range      | 553           | 1      |
| pH                        | 3.4–10.0           | 7.8           | 2      |
| Concentration of HTAR, mol L^{-1} | (0.1–4) × 10^{-5} | 1.0 × 10^{-5} | 3      |
| Mass fraction of TX-114, %| 0.2–2.4            | 1.64          | 4      |
| Incubation time at 50 °C, min | 5–40              | 20            | 5      |

2.2. Composition of the Complex, Formula, Extraction Equation, and Equilibrium Constant

The complex stoichiometry was determined by the mole-ratio method [62] and the mobile equilibrium method [63] at two different pH values (7.8 and 4.7). A molar ratio of 1:2 (Co:HTAR) was found regardless of pH (Figures 7 and 8).

Figure 7. Determination of the HTAR-Co molar ratio by the mole-ratio method: 1.6% of TX-114, 3.2 × 10^{-6} mol L^{-1} of Co, pH 7.8, t = 20 min at 50 °C, λ = 553 nm.

Based on the electroneutrality requirement and the lack of indications that the components of the buffer are included in the complex, one can suggest the following formula of the extracted coordination compound: [Co^{II}(HL^{-})(L^{2-})]^0. Complexes with such a general formula, containing one deprotonated (L^{2-}) and one monoprotonated (HL^{-}) azo dye, have been partially extracted in water–chloroform systems involving similar thiazolylazo dyes, such as 4-(2-thiazolylazo)resorcinol (TAR) [55] and 5-methyl-4-(2-thiazolylazo)resorcinol (MTAR) [58].
Figure 7. Determination of the HTAR:Co molar ratio by the mobile equilibrium method: 1.6% of TX-114, 3.2 × 10^{-6} \text{ mol L}^{-1} \text{ of Co, pH 4.7, } t = 20 \text{ min at } 50 \degree \text{ C, } \lambda = 553 \text{ nm.}

The extraction process in the studied system (at the optimum pH range, 6.0–8.3) can be expressed by the following equation, involving oxidation of Co(II) to Co(III):

\[
\text{Co}^{II}_\text{(aq)} + 2 \text{HL}^-_\text{(aq)} \rightarrow \text{[Co}^{III}\text{(HL}^-_\text{)}\text{(L}^{2-})_\text{(SRP)}] + \text{H}^+_\text{(aq)} + \text{e}^- \\
\]

At lower pH values, the complexation is hampered by HL^- deficiency (H_2L is the dominant species) and incomplete Co(II) oxidation. At higher pH values, the main obstacles may be hydrolysis [64] and HL^- deficiency (due to an increase in the L^{2-} fraction).

The equilibrium constant characterizing this equation was calculated by the Harvey–Manning method [65], \log K_{ex} = 12.1 \pm 0.2 \text{ (mean \pm standard deviation).}

2.3. Analytical Characteristics, Effect of Foreign Ions and Application

The relationship between the absorbance and concentration of Co(II) was investigated under the optimal conditions given in Table 1. Good linearity was found in the range of 5.4–189 \text{ ng mL}^{-1}, R^2 = 0.9992 (n = 8). The regression equation was \(A = 4.460 \gamma + 0.0047\), where \(\gamma\) is the concentration in \(\mu\text{g mL}^{-1}\). The standard deviations of the slope and intercept were 0.052 and 0.0049, respectively. The molar absorption coefficient was \(2.63 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}\), and the limits of detection (LOD) and quantitation (LOQ), calculated as 3- and 10-times standard deviation of the blank divided by the slope, were 1.64 \text{ ng mL}^{-1} and 5.4 \text{ ng mL}^{-1}, respectively. The preconcentration factor defined as the ratio between the masses of the sample (50 g \approx 50 mL; the density is close to unity) and the diluted SRP phase (5 g) was 10.0. A similar value (10.8) was calculated by dividing the slopes obtained in the presence and absence of TX-114.

The effect of foreign ions is shown in Table 2. The most serious interferences are caused by Cu(II), Ni(II), and Zn(II). Under the established optimum conditions, these ions form colored complexes with absorption maxima at 547 nm (Cu), 554 nm (Ni), and 533–543 nm (Zn). The interfering effect of V(V) and Fe(III) is smaller. If necessary, Fe(III) can be masked with HPO_4^{2-} [66] or separated by the fluoride method [67], as described below.

The developed procedure was used to determine Co in artificial mixtures and real samples. As a first step, artificial mixtures imitating cobalt-based dental and super-alloys were analyzed: Marranium CC, EOS CobaltChrome SP2, Vitalium, and Stellite 6. The results were statistically identical to those obtained with the same amount of Co(II) (4.7 \mu g) and the absence of ions corresponding to the alloying elements. The relative standard deviation (RSD) was in the range of 1.2–1.7% (n = 4).
Table 2. Effect of foreign ions on the determination of 4.7 µg Co(II).

| Foreign Ion (FI) Added | Added Salt Formula | Amount of FI Added, mg | FI: Co Mass Ratio | Amount of Co found, µg | R, % |
|------------------------|--------------------|------------------------|-------------------|------------------------|------|
| Al(III)                | Al(NO₃)₃·9H₂O       | 0.47                   | 100               | 4.6                    | 98.2 |
| Ba(II)                 | Ba(NO₃)₂           | 47                     | 10,000 *          | 4.9                    | 105  |
| Ca(II)                 | Ca(NO₃)₂           | 47                     | 10,000 *          | 4.7                    | 99.5 |
| Cd(II)                 | CdCl₂              | 0.094                  | 20                | 4.9                    | 105  |
| Cr(III)                | Cr₂(SO₄)₃          | 0.094                  | 20                | 4.5                    | 95.0 |
| Cr(VI)                 | K₂CrO₄             | 4.7                    | 1000 *            | 4.5                    | 95.8 |
| Cu(II)                 | CuSO₄·5H₂O         | 0.0024                 | 0.5               | 4.7                    | 100  |
| F⁻                     | NaF                | 47                     | 10,000 *          | 4.9                    | 105  |
| Fe(III)                | Fe₂(SO₄)₃          | 0.024                  | 5                 | 4.8                    | 103  |
| HPO₄²⁻                  | Na₂HPO₄·12H₂O      | 9.4                    | 2000              | 4.6                    | 97.6 |
| Hg(II)                 | Hg(NO₃)₂           | 0.235                  | 50                | 4.6                    | 97.1 |
| Mg(II)                 | MgSO₄·7H₂O         | 47                     | 10,000 *          | 4.8                    | 101  |
| Mn(II)                 | MnSO₄·H₂O          | 0.094                  | 20                | 4.8                    | 102  |
| Mo(VI)                 | (NH₄)₂MoO₇O₂·4H₂O  | 2.35                   | 500 *             | 4.8                    | 101  |
| Na⁺                    | NaCl               | 47                     | 10,000 *          | 4.7                    | 99.0 |
| Ni(II)                 | NiSO₄·7H₂O         | 0.0047                 |                   | 4.9                    | 105  |
| NO₂⁻                   | NH₄NO₂            | 47                     | 10,000 *          | 4.6                    | 97.2 |
| Re(VII)                | NH₄ReO₄           | 4.7                    | 1000 *            | 4.5                    | 96.0 |
| Pb(II)                 | Pb(NO₃)₂          | 0.047                  | 10                | 4.5                    | 95.8 |
| V(V)                   | NH₄VO₃            | 0.0094                 | 2                 | 4.9                    | 103  |
| W(VI)                  | Na₂WO₄·2H₂O       | 2.35                   | 500 *             | 4.7                    | 100  |
| Zn(II)                 | ZnSO₄·7H₂O        | 0.0047                 | 1                 | 4.7                    | 100  |

* Higher FI: Co mass ratios have not been investigated.

Tables 3 and 4 show the results of the analysis of real samples: steel, dental alloy, and injection ampoules of Vitamin B₁₂. They characterize the developed procedure as accurate and precise. The results for the cobalt content of the injection ampoules were additionally confirmed by ICP-MS.

Table 3. Determination of cobalt in steel and dental alloy (n = 4).

| Sample                      | Cobalt Content, % | Content of Other Elements (Manufacturer’s Data) | Cobalt Found *, % |
|-----------------------------|-------------------|-------------------------------------------------|-------------------|
| 1 Steel                     | 4.71              | 17.7% W, 4.21% Cr, 1.58% V, 0.35% Mn, 0.081% C, 0.18% Si, and the balance Fe | 4.77 ± 0.08      |
| 2 Dental alloy (Wirobond®C) | 63.3              | 24.8% Cr, 5.3% W, 5.1% Mo, 1.0% Si, and Ce       | 62.8 ± 0.9       |

* Mean ± standard deviation (SD).

Table 4. Determination of cobalt in 1-mL Vitamin B₁₂ (1000 µg) injection ampoules * (n = 6).

| Sample          | Present Method, µg Co per ampoule | RSD, % | ICP-MS **, µg Co per ampoule | RSD, % |
|-----------------|-----------------------------------|--------|------------------------------|--------|
| Sample 1        | 44                                | 4.4    | 45                           | 5.5    |
| Sample 2        | 44                                | 5.9    | 45                           | 5.5    |

* The calculated content of Co in 1000 µg of Vitamin B₁₂ (C₆₃H₈₆CoN₁₄O₁₄P) is 43.5 µg. ** The analysis was performed in another laboratory.

Table 5 includes the results of analysis of rainwater and saline solution for intravenous infusion obtained by the addition–recovery method. The RSD in these determinations ranged from 2.4% to 27%, and the recoveries were between 98.2% and 106%.
Table 5. Addition–recovery of Co(II) from the rainwater and saline solution for intravenous infusion (n = 4).

| Sample                  | Co(II) Concentration, ng mL⁻¹ | Recovery, % |
|-------------------------|-------------------------------|------------|
|                         | Added | Found * |          |          |
| Rainwater               |       |         |          |          |
| 0                       | <LOQ  |         |          |          |
| 20                      | 21.5 ± 5.6 | 106  |          |          |
| 40                      | 41.1 ± 3.9 | 103  |          |          |
| 60                      | 59.3 ± 6.3 | 98.2 |          |          |
| Saline solution for infusion |       |         |          |          |
| 0                       | <LOQ  |         |          |          |
| 20                      | 20.8 ± 5.7 | 104  |          |          |
| 40                      | 40.9 ± 1.0 | 102  |          |          |
| 60                      | 59.6 ± 2.1 | 99.4 |          |          |

*Mean ± standard deviation (SD).

2.4. Comparison with Existing Methods

Table 6 summarizes data on CPE–spectrophotometric procedures for the determination of cobalt. The present CL-CPE procedure can be described as simple, cheap, sensitive, convenient, and environmentally friendly. The reagent is commercially available and does not need to be synthesized. It is not necessary to add electrolyte to increase the extraction efficiency [36,37,40,41] or organic solvent to provide synergistic extraction [33] or to reduce the viscosity of the SRP [31–41].

Table 6. Comparison with reported CPE–spectrophotometric procedures for the determination of cobalt(II).

| Reagent(s) Surfactant | SRP Diluting Agent | Sample | Wavelength, nm | Linear Range, ng mL⁻¹ | LOD, ng mL⁻¹ | Ref. |
|-----------------------|--------------------|--------|----------------|------------------------|--------------|------|
| ACDA                  | TX-114             | DMF    | 452           | 20–200                 | 7.5          | [31] |
| APDC + C₆H₁₅MeImCl    | TX-114             | Ethanol| 598           | 150–2000               | 70           | [41] |
| BTANP 15-Crown-5      | TX-114             | Methanol| 549          | 10–300                 | 1.5          | [35] |
|                        | TX-114             | Ethanol| 290           | 500–5000               | 400          | [38] |
|                        |                    |        |               |                        |              |      |
| MSE                   | TX-100             | Ethanol| 292           | 500–10,000             | 12           | [40] |
| NaSCN                 | CTAB + SDS         | Methanol| 618          | 5890–35,400            | 6.18         | [39] |
| N-BAEH                | TX-100             | Ethanol| 294           | 500–10,000             | 12.7         | [37] |
|                        |                    |        |               |                        |              |      |
| PAN                   | TX-114             | Ethanol| 621           | 5–250                  | 6           | [32] |
|                       | TX-114 + octanol   |        | 450           | 2–300                  | 0.6          | [33] |
|                        |                    |        |               |                        |              |      |
| Salen                 | TX-100             | Ethanol| 378           | 10–70                  | 2.2          | [34] |
|                        | TX-114 + DOSS      | Methanol| 514          | 3140–18,960            | 4.54         | [36] |
|                       |                    |        |               |                        |              |      |
| HTAR                  | TX-114             | Water  | 553           | 5.4–189                | 1.64         | This work |

Abbreviations: ACDA, 2-amino-cyclopentene-1-dithiocarboxylic acid; APDC, ammonium pyrrolidine dithiocarbamate; BTANP, 2-(benzothiazolylazo)-4-nitrophenol; C₆H₁₅MeImCl, 1-hexadecyl-3-methylimidazolium chloride; CTAB, cetyltrimethylammonium bromide; DOSS, docusate sodium salt; MSE, methyl stearate ester; N-BAEH, N-benzoyl-L-arginine ethylester hydrochloride; PAN, 1-(2-pyridylazo)-2-naphthol; SDS, sodium dodecyl sulphate; TPY, 2,2',6',2"-terpyridine; TX-100, Triton X-100; TX-114, Triton X-114.

3. Materials and Methods

3.1. Chemicals and Instrumentation

The chemicals were purchased from Merck (Germany). The stock Co(II) solution (1000 mL, 1 mg mL⁻¹) was prepared by dissolving cobalt(II) sulfate heptahydrate in water containing 2 mL of conc. H₂SO₄ [24]. Working 4 × 10⁻⁴ mol L⁻¹ Co(II) solutions were obtained by appropriate dilution with water. An aqueous solution of HTAR (2 × 10⁻⁵ mol L⁻¹) was prepared in the presence of KOH [52]. Laboratory grade TX-114
was used. It was diluted with water at a mass fraction of 10%. Buffer solutions were made by mixing appropriate volumes of aqueous solutions (2 mol L\(^{-1}\)) of ammonia and acetic acid. Distilled or deionized (ELGA-Veolia LabWater, UK) water was used during the experiments.

An Ultrospec 3300 pro (United Kingdom), equipped with 1 cm path-length cells, was used for the spectrophotometric measurements. The pH was checked with a WTW InoLab 7110 pH meter (Germany). The samples were heated in a GFL 1023 water bath (Germany). An Ohaus Pioneer PA214C analytical balance (USA) was used to measure the mass.

3.2. Samples and Sample Preparation

A saline solution of 0.9% NaCl for intravenous infusion (1000 mL) and 1.0-mL ampoules of vitamin B\(_{12}\) (solution for injection, 1000 µg vitamin B\(_{12}\)) were purchased from a local pharmacy. A dental alloy (Wirobond®C) was kindly provided by the Research Institute at Medical University of Plovdiv. A standard steel sample (4.71% Co) was supplied by the KCM S.A.–Plovdiv.

Rainwater (pH ca. 6.0) was sampled in the outskirts of Plovdiv, Bulgaria (12 June 2022) during the Mediterranean cyclone called “Genesis”. A PET bottle and a glass funnel were used during sampling. The analysis was performed the next day using 35-mL aliquots.

The saline solution for intravenous infusion was also analyzed using 35-mL aliquots. Vitamin B\(_{12}\) ampoules were prepared for analysis by the procedure [68] involving heating in a mixture of conc. HNO\(_3\) (10 mL) and conc. H\(_2\)SO\(_4\) (1 mL) to dryness on a sand bath. The volume of the final solution was 50 mL, and 2-mL aliquots were taken for the analysis.

The dental alloy was treated as described in Ref. [69]. An accurate amount of the alloy (ca. 0.05 g) was weighed into a 50 mL beaker. Then, 10 mL of aqua regia was added and the sample was heated on an initially cold sand bath to dryness. After cooling, 5 mL of HCl (1:1) was added. The sample was reheated to dryness, and the resulting salts were dissolved in water. The obtained solution was transferred to a 1000-mL volumetric flask, and water was added to the mark. Aliquots of 0.2 mL were used for the analysis.

The steel (ca. 0.5 g) was dissolved by a known procedure [70–72] and collected in a 1000-mL volumetric flask. The fluoride precipitation method [67] (p. 177) was then used to remove Fe(III). For this purpose, a 50-mL aliquot of the steel solution was transferred to a 250-mL beaker and heated on a hot plate. A hot 4% NaF solution (100 mL) was added to the beaker, and the mixture was stirred. The resulting white crystalline precipitate (5NaF·2FeF\(_3\)) was removed by filtration through filter paper. The filtrate and the washings were transferred to a 250-mL volumetric flask and diluted to the mark with water. Aliquots of 1 mL were used to determine the cobalt content.

3.3. CL-CPE–Spectrophotometric Optimization

The following solutions were successively added into a pre-weighed 50 mL conical tube: 1–12 mL of 10% TX-114, up to 1.4 mL of 4 \times 10^{-4} \text{ mol L}^{-1} \text{ Co(II)}, 3 mL of ammonium acetate buffer (with pH between 3.4 and 10.0), and 0.025–1.0 mL of 2 \times 10^{-3} \text{ mol L}^{-1} \text{ HTAR. The resulting solution was diluted to 50 mL with water and heated in a water bath for 5–40 min at ca. 50 °C. Then, the tube was placed in a refrigerator for 20–60 min (at −20 °C) to ensure completion of the precipitation process and easy removal of the supernatant by inverting the tube. After decantation, water was carefully added to the SRP to a total mass (SRP + H\(_2\)O) of 5.00 g (an analytical balance was used for this operation). The mixture was then homogenized by gentle heating (for 1–2 min at 40–45 °C) and shaking. Finally, a portion of the resulting clear solution was poured into the cell, and the absorbance was measured against water or a simultaneously prepared blank.

3.4. Recommended Procedure for the Determination of Co

An aliquot of the analyzed solution (5.4–189 ng mL\(^{-1}\) Co) was placed in a pre-weighed 50-mL conical tube. Then, 8.2 mL of 10% Triton X-114 solution, 3 mL of the buffer with
pH 7.8, and 0.25 mL of $2 \times 10^{-3}$ mol L$^{-1}$ HTAR solution were added. The tube was diluted to the mark (50 mL) with water and heated in a water bath (50 °C) for 20 min. After cooling in a refrigerator (at ca –20 °C) for 30 min, the supernatant was removed by inverting the tube. Water was carefully added to the SRP to a total mass (SRP + H$_2$O) of 5.00 g, and the mixture was homogenized by gentle heating and shaking. A portion of the obtained clear solution was poured into the spectrophotometer cell, and absorbance was measured at 553 nm against a corresponding blank. The unknown cobalt concentration was calculated from a calibration plot.

4. Conclusions

A new extraction–chromogenic system for Co ions was studied. It is based on a novel commercially available hydrophobic azo dye, allowing the determination of trace cobalt. The proposed analytical procedure is simple, cheap, sensitive, and convenient. It is reliable and robust due to the wide optimal intervals of the examined variables. The use of organic solvents is not required, which characterizes it as green and environmentally friendly. Unlike other procedures requiring expensive and sophisticated instruments, the proposed analysis can be performed only with affordable and unpretentious equipment, such as a spectrophotometer, a water bath, and a refrigerator.

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References

1. Kloprogge, J.T.; Ponce, C.P.; Loomis, T. The Periodic Table: Nature’s Building Blocks: An Introduction to the Naturally Occurring Elements, Their Origins and Their Uses; Elsevier: Amsterdam, The Netherlands, 2020; pp. 357–368. [CrossRef]
2. Kabata-Pendias, A. Trace Elements in Soils and Plants, 4th ed.; CRC Press: Boca Raton, FL, USA, 2011; pp. 227–234.
3. Rudnick, R.L.; Gao, S. Treatise on Geochemistry, 2nd ed.; Holland, H.D., Turekian, K.K., Eds.; Elsevier: Oxford, UK, 2014; Volume 4, pp. 1–51. [CrossRef]
4. Dehaine, Q.; Tijsseling, L.T.; Glass, H.J.; Törmänen, T.; Butcher, A.R. Geometallurgy of cobalt ores: A review. Miner. Eng. 2021, 160, 106656. [CrossRef]
5. IMA Database of Mineral Properties. Available online: https://rruff.info/ima/ (accessed on 7 July 2022).
6. Jouini, M.; Perrin, M.; Coudert, L. Chemical leaching of inactive gold mine tailings as a secondary source of cobalt and nickel—A preliminary case study. In Proceedings of the Ni-Co 2021: The 5th International Symposium on Nickel and Cobalt, Online, 15–18 March 2021; Springer: Cham, Switzerland, 2021; pp. 179–194. [CrossRef]
7. Chatterjee, K.K. Uses of Metals and Metallic Minerals; New Age International (P) Ltd.: New Delhi, India, 2007; pp. 149–153.
8. Fröhlich, P.; Lorenz, T.; Martin, G.; Brett, B.; Bertau, M. Valuable metals—Recovery processes, current trends, and recycling strategies. Angew. Chem. Int. Ed. 2017, 56, 2544–2580. [CrossRef] [PubMed]
9. Graedel, T.E.; Miatt, A.U.S. Cobalt: A Cycle of Diverse and Important Uses. Resour. Conserv. Recycl. 2022, 184, 106441. [CrossRef]
10. Fu, X.; Beatty, D.N.; Gaustad, G.G.; Ceder, G.; Roth, R.; Kirchain, R.E.; Bustamante, M.; Babbit, C.; Olivetti, E.A. Perspectives on cobalt supply through 2030 in the face of changing demand. Environ. Sci. Technol. 2020, 54, 2985–2993. [CrossRef] [PubMed]
11. Vitamin B12. Fact Sheet for Consumers. Available online: https://ods.od.nih.gov/factsheets/VitaminB12-Consumer/ (accessed on 7 July 2022).
12. Linhares, D.; Pimentel, A.; Borges, C.; Cruz, J.V.; Garcia, P.; dos Santos Rodrigues, A. Cobalt distribution in the soils of São Miguel Island (Azores): From volcanoes to health effects. Sci. Total Environ. 2019, 684, 715–721. [CrossRef]
13. Dospatliev, L.; Ivanova, M. Spectrophotometric investigations on liquid-liquid extraction systems containing cobalt and tetrazolium salts. Application of the developed method for analysis of the cobalt content of biological samples (mushrooms and tobaccos). Bulg. Chem. Commun. 2017, 49, 787–791.

14. Kosioruk, M.; Wyszowski, M. Effect of cobalt on the environment and living organisms—A review. Appl. Ecol. Environ. Res. 2019, 17, 11419–11449. [CrossRef]

15. Garcia, M.D.; Hur, M.; Chen, J.J.; Bhatti, M.T. Cobalt toxic optic neuropathy and retinopathy: Case report and review of the literature. Am. J. Ophthalmol. Case Rep. 2020, 17, 100806. [CrossRef]

16. Mortada, W.I.; Kenawy, I.M.M.; Abdel-Rhman, M.H.; El-Gamal, G.G.; Moalla, S.M.N. A new thiourea derivative [2-(3-ethylthioureido)benzoic acid] for cloud point extraction of some trace metals in water, biological and food samples. J. Trace Elem. Med. Biol. 2017, 44, 266–273. [CrossRef]

17. Altunay, N.; Elik, A.; Bulutlu, C.; Gürkan, R. Application of simple, fast and eco-friendly ultrasound-assisted-cloud point extraction for pre-concentration of zinc, nickel and cobalt from foods and vegetables prior to their flame atomic absorption spectrometric determinations. Int. J. Environ. Anal. Chem. 2018, 98, 655–675. [CrossRef]

18. Temel, N.K.; Sertakan, K.; Gürkan, R. Preconcentration and determination of trace nickel and cobalt in milk-based samples by ultrasound-assisted cloud point extraction coupled with flame atomic absorption spectrometry. Biol. Trace Elem. Res. 2018, 186, 597–607. [CrossRef] [PubMed]

19. Altunay, N.; Elik, A.; Gürkan, R. Vortex assisted-ionic liquid based dispersive liquid microextraction of low levels of nickel and cobalt in chocolate-based samples and their determination by FAAS. Microchem. J. 2019, 147, 277–285. [CrossRef]

20. Sorouraddin, S.M.; Nouri, S. Simultaneous temperature-assisted dispersive liquid–liquid microextraction of cobalt, copper, nickel and zinc ions from high-volume water samples and determination by graphite furnace atomic absorption spectrometry. Anal. Methods 2016, 8, 1396–1404. [CrossRef]

21. Rohanifar, A.; Rodriguez, L.B.; Devasurendra, A.M.; Alipourasiabi, N.; Anderson, J.L.; Kirchhoff, J.R. Solid-phase microextraction of heavy metals in natural water with a polypyrrole/carbon nanotube/1, 10–phenanthroline composite sorbent material. Talanta 2018, 201, 570–577. [CrossRef]

22. Bartosiak, M.; Jankowski, K.; Giersz, J. Determination of cobalt species in nutritional supplements using ICP-OES after microwave-assisted extraction and solid-phase extraction. J. Pharm. Biomed. Anal. 2018, 155, 135–140. [CrossRef]

23. Han, Q.; Huo, Y.; Yang, X.; He, Y.; Wu, J.; Cai, H. Determination of ultra-trace levels of cobalt in water and wheat flour samples using cloud point extraction coupled with laser thermal lens spectrometry. Anal. Methods 2018, 10, 634–640. [CrossRef]

24. Marczenko, Z.; Balcerzak, M. Separation, Preconcentration and Spectrophotometry in Inorganic Analysis; Elsevier: Amsterdam, The Netherlands, 2000; p. 168.

25. Zezzi-Arruda, M.A.; Poppi, R.J. Encyclopedia of Analytical Science, 2nd ed.; Worsfold, P., Townshend, A., Poole, C., Eds.; Elsevier: Oxford, UK, 2005; pp. 351–358. [CrossRef]

26. Passos, M.L.C.; Saraiva, L.M.F.S. Detection in UV-visible spectrophotometry: Detectors, detection systems, and detection strategies. Measurement 2019, 135, 896–904. [CrossRef]

27. Saoud, A.; Mesdour, S.H.; Nabieva, A.; Hamada, B.; Amrane, A.; Nabiev, M. Liquid-liquid extraction and simultaneously spectrophotometric determination of Co (II) and W (VI) using crown ether (DB-18-C6) in aqueous media and in high speed steel. Int. J. Environ. Anal. Chem. 2022, 102, 1814–1824. [CrossRef]

28. Al-Saidi, H.M.; Alharthi, S. Efficiency enhancement of the spectrophotometric estimation of cobalt in waters and pharmaceutical preparations using dispersive liquid–liquid microextraction and microcells with long optical paths. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2021, 253, 119552. [CrossRef]

29. Mohanna, E.; Moinfar, S.; Mohammad, S.; Khayatian, G. A Continuous sample drop flow-based microextraction method for spectrophotometric determination of cobalt with 1-(2-pyridylazo)-2-naphthol in water samples. J. Anal. Chem. 2021, 76, 172–179. [CrossRef]

30. Torabi, A.; Shirani, M.; Semnani, A.; Akbari, A. Deep eutectic solvent-based ligandless ultrasound-assisted liquid-phase microextraction for extraction of cobalt ions from food samples prior to spectrophotometric determination. J. Iran. Chem. Soc. 2021, 18, 893–902. [CrossRef]

31. Safavi, A.; Abdollahi, H.; Hormozi Nezhad, M.R.; Kamali, R. Cloud point extraction, preconcentration and simultaneous spectrophotometric determination of nickel and cobalt in water samples. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2004, 60, 2897–2901. [CrossRef] [PubMed]

32. Afkhami, A.; Bahram, M. Cloud point extraction simultaneous spectrophotometric determination of Zn(II), Co(II) and Ni(II) in water and urine samples by 1-(2-pyridylazo)2-naphthol using partial least squares regression. Microchim. Acta 2006, 155, 403–408. [CrossRef] [PubMed]

33. Wen, X.; He, L.; Shi, C.; Deng, Q.; Wang, J.; Zhao, X. Application of rapid cloud point extraction method for trace cobalt analysis coupled with spectrophotometric determination. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2013, 115, 452–456. [CrossRef] [PubMed]

34. Dhabir, S.A.; Bakir, S.R. Cloud point extraction spectrophotometric determination of copper, chromium and cobalt by salen as reagent in wastewater of Iraq. Asian J. Chem. 2014, 26, 5305–5310. [CrossRef]

35. Elsheikh, R.; Gouda, A.A.; Elsayed, H.A.; Alamin, E.M. Cloud point extraction, preconcentration and spectrophotometric determination of cobalt in water samples. Int. J. Pharm. Pharm. Sci. 2015, 7, 213–221.
36. Shyamala, P.; Kumar, K.R. Cloud point extraction with mixed micelles of TX-114/DOSS and derivative spectrophotometry for simultaneous determination of Fe$^{3+}$ and Co$^{2+}$ using 2,2′,6′,2″-terpyridine. *Indian J. Chem. Sect. A* 2016, 55, 1471–1475.

37. Hayder, F.H.; Jawad, S.K. Onium system for separation, preconcentration and spectrophotometric determination of Co (II), via cloud point extraction methodology. *J. Glob. Pharma Technol.* 2017, 11, 83–91.

38. Mussa, Y.O.; Ghali, A.A.; Hussein, A.S. Cloud point extraction, preconcentration and spectrophotometric determination of Co(II) and Cu(II) using 15-crown-5. *Indian J. Forensic Med. Toxicol.* 2020, 14, 1146–1152. [CrossRef]

39. Kakitha, R.; Pulipaka, S.; Puranam, D. Simultaneous determination of iron and cobalt using spectrophotometry after catanionic mixed micellar cloud point extraction procedure. *Orient. J. Chem.* 2020, 36, 1168–1172. [CrossRef]

40. Azooz, E.A.; Abd Wannas, F.; Jawad, S.K. Developed cloud point extraction coupled with onium system for separation and determination cobalt in biological samples. *Res. J. Pharm. Technol.* 2021, 14, 594–598. [CrossRef]

41. Habibi, Z.; Bamdad, F. Simultaneous determination of traces of cobalt and iron ions after pre-concentration by surface-active ionic liquid-assisted cloud point microextraction. *Anal. Bioanal. Chem. Res.* 2022, 29, 243–250. [CrossRef]

42. Bazel, Y.; Tupys, A.; Ostapiuk, Y.; Tymoshuk, O.; Matiychuk, V. A green cloud-point microextraction method for spectrophotometric determination of Ni(II) ions with 1-[(5-benzyl-1,3-thiazol-2-yl)diazenyl]naphthalene-2-ol. *J. Mol. Liq.* 2017, 242, 471–477. [CrossRef]

43. Snigur, D.; Barbalat, D.; Chebotarev, A.; Synievyd, A.; Bevziuk, K. A rapid cloud point extraction of molybdenum(VI) with 6,7-dihydroxy-2,4-diphenylbenzopyrylum perchlorate prior to its spectrophotometric determination. *Chem. Pap.* 2021, 75, 1823–1830. [CrossRef]

44. Snigur, D.; Dubovyj, V.; Barbalat, D.; Zhukovetska, O.; Chebotarev, A.; Bevziuk, K. A rapid room-temperature cloud point extraction for spectrophotometric determination of copper (II) with 6,7-dihydroxy-2,4-diphenylbenzopyrylum chloride. *Anal. Sci.* 2022, 38, 949–954. [CrossRef]

45. Gatuszka, A.; Migaszewski, Z.; Namieśnik, J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC Trends Anal. Chem.* 2013, 50, 78–84. [CrossRef]

46. Mortada, W.I. Recent developments and applications of cloud point extraction: A critical review. *Microchem. J.* 2020, 157, 105055. [CrossRef]

47. Lemos, V.A.; Santos, E.S.; Santos, M.S.; Yamaki, R.T. Thiazolylazo dyes and their application in analytical methods. *Microchim. Acta* 2007, 158, 189–204. [CrossRef]

48. Pytłakowska, K.; Kozik, V.; Dabloch, M. Complex-forming organic ligands in cloud-point extraction of metal ions: A review. *Talanta* 2013, 110, 202–228. [CrossRef]

49. amini, Y.; Ghabbarian, M. *Comprehensive Sampling and Sample Preparation*; Pawliszyn, J., Ed.; Academic Press: Oxford, UK, 2012. [CrossRef]

50. Samaddar, P.; Sen, K. Cloud point extraction: A sustainable method of elemental preconcentration and speciation. *J. Ind. Eng. Chem.* 2014, 20, 1209–1219. [CrossRef]

51. Milčheva, N.P.; Genç, F.; Racheva, P.V.; Delchev, V.B.; Andrush, V.; Gavazov, K.B. An environmentally friendly cloud point extraction–spectrophotometric determination of trace vanadium using a novel reagent. *J. Mol. Liq.* 2021, 334, 116086. [CrossRef]

52. Racheva, P.V.; Milčheva, N.P.; Genç, F.; Gavazov, K.B. A centrifuge-less cloud point extraction–spectrophotometric determination of copper(II) using 6-hexyl-4-(2-thiazolylazo)resorcinol. *Spectrochim. Acta A Mol. Biomol. Spectros.* 2021, 262, 121016. [CrossRef] [PubMed]

53. Watanabe, H. Spectrophotometric determination of cobalt with 1-(2-pyridylazo)-2-naphthol and surfactants. *Talanta* 1974, 21, 295–302. [CrossRef]

54. Mochizuki, K.; Inamura, T.; Ito, T.; Fujimoto, M. Bivalent and tervalent cobalt complexes of 4-(2-pyridylazo) resorcinol, 1-(2-pyridylazo)-2-naphthol, and their thiazolyl analogs in aqueous and aqueous dioxane media. Rapid-scan spectral and kinetic studies. *Bull. Chem. Soc. Jpn.* 1978, 51, 1743–1750. [CrossRef]

55. Marić, L.; Široki, M.; Štefanac, Z.; Herak, M.J. Extraction of cobalt-[4-(2-thiazolylazo)resorcinol] complexes. *J. Inorg. Nucl. Chem.* 1981, 43, 3357–3361. [CrossRef]

56. Cucinotta, V.; Caruso, R.; Giuffrida, A.; Messina, M.; Maccarrone, G.; Torrisi, A. Separation and quantitation of metal ions by 4-(2-pyridylazo)resorcinol complexation in capillary electrohoresis–electrospray ionisation mass spectrometry. *J. Chromatogr. A* 2008, 1179, 17–23. [CrossRef]

57. Vyschherovich, I.V.; Kalinichenko, I.E. Photometric determination in drinking water of cobalt and nickel with 4-(2-pyridylazo)-resorcinol. *J. Water Chem. Technol.* 2010, 32, 33–38. [CrossRef]

58. Hristov, D.G.; Racheva, P.V.; Toncheva, G.K.; Gavazov, K.B. Extraction-chromogenic system for cobalt based on 5-methyl-4-(2-thiazolylazo)resorcinol and benzalkonium chloride. *Acta Chim. Slov.* 2021, 68, 37–43. [CrossRef]

59. Özcan, R.; Kasa, N.A.; Bakirdere, E.G.; Bakirdere, S. Cloud point extraction-slotted quartz tube with four-exit holes-flame atomic absorption spectrometry combination for the determination of cobalt at trace levels in fennel tea samples after complexation with a Schiff base ligand. *J. Food Meas. Charact.* 2021, 15, 2943–2950. [CrossRef]

60. Yamaki, R.T.; Nunes, L.S.; De Oliveira, H.R.; Araújo, A.S.; Bezerra, M.A.; Lemos, V.A. Synthesis and application of a new thiazolylazo reagent for cloud point extraction and determination of cobalt in pharmaceutical preparations. *J. AOAC Int.* 2011, 94, 1304–1309. [CrossRef]
61. Jamali, M.R.; Gholinezhad, M.; Balarostaghi, S.; Rahnama, R.; Rahimi, S.H.A. Development of a cloud-point extraction method for cobalt determination in natural water samples. *J. Chem.* 2013, *2013*, *2013*, 615175. [CrossRef]

62. Yoe, J.H.; Jones, A.L. Colorimetric determination of iron with disodium-1,2-dihydroxybenzene-3,5-disulfonate. *Ind. Eng. Chem. Anal. Ed.* 1944, *16*, 111–115. [CrossRef]

63. Zhiming, Z.; Dongsten, M.; Cunxiao, Y. Mobile equilibrium method for determining composition and stability constant of coordination compounds of the form $M_mR_n$. *J. Rare Earths* 1997, *15*, 216–219.

64. Pshinko, G.N. Impact of complexing agents on the processes of sorption treatment of waters containing cobalt. *J. Water Chem. Technol.* 2008, *30*, 197–202. [CrossRef]

65. Harvey, A.E.; Manning, D.L. Spectrophotometric methods of establishing empirical formulas of colored complexes in solution. *J. Am. Chem. Soc.* 1950, *72*, 4488–4493. [CrossRef]

66. Stefanova-Bahchevanska, T.; Milcheva, N.; Zaruba, S.; Andrch, V.; Delchev, V.; Simitchiev, K.; Gavazov, K. A green cloud-point extraction-chromogenic system for vanadium determination. *J. Mol. Liq.* 2017, *248*, 135–142. [CrossRef]

67. Pyatnitskii, I.V. *Analiticheskaya Khimiya Kobal’ta (Analytical Chemistry of Cobalt)*; Vinogradov, A.P., Ed.; Nauka: Moscow, Russia, 1965; p. 177.

68. Divarova, V.V.; Stojnova, K.; Racheva, P.; Lekova, V. Determination of cobalt in the form of an ion associate in vitamin B 12. *Russ. J. Inorg. Chem.* 2018, *63*, 974–977. [CrossRef]

69. Divarova, V.V. Investigation of Ternary Ion-Associated Complexes of Cobalt Involving Tetrazolium Salts. Ph.D. Thesis, Plovdiv University “Paissii Hilendarski”, Plovdiv, Bulgaria, 2015. Available online: https://procedures.uni-plovdiv.bg/docs/procedure/838/5477410121410613880.pdf (accessed on 7 July 2022).

70. Anjaneyulu, Y.; Reddy, M.P.; Kumar, P.V.S.; Kavipurapu, C.; Rao, B. Selective and sensitive extraction spectrophotometric method for the determination of vanadium (V) as a mixed ligand complex withN-phenyl benzohydroxamic acid and 4-(2-pyridylazo)resorcinol in non-aqueous media. *Microchem. Acta* 1990, *100*, 87–94. [CrossRef]

71. Simeonova, Z.; Gavazov, K.; Alexandrov, A. Extraction-spectrophotometric study on the system vanadium(V)—2,3-dihydroxynaphthalene—Iodonitrotetrazolium chloride—Water—Chloroform and its analytical application. *Cent. Eur. J. Chem.* 2006, *4*, 258–266. [CrossRef]

72. Divarova, V.V.; Saravanska, A.D.; Toncheva, G.K.; Milcheva, N.; Delchev, V.B.; Gavazov, K.B. Spectrophotometric determination of molybdenum(VI) as a ternary complex with 4-nitrocatechol and benzalkonium chloride. *Molecules* 2022, *27*, 1217. [CrossRef]