

*Original Paper*

## Solid Phase Extractive Preconcentration of Cobalt and Nickel in Hair Samples Using Ethyl Xanthate Modified Benzophenone

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**Abstract.** The ethyl xanthate complexes of cobalt and nickel are quantitatively retained on benzophenone in the pH range of 8.0–10.0. The solid mixture consisting of metal complex together with benzophenone is dissolved in 5 mL of dimethyl formamide, and the cobalt and nickel contents were determined by flame atomic absorption spectrometry. The calibration graphs are rectilinear over the concentration range of 0–75 (Co) and 0–100 (Ni)  $\mu\text{g L}^{-1}$ . The detection limits of flame AAS for cobalt and nickel are lowered by a factor of 200 due to the solid phase extraction preconcentration procedure. NIES CRM Human Hair No. 5 and IAEA Reference Hair HH-1 certified reference materials were used in order to verify the accuracy of the developed preconcentration method in analysing human hair samples. The results obtained were found to be in excellent agreement with certified values. Furthermore, hair samples collected from various inhabitants of different age groups and sex living in the Indian states of Andhra Pradesh and Tamil Nadu were successfully analysed by flame AAS in conjunction with the solid phase extraction preconcentration procedure developed for cobalt and nickel.

**Key words:** Solid phase extraction; flame AAS; benzophenone; cobalt and nickel; hair.

Flame AAS offers low blank values, better reproducibility, low cost and exists in almost all analytical/clinical laboratories. However, this technique is less sensitive compared to electrothermal AAS, ICP-AES or ICP-MS. This can be circumvented by adopting various preconcentration techniques [1, 2] prior to determination. Solid phase extraction (SPE) [3, 4] is replacing liquid–liquid extraction (LLE) due to several advantages such as reduced costs, higher enrichment factors, better precision and accuracy, high flexibility and – most importantly – environmental compatibility.

The preconcentration procedures developed for cobalt and nickel using benzophenone/naphthalene sorbents are summarized in Table 1. Most of these preconcentration procedures utilize molten benzophenone or naphthalene as extractant and subsequent determination by spectrophotometry. These procedures are not precise and not sufficiently sensitive to determine cobalt or nickel in biological samples even after preconcentration. The interest in human hair as a clinical sample has increased in recent years in contrast to blood or urine samples, [11–13] since (i) the concentrations of most of the trace elements are higher in hair than in other human materials, (ii) specimens can be collected more quickly and easily than specimens of blood, urine or any other tissue and special storage conditions are not needed, (iii) unlike

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**Table 1.** Summary of preconcentration procedures developed for Co and Ni using benzophenone/naphthalene as collector

S. no.	Nature of preconcentration	Reagent	Metal ion	Technique	pH	Linear range ( $\mu\text{g mL}^{-1}$ )	Application
1.	molten benzophenone	nitroso-R-salt CTAB[5]	Co	spectrophotometry	–	–	N.A
2.	Naphthalene	tetrahydrofurfuryl xanthate[6]	Co & Ni	spectrophotometry	2.2–9.5 and 3.7–8.6	0–3 and 1–8	N.A
3.	molten naphthalene	ethyl xanthate[7]	Co & Ni	spectrophotometry	7.5–9.0 and 5.5–7.5	2.0–21.2 and 2.4–51.6	N.A
4.	molten naphthalene	ethyl thio xanthate[8]	Co	spectrophotometry	5.0–9.5	0.5–14	alloys
5.	Naphthalene	butyl xanthate[9]	Co & Ni	spectrophotometry	4.3–8.1 and 4.1–6.8	0.5–5.5 and 0.8–8.0	steel
6.	molten naphthalene	isoamyl xanthate[10]	Co & Ni	spectrophotometry	–	–	alloys
7.	benzophenone	potassiumethyl xanthate (present method)	Co & Ni	AAS	8–10 and 8–10	0.3–75 $\mu\text{g L}^{-1}$ and 0.5–100 $\mu\text{g L}^{-1}$	hair

N.A. Not applied to real samples.

blood, hair is an inert and chemically homogeneous sample and (iv) concentrations in hair provide a retrospective index of trace element supplies unlike serum and urine which provide only an acute index, and this only over a relatively short period. Recently, Zhang et al. [14] described a liquid–liquid extractive preconcentration procedure using diethyl dithiocarbamate as extractant into MIBK followed by determination of cobalt in hair samples using flame AAS.

Cobalt is associated with the synthesis of vitamin B<sub>12</sub>, and a lack of it causes anaemia. Several cases of lung, nose and nasopharynx cancer were reported of workers of a nickel refinery located in England in the 1930s. Nickel has been found in the hair of persons exposed to nickel oxide dust in the alkali–cadmium–nickel storage battery industry.

In the method described in this paper, cobalt and nickel contents in hair samples of residents of India were determined by using simple and readily available flame AAS in conjunction with the newly developed SPE preconcentration procedure based on enrichment of cobalt and nickel onto ethyl xanthate modified benzophenone.

## Experimental

### Apparatus

Measurements were performed with a Perkin Elmer Analyst-100 Flame AAS (Perkin Elmer, USA). Instrument settings were made

according to the manufacturer's recommendation. A LI-120 digital pH meter (ELICO, India) was used for pH measurements.

### Reagents

AR grade HNO<sub>3</sub> and HClO<sub>4</sub> were used in the preparation of hair solutions. The standard solutions of cobalt and nickel and hair sample solutions were prepared using deionised water. Other reagents used in this study including the synthesis of potassium ethyl xanthate were of analytical reagent grade. Hair reference materials CRM No. 5, supplied by the National Institute of Environmental Studies (NIES, Japan), and reference hair HH-I, supplied by the International Atomic Energy Agency (IAEA, Vienna), were used for validation. For quantitative analysis, cobalt and nickel were prepared from 1 g L<sup>-1</sup> commercial AAS standards and stored in polyethylene bottles.

### General Procedure

The stock solution was diluted to 1 litre to give final concentrations of 0–75  $\mu\text{g}$  of Co and 0–100  $\mu\text{g}$  of Ni, and the pH was adjusted to  $9.0 \pm 1.0$  by adding 10 mL of 1 M ammonia–ammonium chloride buffer and transferred to a 2 litre beaker. 10 mL of 10% potassium ethyl xanthate and 2 mL of 10% benzophenone in acetone were added to the above solution and stirred for 10 min. The residue obtained after filtration was dissolved in 5 mL of DMF and determined by flame AAS.

### Procedure for Analysis of Hair Samples

0.5 g of hair sample was dissolved by adding 10 mL of conc. HNO<sub>3</sub> and 1 mL of conc. HClO<sub>4</sub>, then heated to white fumes (avoid complete drying) and made up to 50 mL. The pH of the above sample was adjusted to  $9.0 \pm 1.0$  after addition of 10 mL each of 1 M NH<sub>3</sub>–NH<sub>4</sub>Cl buffer and 1% potassium ethyl xanthate. Solid phase extraction of cobalt and nickel was carried out after addition of

2 mL of 10% benzophenone in acetone and determined by flame AAS following the procedure described above.

## Results and Discussion

The SPE of 10 µg each of cobalt and nickel present in 1 litre of solution was investigated as a function of pH, xanthate and benzophenone concentrations, time of stirring, aqueous phase volume and kind of solvent.

### *Examination of Main Experimental Variables*

#### Effect of pH

The effect of pH on the preconcentration of 10 µg each of cobalt and nickel present in 1 litre of solution was studied. The enrichment of cobalt and nickel was found to be constant and to reach its maximum in the pH range of 8.0–10.0. In all subsequent work, the pH was adjusted to  $9.0 \pm 1.0$  by adding 10 mL of 1 M  $\text{NH}_3\text{-NH}_4\text{Cl}$  buffer.

#### Effect of Potassium Ethyl Xanthate and Benzophenone

The concentration of potassium ethyl xanthate was varied in the range of 0.01–1% in a total volume of 1 litre. A minimum of 0.1% potassium ethyl xanthate was enough for quantitative enrichment of cobalt and nickel. Hence, 10 mL of 10% potassium ethyl xanthate was used in all subsequent studies. Since a minimum of 0.2 g of benzophenone was sufficient for quantitative enrichment of cobalt and nickel from 1 litre of solution, 2 mL of 10% benzophenone in acetone solution was added for enrichment of cobalt and nickel in subsequent studies.

#### Effect of Stirring Time and Aqueous Phase Volume

The stirring time was varied from 5–60 min. during preconcentration of 10 µg each of cobalt and nickel from 1 litre of solution. The results obtained show that 10 minutes of stirring were enough for quantitative enrichment of cobalt and nickel. Ten minutes of stirring was used in all subsequent investigations. The effect of aqueous phase volume on the preconcentration of 10 µg each of cobalt and nickel was studied in the range of 25–100 mL. The results obtained indicate that quantitative preconcentration of cobalt and nickel took place up to 1000 mL, thus enabling an enrichment factor of 200.

### Choice of Solvent

Various solvents were tested for the dissolution of xanthate chelates of cobalt and nickel together with benzophenone. These materials were found to be soluble in dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), propylene carbonate and acetonitrile. DMF was preferred because of the high solubility and stability. Though it was found that 2–3 mL of DMF was sufficient to dissolve the benzophenone or metal enriched benzophenone, 5 mL of DMF was chosen for convenience.

### Linearity, Sensitivity and Precision

Under the optimal conditions described above, the calibration curves were linear over the concentration range of 0–75 (Co) and 0–100 (Ni) µg present in 1 litre of solution. Five replicate determinations of 10 µg of cobalt or nickel present in 1 litre of solution gave mean absorbances of 0.102 and 0.06 with relative standard deviations of 2.1 and 2.5%, respectively. The detection limits corresponding to 3 times the standard deviation of the blank were found to be 0.3 and 0.5 µg L<sup>-1</sup> for cobalt and nickel, respectively. The linear equations with regression (R) are as follows

$$\begin{aligned} A_1 &= 0.0184 C_{\text{Co}} - 0.005 & R_{\text{Co}} &= 0.9996 \\ A_2 &= 0.0069 C_{\text{Ni}} - 0.0015 & R_{\text{Ni}} &= 0.9986 \end{aligned}$$

where A is the peak height absorbance and C is the concentration in µg L<sup>-1</sup>. All the statistical calculations are based on the average of triplicate readings for each standard solution in the given range.

### Effect of Neutral Electrolytes

Sample solutions containing 10 µg each of cobalt and nickel in the presence of 0.1 M of NaCl, NaNO<sub>3</sub>, NaClO<sub>4</sub>, Na<sub>2</sub>SO<sub>4</sub> and Na<sub>3</sub>PO<sub>4</sub> were determined by subjecting them to the general procedure experimental. It was found that none of the neutral electrolytes affects the preconcentration and AAS determination of cobalt or nickel.

### *Analysis of Standard Reference Materials*

The standard reference materials (NIES CRM – Human Hair No. 5 and IAEA Reference Hair HH-1) were placed into the solution and analysed following the procedure experimental. The results were found to be in good agreement with the certified values (see Table 2) [15, 16].

**Table 2.** Elemental concentrations in standard reference materials<sup>a</sup>

Element	NIES CRM Human hair No. 5		IAEA reference hair HH-I	
	Found <sup>b</sup>	Certified	Found <sup>b</sup>	Certified
Cobalt	0.11 ± 0.01	0.1 ± 0.01	5.8 ± 0.5	6.0 ± 0.4
Nickel	1.7 ± 0.1	1.8 ± 0.1	2.5 ± 1.0	2.3 ± 1.1

<sup>a</sup> Concentration in  $\mu\text{g g}^{-1}$ , <sup>b</sup> average of 5 determinations.

### *Analysis of Human Hair Samples of a Few Residents of India*

Sampling, the sample protocol and washing procedures were carried out as described by Airey [17], Ryabukhin [18] and Assarian and Oberleas [19], respectively. The factors that influence trace elements of hair samples are i) the distance of the sample from the scalp; ii) age and sex of the donor, iii) the geographical habit of the donor and iv) his or her dietary supplements and medication. However, factors i) and iv) were eliminated by the careful sampling procedure adopted in the present study. Hair samples were collected covering different ages, sex and geographical location within India, washed, dissolved, preconcentrated and determined by flame AAS following the procedure experimental. The results obtained for cobalt and nickel contents of the hair samples are given in Table 3 along with the standard deviation ( $\pm 2\sigma$ ) for a triplicate of each measurement. Although

the number of hair samples analysed is only 20, the following conclusions may be drawn from the data compiled in Table 3.

- i) The cobalt content in hair samples of Indian residents is around  $0.30 \mu\text{g g}^{-1}$ , except for samples 1, 6 and 15. These values are independent of age and sex.
- ii) The cobalt contents established by the present procedure agree well with ICP-AES values obtained earlier [20] and also lie in the normal range ( $0.2\text{--}1.0 \mu\text{g g}^{-1}$ ) given for trace and minor elements in human hair [21].
- iii) The nickel content in hair samples of Indian residents lies in the range of  $0.60\text{--}1.33$ , except in samples 3, 4, 13 and 19. Again, these values are not much dependent on age or sex.
- iv) The nickel contents established by the present procedure agree well with ICP-AES values reported by Rao et al. [20] and lie in the range ( $1.0\text{--}2.0$ ) established by Minera lab [21].

**Table 3.** Elemental concentrations in hair samples ( $\mu\text{g g}^{-1} \pm 2\sigma$ )\*

Sample no.	Sample (age)	Location (AP/TN)	Cobalt	Nickel
1	B(5)	Tirupati, AP	<0.06	<0.13
2	G(6)	Tirupati, AP	$0.30 \pm 0.05$	$0.60 \pm 0.05$
3	B(10)	Tirupati, AP	$0.31 \pm 0.06$	$2.40 \pm 0.10$
4	F(20)	Tirupati, AP	$0.32 \pm 0.07$	$1.80 \pm 0.11$
5	M(22)	Tirupati, AP	$0.30 \pm 0.05$	$0.60 \pm 0.08$
6	M(25)	Renigunta, AP	<0.06	$0.62 \pm 0.06$
7	M(30)	Vellore, TN	$0.31 \pm 0.07$	$0.60 \pm 0.05$
8	F(30)	Chennai, TN	$0.32 \pm 0.06$	$0.61 \pm 0.10$
9	M(32)	Tirupati, AP	$0.63 \pm 0.08$	$1.33 \pm 0.20$
10	F(32)	Vijayawada, AP	$0.31 \pm 0.06$	$0.94 \pm 0.15$
11	F(32)	Madhurai, TN	$0.60 \pm 0.05$	$0.60 \pm 0.12$
12	M(35)	Tirupati, AP	$0.33 \pm 0.05$	$0.62 \pm 0.10$
13	F(35)	Tirupati, AP	$0.30 \pm 0.06$	$3.05 \pm 0.15$
14	M(37)	Tirupati, AP	$0.30 \pm 0.05$	$1.20 \pm 0.10$
15	F(40)	Tirupati, AP	<0.06	$0.60 \pm 0.09$
16	F(47)	Chennai, TN	$0.32 \pm 0.05$	$1.25 \pm 0.06$
17	M(48)	Tirupati, AP	$0.31 \pm 0.06$	$1.22 \pm 0.07$
18	M(55)	Erode, TN	$0.32 \pm 0.06$	$1.21 \pm 0.08$
19	F(74)	Tirupati, AP	$0.30 \pm 0.05$	$1.80 \pm 0.07$
20	M(77)	Tirupati, AP	$0.30 \pm 0.06$	$0.60 \pm 0.05$

\* Average of 3 determinations.

B Boy, G Girl, M Male, F Female, AP Andhra Pradesh, TN Tamil Nadu.

## Conclusions

The preconcentration procedure developed for cobalt and nickel in this paper offers the advantages of higher enrichment factors ( $\sim 200$ ), reduced consumption of reagents, and environmental friendliness. Application to the determination of cobalt and nickel in human hair samples in the range of 0.30–0.60 and 0.60–1.33  $\mu\text{g g}^{-1}$ , respectively, was possible. The accuracy and precision of the preconcentration procedure is very good as is evident from the results obtained by analysing standard reference materials (NIES CRM Human Hair No. 5 and IAEA Reference Hair HH-I). The results obtained for cobalt and nickel by flame AAS in the present study agree well with ICP-AES [20] and NAA techniques [21].

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## References

- [1] Mizuike A (1983) Enrichment techniques for inorganic trace analysis. Springer, Berlin Heidelberg
- [2] Prasada Rao T, Gladis J M (2001) *Rev Anal Chem* 20: 145
- [3] Gladis J M, Prasada Rao T (2002) *Anal Lett* 35: 501
- [4] Preetha C R, Gladis J M, Prasada Rao T (2002) *Talanta* 58: 701
- [5] Oh H, Choi H (2000) *Anal Sci* 16: 183
- [6] Hussain M F, Bansal R K, Puri B K, Satake M (1985) *Analyst* 110: 1131
- [7] Gautam M, Puri B K (1979) *Mikrochim Acta I*: 515
- [8] Puri B K, Gautham M (1980) *J Chem Technol & Biotechnol* 30: 244
- [9] Puri B K, Gupta A K, Rao A L J, Satake M (1984) *Chim Acta Turc* 12: 245
- [10] Malik A K, Rao A L J (2000) *J Anal Chem* 55: 746
- [11] Laker M (1982) *Lancet* 2: 260
- [12] Wasiak W, Ciszewska W, Ciszewski A (1996) *Anal Chim Acta* 335: 201
- [13] Burguera J L, Burguera M, Rondon C E, Rivas C, Burguera J A, Alarcon O M (1987) *J Trace Elem Electrolytes Health Dis* 1: 21
- [14] Zhang X G, Zhang X L, Zhang H X (1997) *Lithua Jianyan, Huaxue Fence* 33: 25
- [15] Okamoto K, Morita M, Quan H, Uehiro T, Fuwa K (1985) *Clin Chem* 31: 1592
- [16] Coetzee P P, Pieterse H (1986) *S Afr J Chem* 39: 85–88
- [17] Airey D (1983) *Environ Health Perspect* 52: 303
- [18] Ryabukhin Y S (1978) IAEA report, IAEA/RL/50. IAEA, Vienna
- [19] Assarian G S, Oberleas D (1977) *Clin Chem* 23: 1771
- [20] Rao K S, Balaji T, Prassada Rao T, Babu Y, Naidu G R K (2002) *Spectrochim Acta Part B* 57: 1333
- [21] Chatt A, Katz S A (1988) VCH Publishers Inc., New York