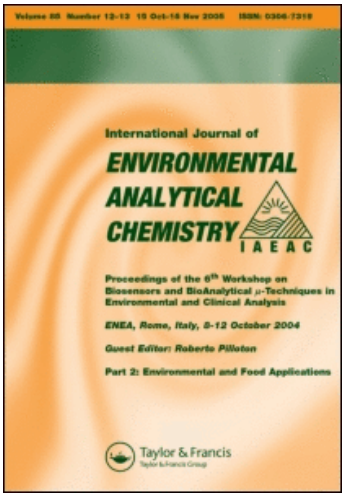

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Cobalt in Bilogical Materials by Voltammetry and Electrothermal Atomic Absorption Spectrometry

Regine Heinrich[a]; Jürgen Angerer[a]
[a] Zentralinstitut für Arbeitsmedizin der Gesundheitsbehörde und Ordinariat für Arbeitsmedizin der Universität (Direktor: ord. Prof. Dr. med. G. Lehnert), Adolph-Schönfelder-Strañe 5, Hamburg, Federal Republic of Germany

To cite this Article Heinrich, Regine and Angerer, Jürgen(1984) 'Determination of Cobalt in Bilogical Materials by Voltammetry and Electrothermal Atomic Absorption Spectrometry', International Journal of Environmental Analytical Chemistry, 16: 4, 305 — 314
To link to this Article: DOI: 10.1080/03067318408076960
URL: http://dx.doi.org/10.1080/03067318408076960

Intern. J. Environ. Anal. Chem. 1984, Vol. 16, pp. 305-314
0306-7319/84/1604-0305 $18.50/0

Printed in Great Britain

Determination of Cobalt in Biological Materials by Voltammetry and Electrothermal Atomic Absorption Spectrometry†

REGINE HEINRICH and JÜRGEN ANGERER

Zentralinstitut für Arbeitsmedizin der Gesundheitsbehörde und Ordinariat für Arbeitsmedizin der Universität (Direktor: ord. Prof. Dr. med. G. Lehnert), Adolph-Schönfelder-Straße 5, D-2000 Hamburg 76, Federal Republic of Germany

(*Received September 9, 1983*)

For industrial purposes cobalt is used to a large extent. About 2,300 persons are occupationally exposed to this carcinogenic metal in Western Germany. As reliable analytic methods for biological monitoring are not available we developed procedures for analyzing cobalt in whole blood and urine by using two independent methods, voltammetry and ETAAS.

For ETAAS-analysis of urine the cobalt content is chelatized and extracted in an organic solvent. This clean-up step enables us to calibrate with aqueous standards.— Samples of whole blood are directly injected into the graphite tube after being diluted with a homogeniziser. For their gentle thermal decomposition we use a temperature/time programme containing six steps. Both kinds of sample treatments are uncomplicated and permit routine applications.

For voltammetric determination of cobalt in urine and blood the biological material must be completely mineralized. The dry residue is dissolved in a

†Presented at the Workshop on "Carcinogenic and/or Mutagenic Metals (Environmental Chemistry, Analytics, Biological Effects)", Geneva, 13th Sept. 1983.

NH_4Cl/NH_3 solution. Cobalt is chelatized with 2,3-butanedione-dioxime and preconcentrated by adsorption at the hanging mercury electrode. By scanning the potential into negative direction the cobalt complex is reduced. The resulting signal can be used for the quantitative determination.

For comparison of both methods we have analyzed blood and urine samples of occupationally exposed persons. We found very good correlations with a statistical significance at the level of 0.01%.

KEY WORDS: Cobalt, Body fluids, Voltammetry, ETAAS.

INTRODUCTION

Recently, interest of hygienists has focussed on health surveillance of persons occupationally exposed to cobalt or its compounds. About 2,300 persons in the Federal Republic of Germany have contact to this carcinogenic metal in the daily work.[1] On the other side, cobalt is an essential trace element and as such takes part in the blood production. Before epidemiological studies for estimating the health risks combined with cobalt can be applied we had to develop analytical methods that reach the demands of

—reliability
—practicability
—and sensitivity.

As generally agreed upon the most suitable techniques today for analyzing ppb-traces of metals are electrothermal atomic absorption spectrometry (ETAAS) and voltammetry. Both of them were used for determining cobalt in blood and urine.

COBALT IN BLOOD BY ETAAS[2]

Principle

Eightfold dilution of the blood sample with a homogenizer and a gentle thermal decomposition before atomization allow a complete compensation of the interferences due to the biological matrix. Pyrocoated graphite tubes combined with a very fast heating rate up to the atomization temperature lead to an increase in sensitivity so that concentrations above the normal level can be easily analyzed.

Sample treatment

125 μl whole blood is pipetted into each of four carefully cleaned polyethylene tubes each of which containing 850 μl Triton-X-100 solution (0.001%) and one drop of n-octanol. The solutions are mixed and 25 μl 0.01 M nitric acid is added, containing no or different amounts of cobalt, corresponding to spiked blood concentrations of 20, 50 and 80 μg/l. After mixing again, 25 μl of each sample solution is injected into a pyrocoated graphite tube.

Operational parameters

Atomic absorption spectrometer:	Type 4000 by Perkin Elmer
Wavelength:	240.7 nm
Spectral slit width:	0.2 nm
Lamp current:	30–40 mA
Background compensation:	Deuterium lamp
Graphite furnace:	Type HGA 500 by Perkin Elmer
Tube:	Pyrocoated
Inert gas:	Purified argon

TABLE I
Temperature/time program of the graphite furnace (blood):

	Drying	Mineralization I	II	III	Atomization	Conditioning
Temperature (°C)	100	350	500	1000	2650	2700
Ramp time (s)	10	15	20	5	0	1
Hold time (s)	25	15	15	15	15	2
Internal flow (ml/min)	300	300	300	300	30	300

Detection limit

The detection limit was checked to lie at 2 μg/l (1% absorbance). In reagent blanks where we used double distilled water instead of blood we could not observe any cobalt signal.

Normal value

Twenty persons of different age and sex but with no occupational contact to cobalt were analyzed for their blood concentrations. All values were at or below 2.0 μg/l (detection limit).

Between-day imprecision

Self-made samples were applied as commercially available control material is still missing. They consisted of human blood spiked with cobalt. Analyzing this material at eight different days we found a mean concentration of 11.3 μg/l with a relative standard deviation of 6.2%.

COBALT IN URINE BY ETAAS[3,4]

Principle

Cobalt in urine is chelatized with the hexamethylene derivative of dithiocarbamic acid and extracted into an organic solvent. The extract is injected into the graphite tube. This clean-up step separates cobalt from interfering urinary constituents and simultaneously enriches it. So aqueous standards can be applied for calibration. It also results in a very low detection limit combined with high sensitivity so that normal cobalt excretions can be determined.

Sample treatment

In a carefully cleaned polyethylene centrifuge tube 4 ml urine (acidified with glacial acetic acid, 1 ml to 100 ml urine) is pipetted, and 0.5 ml formate buffer (pH 4.4) followed by 1 ml extraction solution (0.05 M hexamethylene-ammonium hexamethylene dithiocarbamidate in a 30/70 per cent solution of xylene and diisopropyl ketone) is added. The phases are mechanically shaken and separated by centrifugation. 50 μl organic extract which contains the complexed cobalt is injected into the graphite tube.—For an increased sensitivity in the lower concentration range (normal values) an eightfold enrichment can be applied (4 ml urine and 0.5 ml extraction solution).

Operational parameters

Atomic absorption spectrometer:	Type 4000 by Perkin Elmer
Wavelength:	240.7 nm
Spectral slit width:	0.2 nm
Lamp current:	30–40 mA
Background compensation:	Deuterium lamp
Graphite furnace:	Type HGA 500 by Perkin Elmer
Tube:	Normal type
Inert gas:	Purified argon

TABLE II
Temperature/time program of the graphite furnace (urine):

	Drying	Mineralization	Atomization	Conditioning
Temperature (°C)	120	1000	2600	2700
Ramp time (s)	25	35	0	1
Hold time (s)	25	25	15	2
Internal flow	300	300	30	300

Detection limit

The detection limit was estimated from the threefold standard deviation of the reagent blank to lie at 0.1 μg/l.

Normal value

Twenty persons of different age and sex were analyzed for their cobalt excretions in the course of 24 hours. The concentrations ranged between $\leqq$0.1 and 0.7 μg/l with a median value of 0.2 μg/l.

Between-day imprecision

Commercially available control material (Behring, Lanonorm) was analyzed at ten different days. At mean concentrations of 5.2, 30.7 and 147 μg/l respectively, we found relative standard deviations of 11.2, 5.3 and 5.3% respectively.

COBALT IN BLOOD AND URINE BY VOLTAMMETRY[5]

Principle

The biological material is completely mineralized by wet digestion. The residue is dissolved in a NH_4Cl/NH_3 solution. The cobalt content is chelatized with 2,3-butanedion dioxime and the complex preconcentrated by adsorption at the hanging mercury drop electrode. By scanning the potential into negative direction cobalt is reduced resulting in a current maximum in the differential pulse voltammogram. Using standard addition this signal leads to the cobalt concentration of the biological material.

Sample treatment

All glass containers used for digestion and voltammetric measurement must be carefully precleaned with nitric acid (1M) and with double distilled water.

1 ml blood or 5 ml urine are pipetted into round quartz flasks of the digestion apparatur (Büchi No. 445). This apparatus consists of six flasks which rotate in or above a salt bath of 300°C. The angle of the air condensors and the duration of rotating in or above the salt bath can be adjusted.

At first samples are gently heated after addition of 2 ml nitric acid (65%) and, for blood, 0.4 ml sulfuric acid (96%). After 30 minutes their colour has changed to a light yellow. To the reduced volume of the urine samples (2 ml) are pipetted 0.4 ml sulfuric and 2 ml nitric acid, to the blood samples 4 ml nitric acid only. Now the duration of rotating in the salt bath is stepwise increased till the samples stay completely in it for 5 minutes. For urine, after a second addition of 2 ml nitric acid, this procedure is repeated. An excess of nitric acid is then removed by azeotropic distillation after addition of 3×5 ml double distilled water. Sulfuric acid is neutralized with an excess of ammonia (25%) which is removed by azeotropic distillation as well. The dry residue is white or yellow (blood).

It is dissolved in 7 ml 0.1 M NH_4Cl solution and transferred into a glass container for the voltammetric measurement. After addition of 300 μl 0.05 M ethanolic 2,3-butanedion dioxime solution, a pH value of 7.5 is adjusted with ammonia and the voltammetric determination is carried out. Standard addition is applied for

calibration.—Especially for urine determinations other sample volumes than 5 ml may be advantageous. In the lower concentration range (normal values) we used 10 ml and 1 ml for occupationally exposed persons.

Operational parameters

Apparatus:	Polarograph 1E 506, VA-Dopplestand E 607, VA-Controller E 608, Dosimat E 535 and Dosifix E 542 by Metrohm
Operation mode:	Differential pulse
Working electrode:	Hanging mercury drop electrode (HMDE)
Reference electrode:	Ag/AgCl/sat. KCl
Auxiliary electrode:	Platinum
N_2 purge time:	400 s
Preconcentration:	200 s with, 30 s without stirring at −0.7 V
Initial potential:	−0.6 V
Final potential:	−1.2 V
Scan rate:	−10 mV/s
Pulse amplitude:	25 mV
Current registration:	4.10^{-9} A/mm

Detection limit

The detection limit was estimated from the threefold standard deviation of the reagent blank. For blood it is 0.8 μg/l and 0.2 μg/l for urine respectively.

Normal value

Thirty-one persons of different age and sex but with no exposure to cobalt were analyzed for their cobalt content in blood, twenty-two persons for their cobalt excretion in urine. For blood, all values were at or below the detection limit. For urine, we found a median value of 0.6 μg/l and a range from <0.2 to 2.9 μg/l.

Between-day imprecision

Spiked urine and blood samples at concentrations of 5.0 and 19.8 μg/l (urine) and 5.1 and 49.0 μg/l (blood) were analyzed at eleven days. The relative standard deviations were 13.0 and 7.8, and 11.8 and 5.8% respectively.

ACCURACY OF THE METHODS

To assess the accuracy biological materials of a group of occupationally exposed persons were analyzed by both independent methods. For blood and urine we found highly significant linear correlations (blood: $n=25$; $y=0.95\ x+0.68$; $r=0.986$; $P<0.0001$; Fig. 1; urine; $n=22$; $y=1.02\ x+1.75$; $r=0.994$; $P<0.0001$; Fig. 2). This indicates that both procedures for ETAAS and voltammetry lead to the same accurate results within the ranges of their imprecisions.

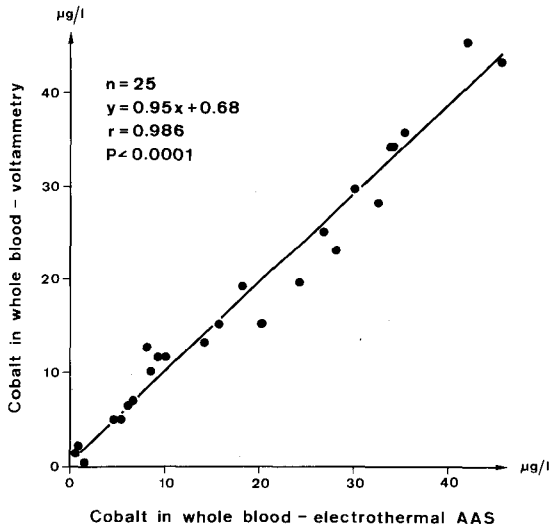

FIGURE 1 Diagram of correlation for the parallel analysis of blood samples from a group of occupationally exposed persons by electrothermal AAS and voltammetry.

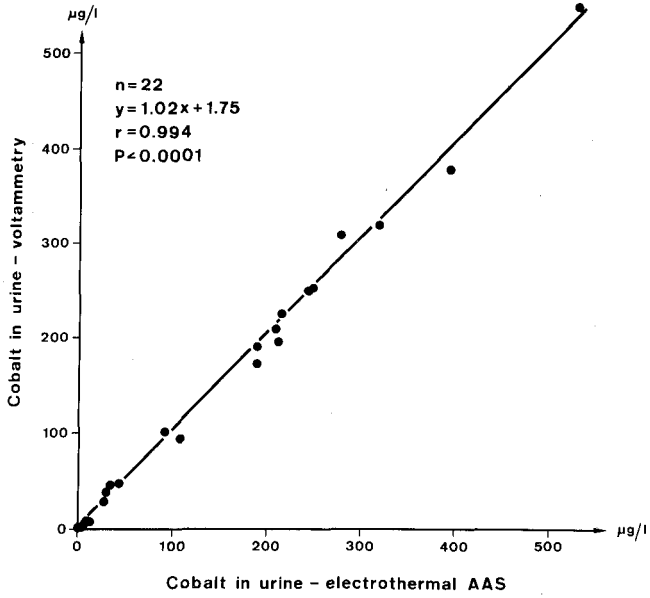

FIGURE 2 Diagram of correlation for the parallel analysis of urine samples from a group of occupationally exposed persons by electrothermal AAS and voltammetry.

DISCUSSION

As we have shown, both methods for blood, and urine respectively, fulfill the requirements concerning *accuracy*. Additionally, in all four cases we found between-day imprecisions ranging from 5.3 to 13.0%. Thus, the derived results show a high degree of *precision*.

Besides these criteria of reliability, methods suitable for health surveillance in occupational medicine have to show a high degree of *practicability* as large series of samples have to be analyzed. Discussing this point, the voltammetric determinations fail to reach this requirement mainly because of their time consuming digestion procedure. On the contrary, sample treatments for ETAAS-analysis of urine and blood are uncomplicated. This is especially true for the urine method where calibration by aqueous standards is obtained and about forty samples per day and technical assistant can easily be managed. Both procedures were proved for routine application for

more than one year in our laboratory.—Thus, the described ETAAS procedures are most suitable for purposes of health surveillance whereas one has to look at the voltammetric procedures as accurate and independent reference methods.

Besides, analytical procedures for estimating the cobalt content in body fluids of normal unexposed persons have to show a high *sensitivity* combined with a low detection limit. For blood, both methods failed to establish a normal value as it lies at or below the detection limit. Decreasing reagent blanks for the voltammetric determination and an alternative sample treatment for ETAAS (by extraction?) would give more informations. However, the normal blood level estimated by Barfoot and Pritchard[6] to range from 2 to 2.8 μg/l might be too high.—For urine, both methods allow determinations in the normal concentration range and confirm that the median normal level lies right below 1 μg/l. This agrees quite well with recent data of Lidums[7] and Wester[8] who found median values of 0.4 μg/l and 0.73 μg/24 h respectively.

References

1. Berufsgenossenschaften der chemischen Industrie, *Sich. Chemiearb.* **8/1982,** 60
2. J. Angerer and R. Heinrich, *Fresenius' Z. Anal. Chem.*, in press.
3. E. Schumacher-Wittkopf and J. Angerer, *Int. Arch. Occup. Environ. Health* **49,** 77 (1981).
4. J. Angerer and K. H. Schaller, *Analyses in Biological Materials* (Verlag Chemie, Weinheim/Deerfield Beach, 1984), in press.
5. R. Heinrich, *Fresenius' Z. Anal. Chem.*, in press.
6. R. A. Barfoot and J. G. Pritchard, *Analyst* **105,** 551 (1980).
7. V. V. Lidums, *At. Absorpt. Newsl.* **18,** 71 (1979).
8. P. O. Wester, *Acta Med. Scan.* **194,** 505 (1973).