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DETERMINATION OF NICKEL IN URINE AND BLOOD SERUM BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

Marina Stefova[1], **Trajče Stafilov**[1] and **Elisaveta Stikova**[2]

[1]*Institute of Chemistry, Faculty of Natural Sciences and Mathematics, The "Sv. Kiril & Metodij" University, P.O. Box. 162, 91001 Skopje, Republic of Macedonia*
[2]*Institute for Public Health "Skopje", 91000 Skopje, Republic of Macedonia*

In this work a determination of nickel in urine and blood serum samples by electrothermal atomic absorption spectrometry is performed using the IUPAC reference method with several modifications. These modifications refer to the concentration of the sample and to the instrumental parameters. Nickel is extracted as *bis*-(1-pyrrolidinecarbodithioato)nickel(II) complex in 4-methyl-pentane-2-one (methyl isobuthyl ketone, MIBK) as a solvent. Samples are first digested with mixture of acids (HNO_3, H_2SO_4, $HClO_4$) up to 200 °C to remove the organic matter. The residue is then adjusted to pH 7 and ammonium pyrrolidine dithiocarbamate (APDC) solution is added. The *bis*-(1-pyrrolidinecarbodithioato)nickel(II) complex is extracted in MIBK. The concentration of nickel extracted in MIBK is measured by electrothermal atomic absorption spectrometry. The method was tested with standard additions of nickel to samples of urine and serum and satisfactory results were obtained for the recovery values. Determinations of nickel concentration performed on 70 samples of urine and 68 samples of serum from workers exposed to nickel compounds showed that in the most cases nickel concentrations are slightly higher than the ones found in the control group. This fact indicates the need of permanent control and action to protect the workers from the acute or chronic intoxication with nickel and its compounds.

Key words: nickel; blood serum; urine; determination; electrothermal atomic absorption spectrometry

INTRODUCTION

Nickel is a ubiquitous trace metal and occurs in soil, water, air and in the biosphere. It is used for a wide variety of purposes in modern industry and, because it is a constituent of stainless steel, virtually every individual in the industrialized world is in daily contact with the metal. Nickel is an allergenic and in some of the compounds a carcinogenic element. High risks of lung and nasal cancer have been reported in nickel refinery workers employed in the high-temperature roasting of sulfide ores and in processes of electrolysis and hydrometallurgy [1]. Because of this, the need for reliable analytical procedures for monitoring nickel in human body fluids, tissues and excreta has been emphasized during past two decades.

Several methods for nickel analysis in biological materials by atomic absorption spectrometry (AAS) have been reported [2–10]. They can be classified into two groups: direct determination by flameless AAS [2–5] and determination that involves preliminary wet or dry ashing and extraction step before flameless AAS [6–10]. Different chelating agents are recommended for complexing nickel before extraction, such as: dimethylglyoxime [6], furildioxime [7] and ammonium pyrrolidine dithiocarbamate – APDC [8–10].

Methods for nickel analysis in biological materials are reviewed in a paper published by the IUPAC Subcommittee on Environmental and Occupational Toxicology of Nickel with emphasis on preliminary steps for oxidation or removal of organic matter, pre-concentration and separation procedures, instrumental techniques for quantitation of nickel and reference values for nickel concentrations in human body fluids, tissues and excreta [11]. Electrothermal atomic absorption spectrome-

try – ETAAS, is recommended as currently the most sensitive, convenient and reliable technique for determining nickel in biological materials. This Committee also recommended a reference method for analysis of nickel in serum and urine by ETAAS [12] including acid digestion and extraction of nickel as *bis*-(1-pyrrolidine-carbodithioato)nickel(II) complex in 4-methyl-pentane-2-one (methylisobutyl ketone, MIBK).

In this work the modified IUPAC reference method was used for nickel analysis in samples of blood serum and urine from workers occupationally exposed to nickel and its compounds.

EXPERIMENTAL

Apparatus

A Perkin-Elmer Model 1100B Atomic Absorption Spectrometer equipped with a deuterium background corrector and an HGA-700 graphite furnace were used. Nickel hollow cathode lamp was used as a source, argon as an inert gas. The graphite tubes used in the furnace were pyrolytically coated. The instrument parameters are given in Table I.

Table I

Operation parameters of the atomic absorption spectrometer

Wavelength	231.8 nm
Slit width	0.2 nm
Hollow cathode light current	25 mA
Integration time	5 s
Calibration mode	Absorbance, peak height
Background correction	Deuterium lamp
Gas	Argon

Reagents and solutions

Acid digestion mixture. 120 ml of nitric acid, 40 ml of sulfuric acid and 40 ml of perchloric acid are placed successively into a glass-stoppered borosilicate glass bottle (250 ml capacity) and thoroughly mixed. Ultra-pure acids are essential.

Concentrated ammonium hydroxide solution-ultra-pure.

Bromothymol blue indicator solution. 20 mg of bromothymol blue (certified reagent) and 1 ml of dilute ammonium hydroxide solution are placed into a screw-cap polyethylene drop-dispenser bottle (60 ml capacity). The contents are diluted to 50 ml with ultra-pure water.

4-Methyl-pentane-2-one – ultra-pure.

Ammonium pyrrolidinedithiocarbamate (APDC) and its solution. APDC was synthesized by a method described elsewhere [13]. Its solution is prepared by dissolving 0.5 g APDC in 25 ml of ultra-pure water and the solution is then extracted three times with 1.4 ml portions of MIBK. The last washing is analyzed by electrothermal AAS to verify that it contains no detectable nickel.

Potassium phosphate buffer (1 mol/l, pH = 7). 17 g anhydrous KH_2PO_4 and 21.8 g anhydrous K_2HPO_4 are placed into a 250 ml volumetric flask and dissolved in ultra-pure water. The solution is then transferred in a 250 ml separatory funnel, 5 ml APDC solution is added and the mixture is extracted three times with 10 ml portions of ultra-pure chloroform. The last washing is analyzed by electrothermal AAS to verify that it contains no detectable nickel.

Nickel stock solution and calibration solutions. 1 g of nickel metal is weighed and transferred into 1000 ml volumetric flask. Nickel is dissolved in small volume of HNO_3 (1:1) and diluted to volume with 1 % (vol.) HNO_3. Calibration solutions with concentrations: 1, 2, 4, 5, 10, 20, 30, 40 and 50 μg Ni/l are prepared by diluting this solution with ultra-pure water in volumetric flasks.

Procedures

Urine. 10 ml samples of urine are first digested with 5 ml of mixture of acids in screw-cap glass erlenmeyer flasks (50 ml capacity) on a sand bath (half an hour at 110 °C and one hour at 250 °C) to remove the organic matter. The residue (colorless, volume approximately 1 ml-corresponding to the volume of H_2SO_4) is then dissolved in 2 ml of water, bromothymol blue solution is added and pH is adjusted to neutral with 2 ml of phosphate

buffer and a few drops of concentrated ammonium hydroxide solution (light blue-green colour). Then 1 ml of APDC solution is added and the *bis*-(1-pyrrolidinecarbodithioato) nickel(II) complex is extracted in 1 ml of MIBK. 50 μl of each extract is then introduced in the graphite furnace for analysis.

Serum. The procedure for serum analysis is similar to that for urine, differing from it only in the sample volume (5 ml) and the volume of the APDC solution (0.5 ml) and MIBK (0.5 ml).

The same procedure is used for the standard calibration solutions. 10 ml of each standard solution is treated in the same way as the samples of urine for nickel analysis in urine. 5 ml of each standard solution is treated as the samples of serum for nickel analysis in serum.

RESULTS AND DISCUSSION

Optimal conditions for the ETAAS determination of nickel extracted as *bis*-(1-pyrrolidinecarbodithioato)nickel(II) complex in MIBK were established by extensive testing. The time-temperature programme is given in Table II. The dependence of the absorbance of nickel on different charring and atomization temperatures is given in Fig. 1 and Fig. 2, respectively. Temperature of 1400 °C is chosen as optimal charring temperature because it's the highest temperature (necessary for complete charring of the sample introduced in the furnace) at which the highest absorbance of nickel is measured. It's higher than the temperature proposed in the reference method (1000–1200 °C) [12]. Temperature of 2400 °C is chosen as optimal atomization temperature because it's the lowest temperature at which maximal absorbance of nickel is measured. The atomization temperature we used is lower than the proposed in the reference method (2600–2700 °C).

Table II

Time-temperature programme for HGA-700 for nickel analysis

Step	Temperature °C	Ramp time s	Time s	Gas flow $ml \cdot min^{-1}$
Dry	90	10	20	300
	120	1	10	300
Char	1400	5	30	300
Atomize	2400	0	5	50
Clean	2650	1	3	300

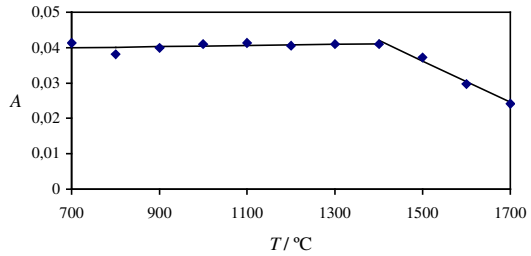

Fig. 1. Dependence of the absorbance of nickel on different charring temperatures

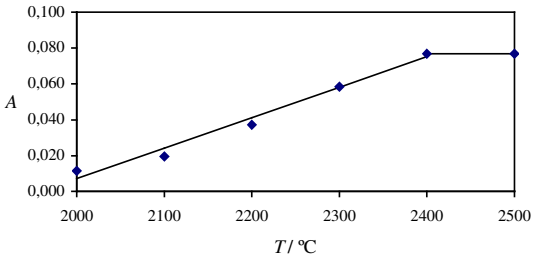

Fig. 2. Dependence of the absorbance of nickel on different temperatures of atomization

The interference of sodium, potassium, calcium and magnesium, present in the samples in considerably higher concentrations than nickel, was studied. Solutions with the same concentration of nickel but with various concentrations of the interfering elements (similar to those in samples) were prepared and the absorbance of nickel was measured. No interference from these elements on absorbance of nickel was found.

The extraction of nickel was investigated using different volumes of MIBK. 1 ml of MIBK was found to be the optimal volume for nickel analysis in urine (sample volume 10 ml) and 0.5 ml of MIBK for nickel analysis in serum (sample volume 5 ml). In this way, tenfold concentration of the

nickel content in samples can be achieved, which enables determination of lower concentrations than the reference method (2 ml sample volume and 0.7 ml MIBK) [12].

This method, modified in the instrumental conditions and sample treatment, was tested with standard additions of nickel to samples of urine and serum. The results are given in Table III. It can be seen that the recovery values are satisfactory and the method can be used for determination of nickel in samples of urine and serum.

Table III

Results of determination of nickel in urine and serum by method of standard additions

γ(Ni) added/μg·l^{-1}	Urine, γ(Ni) / μg·l^{-1}		R (%)	Serum, γ(Ni) / μg·l^{-1}		*R* (%)
	calculated	determined		calculated	determined	
–	–	0.60	–	–	2.80	–
2	2.60	2.58	99.23	4.80	4.86	101.25
4	4.60	4.62	100.43	6.80	6.67	98.08
10	10.60	10.90	102.83	12.80	13.34	104.21
20	20.60	21.18	102.81	22.80	22.06	96.75

A calibration curve (for organic solutions containing up to 20 ng Ni) was prepared using the proposed extraction procedure for standard nickel solutions. The standard deviation (SD) for 1.0 ng nickel was 0.01 ng and the relative standard deviation for this method ranged from 1.0–3.5 %. The detection limit of the method, calculated as 3 SD of the blank, was found to be 0.10 μg/l.

After establishing the optimal instrumental parameters and testing the method, analyses of nickel in 70 samples of urine and 68 samples of serum of workers occupationally exposed to nickel compounds were performed. At the same time the quantity of total and respiratory dust in the working area and their nickel content was measured. These results are given in Table IV.

Table IV

Results from the nickel determination in urine and blood serum of subjects occupationally exposed to nickel compounds and data about nickel content in working area

Dept[1]	Dust, total mg/m^3	Ni in total dust, mg/m^3	Respiratory dust, mg/m^3	Ni in respiratory dust, mg/m^3	No. of subjects urine, serum	Ni in urine, μg/l Mean ± SD	Ni in serum, μg/l Mean ± SD
1	–	–	–	–	9; 10	1.33 ± 1.03	0.76 ± 0.47
2	12.2–16.6	0.08–0.17	2.0–8.25	0.005–0.023	10; 9	12.05 ± 10.44	2.08 ± 0.64
3	2.33–40.8	0.02–0.57	1.6–12.3	0.03–0.48	13; 13	5.36 ± 4.65	1.88 ± 0.47
4	1.66–16.8	0.006–0.81	2.0–4.5	0.019–0.25	28; 27	6.56 ± 4.5	1.87 ± 0.72
5[2]	–	–	–	–	10; 9	3.44 ± 3.70	0.84 ± 0.43
MPV[3]	6.0	0.5	2.0	0.5		–	–
					Total 70; 68	mean: 5.92 range: 0.6–24.4	mean: 1.60 range: 0.15–3.25

[1] Production department; [2] Control group; [3] Maximum permitted values

It can be seen that the quantity of total and respiratory dust is above the maximum permitted values and the nickel content in total dust is also above the permitted limit in some departments.

This is probably the main reason for higher concentrations of nickel in urine and serum of some workers compared to the concentration found in samples from the control group (mean 0.84 μg/l for serum and 3.44 μg/l for urine). In the literature the normal nickel concentration in specimens from healthy non-exposed adults in serum ranges from 0.05–1.08 μg/l and in urine from 0.5–8.8 μg/l [1]. Higher concentrations found in urine (Table IV) show that nickel, which is brought into the human body, is excreted through urine. This implies that nickel concentration in serum can be brought back to normal by excreting it through urine if the human body is no longer exposed to nickel compounds.

CONCLUSION

The proposed method for determination of nickel in urine and serum extracted as *bis*-(1-pyrrolidine-carbodithioato)nickel(II) complex in MIBK gives satisfactory results, which is proved by the method of standard additions.

Determinations of nickel concentration performed on 70 samples of urine and 68 samples of serum from workers exposed to nickel compounds showed that in the most cases nickel concentrations are slightly higher than the ones found in the control group. This fact indicates the need of permanent control and action to protect the workers from the acute or chronic intoxication with nickel and its compounds.

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Резиме

ОПРЕДЕЛУВАЊЕ НА НИКЕЛ ВО УРИНА И КРВЕН СЕРУМ СО ЕЛЕКТРОТЕРМИЧКА АТОМСКА АПСОРПЦИОНА СПЕКТРОМЕТРИЈА

Марина Стефова[1], **Трајче Стафилов**[1] и **Елисавета Стикова**[2]

[1]Институт за хемија, Природно-математички факултет, Универзитет "Св. Кирил и Методиј", п. фах 162, 91001 Скопје, Република Македонија
[2] Здравствен дом на Скопје, 91000 Скопје, Република Македонија

Клучни зборови: никел; серум; урина; определување; електротермичка атомска апсорпциона спектрометрија

Во трудов се презентирани резултатите од определувањето на никел во примероци од урина и серум со електротермичка атомска апсорпциона спектрометрија, користејќи го референтниот метод од IUPAC со неколку модификации. Овие модификации се однесуваат на концентрирањето на образецот и на инструменталните параметри. Никелот е екстрахиран како *bis*-(1-пиролидинкарбодитиоато)никел(II) комплекс во 4-метил-пентан-2-он(метил изобутил кетон, MIBK) како растворувач. Примероците се загреваат со смеса од

киселини (HNO_3, H_2SO_4, $HClO_4$) до 200 °C за отстранување на органските материи, а остатокот потоа се неутрализира до pH = 7. Потоа се додава растворот од амониумпиролидиндитиокарбамат (APDC) и формираниот *bis*-(1-пиролидинкарбодитиоато)никел(II) комплекс се екстрахира во MIBK. Концентрацијата на никел во екстрактот се определува со електротермичка атомска апсорпциона спектрометрија. Постапката е проверена со методот на стандардни додатоци, при што се добиени задоволителни резултати за аналитичкиот принос. Извршени се определувања на застапеноста на никел во 70 примероци од урина и 68 примероци од серум земени од работници изложени на соединенијата на никел. Добиените резултати во повеќето случаи имаат малку повисоки вредности од оние во контролната група, што ја наметнува потребата за постојана контрола и преземање мерки за заштита на работниците од акутни или хронични труења со никел и неговите соединенија.