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)

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The study presents a newly-developed method for extraction-spectrophotometric determination of Co (II) in samples by means of iodnitrotetrazolium chloride (INT). According to our studies, the electronic spectrum of the cobalt ion associate has a peak at 630 nm. The INT:$[Co(SCN)_4]$ ratio in the triple ion-associate complex was 2:1, and due to the good solubility of the Co(II) ion associate and INT in 1,2-dichloroethane, maximum extraction into the organic phase was achieved for 30 s. The molar absorptivity of the studied ion associate $INT_2[Co(SCN)_4]$ was $\varepsilon_{630} = 0.6\times10^3$ L/mol cm. Sandell's sensitivity of the method was 9.8×10^{-2} μg/cm^2. The maximum quantity of Co was extracted with INT at pH 2-7. Maximum absorption of the extracts was registered at thiocyanate ions concentration ($\geq 1M$) in the aqueous phase. The ion associate displayed the highest absorption at INT concentration $\geq 6\times10^{-4}$ M. The relationship between Co (II) concentration and absorption was linear in the range of 6 – 125 μg Co(II) in 10 ml aqueous solutions. The content of Co in *Amanita calsarea* was 1.543 ± 0.158 mg/kg dry weight, in *Boletus pinophilus* - 0.257 ± 0.042 mg/kg dry weight and in *Burley tobacco* - 1.967 ± 0.229 mg/kg dry weight. The extraction-spectrophotometric method for cobalt determination in biological samples with iodnitrotetrazolium chloride, developed in the present study, is characterized with high speed, selectivity and satisfactory accuracy.

Keywords: Co, INT, spectrophotometry, application, mushrooms and tobaccos.

INTRODUCTION

It is known that long-term cobalt deficiency can result in demyelination of large nerve trunks and spinal cord, reduced white blood cells, pernicious anemia and reduced resistance to parasite and microbial infections. On the other hand, cobalt can be toxic in high doses, causing polycythemia, bone marrow hyperplasia, pancreatic failure, congestive heart failure and cardiomyopathy [1, 2]. That is why its content in various systems should be monitored [3–5].

It is widely known that Co content in agrochemical objects is insignificant [3–8]. The scientific literature presents many methods for separation, preconcentration and determination of cobalt in samples [2–20]. The widely applied methods for determination of the element content are photocolorimetric, polarographic and spectral. Extraction-photometric methods are applied for determination of ultra-small Co quantities in agrochemical objects. Their major advantages are simplicity, rapidity and accessibility. In the analytical practice, the reaction between Co (II) and nitroso-R-salt is widely used. One of the base factors that reduce the precision of the photometric determination of Co microquantities in soil samples is the presence of colored organic compounds, as well as of Fe, Ni and Cu salts.

Till now the literature presents only a few methods for Co determination in soil and plants. The following reagents are used: 1,10–phenantroline, eosin, thiodibenzoylmethane, picraminazo-4-cyclohexylresorcinol, diantipyryl-methane, and 2-(2-benzthiazolylazo)-5-dimethyl-aminobenzoic acid. Some of these methods require exact working pH and preliminary stay of the samples before Co determination. Other methods are characterized with low sensitivity and selectivity. Another method for quantitative determination of Co includes heating to 80º C. However, the complex formed is stable for only 20 min [14-26].

The aim of the present study was to develop an extraction-spectrophotometric method for Co (II) determination in biological samples (mushrooms and tobaccos), with selectivity and speed exceeding those of most up-to-date applied methods. The structure formula of INT is presented in Fig. 1.

IUPAC name: 3-(4-iodophenyl)-2-(4-nitro-phenyl)-5-phenyl-2H-tetrazol-3-ium chloride. Other names: 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-tetrazolium chloride; Iodnitrotetrazolium chloride, INT.

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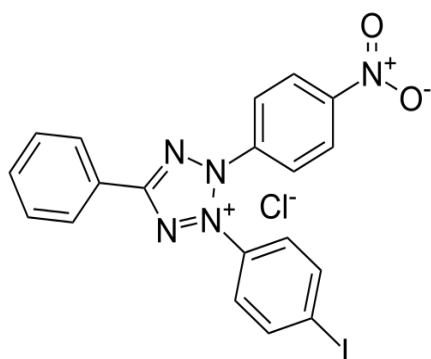E-mail: lkd@abv.bg

Fig. 1. Structure formula of INT

The following objectives have to be studied:

- the solubility of the ion-associated thiocyanate complex of Co(II) with iodnitrotetrazolium chloride (INT) in different organic solvents and to select the most appropriate of them;
- the effect of pH;
- the time necessary for Co extraction, and to estimate the time necessary for complete metal extraction as an ionic associate in the organic phase;
- the effect of the concentrations of the reagents participating in the extraction equilibrium of the system. The optimal established quantity of each of them would guarantee the complete extraction of Co (II) using the investigated monotetrazolium salt;
- the effect of side ions on Co extraction and determination in order to assess the selectivity of the developed method.

EXPERIMENTAL

Reagents and apparatus

Apparatus

Absorbance measurements were made on a UV - Vis spectrophotometer, Germany, with a 1 cm quartz cuvette at 630 nm.

Reagents

The reagents used were of analytical grade (p.a., Merck and Fluka). All solutions were prepared with distilled demineralized water.

Extraction procedure

In a separating funnel of 100 ml the following solutions were added: 1 ml of 1×10^{-3} M Co (II), 1 ml of 4M potassium thiocyanate and 7 ml of 1.5×10^{-3} M iodnitrotetrazolium chloride (INT). Distilled water was added to the aqueous phase to 10 ml volume. The following step was extraction with 3 ml of 1,2-dichlorethane for 30 s. After phase separation, the organic phase was transferred through a paper filter

into a cuvette b = 1 cm and subjected to photometric analysis at 630 nm on a UV - Vis spectrophotometer.

RESULTS AND DISCUSSION

Optimal Conditions

Solvent

The extraction of the Co ion associate in different organic solvents: methyl ethyl ketone, 1,2-dichlorethane, chloroform, benzene, toluene, tetrachloromethane and diethyl ether, was studied.

1,2-dichloroethane was found as the most suitable solvent. The electronic spectrum of the cobalt ion associate has a maximum at 630 nm. The maximum absorption was detected at this wavelength.

The bond in the molecule of the obtained ion associate, formed between $[Co(SCN)_4]^{2-}$ and the tetrazolium salt was electrostatic.

Composition of the triple ion-associated complex

The composition of the triple ion-associated complex was determined by the Ostromislensky - Job method [26]. It was found that the ratio INT:$[Co(SCN)_4]$ was 2:1.

Extraction time

The effect of time as a factor for achieving extraction equilibrium between the two phases was studied. Due to the good solubility of the ion-associated complex of cobalt (II) and INT in 1,2-dichloroethane, maximum extraction of the organic phase was achieved for 30 s.

Determination of the molar absorbance of the ion triple associated thiocyanate complex of cobalt (II) and INT

Molar absorbance of the ion associate $INT_2[Co(SCN)_4]$ was determined by the method of Komar-Tolmachov [26] by measuring the light absorbance of solutions with different concentration of the reagents at constant stoichiometry. The obtained results are presented in Table 1.

The molar absorbance of the studied ion associate $INT_2[Co(SCN)_4]$ was $\varepsilon_{630} = 0.6\times10^3$ L/mol cm. The sensitivity of the method according to Sandell [4] was 9.8×10^{-2} μg/cm².

Table 1. Determination of the molar absorptivity of the ion-associated complex.

ml Co(II) 1.10^{-3} M	$C_{Co(II)}$	A	$\frac{C.l}{A} = \frac{1}{\varepsilon} \frac{1}{\varepsilon}.10^{-3}$	$1/^{n+1}\sqrt{A}$
0.1	0.00001	0.020	0.0005	0.50	7.09
0.2	0.00002	0.030	0.0007	0.76	5.78
0.8	0.00008	0.080	0.001	1.00	3.78
2	0.0002	0.160	0.00125	1.25	2.67

Study of the medium acidity

The acidity of the medium is a factor that affects the amount of cobalt uptake by the organic phase. There is a very large pH range in which it has constant values. Experimental data showed that light absorption in neutral and acidic medium to pH 2 has a constant (maximum) value. At pH < 2, it begins to decrease and the determination becomes impossible. Maximum Co(II) uptake was achieved at pH 2-7. The wide pH range makes it possible to work without precisely controlling the pH, or using buffer solution. This makes the method less pretentious.

Reagents quantity

To find out the optimal conditions for the extraction of the thiocyanate complex of Co (II) with INT the influence of tetrazole salt and thiocyanate ions was examined. The formation of the ion associate significantly depends on the concentration of the thiocyanate ions. In order to shift the equilibrium to the formation of the thiocyanate complex of Co (II) an excess of thiocyanate ions was required. This excess had no effect on the extraction equilibrium and facilitated the quantitative extraction of cobalt in the organic phase. By varying the amount of thiocyanate ions in the aqueous phase the highest light absorbance of the extracts was found at a concentration of ≥ 1 M.

An important factor in the photometric determination of cobalt is the concentration of the tetrazolium salt. In order to maximize the recovery of the metal from the aqueous phase, the influence of different INT concentrations was investigated. The light absorption of the ion associate has a maximum value at a concentration of 6×10^{-4} M INT. With increasing concentrations of the tetrazolium salt, the light absorption of the associate remains constant.

Effect of Co(II) concentration on the light absorption

When INT was added to the thiocyanate solution of cobalt (II) a pale green precipitate soluble in the organic solvent was obtained.

$$INT + [Co(SCN)_4]^{2-} \rightarrow [INT]_2 [Co(SCN)_4]$$
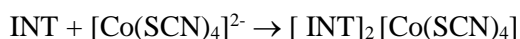
The intensity of the precipitate color increased with increasing cobalt (II) concentration. The relationship between cobalt (II) concentration and light absorption is linear in the range from 6 mg to 125 mg Co (II) in 10 ml aqueous phase.

Effect of side ions

To get a more complete picture of cobalt (II) extraction and to characterize the selectivity of the extraction, the influence of some ions, which would probably be present in the analyzed samples, was studied. Experimental data are given in Table 2. The results qualify the tetrazolium salt used as a selective reagent for the determination of cobalt. Among all examined ions, only Fe (III) interferes with the determination. The interference is removed by the addition of ascorbic acid as a masking agent whuch has pronounced reduction properties.

Table 2. Effect of some ions on Co(II) extraction with INT: $C_{Co(II)} = 0.5 \times 10^{-4}$ M, $C_{INT} = 4.5 \times 10^{-4}$ M, $C_{SCN} = 0,8$ M

Side ion	Concentration limit of the side ion, mg	Limit ratio $C_x/C_{C_{o(II)}}$, mg
K^+	300	10204:1
Na^+	100	3401:1
Sr^{2+};$S_2O_3^{2-}$	50	1700:1
$C_2O_4^{2-}$	20	680:1
Hg^{2+}	15	510:1
Al^{3+};Ca^{2+};Ni^{2+};BO_3^{3-}	10	340:1
Pb^{2+}	8	272:1
W^{6+}	7.4	251:1
Cd^{2+}	5	170:1
V^{5+}	0.5	17:1
Cr^{6+}	0.3	10:1
Br^-	0.2	6.8:1
$EDTA$;NO_3^-;J^-;SO_4^{2-}	0.1	3.4:1
ClO_4^-	0.05	1.7:1
Fe^{3+}	0.01	interferes

Application of the developed method for analysis of the cobalt content in biological samples (mushrooms and tobaccos).

Accuracy and precision

In order to check the accuracy of the method a reference material CTA-VTL-2 (Virginia tobacco leaves) was used. For evaluation of the accuracy of the digestion and measuring procedures, we have used the **R** criterion showing the percent extraction of the element from the certified value. When the measured value X is within the limits of $X_{CRM} \pm U_{CRM}$, where U_{CRM} is the indefiniteness of the certified value, we accept the extent of extraction to be 100%. In all remaining cases, the extent of extraction is equal to $X / X_{CRM} \cdot 100$. As can be seen from Table 3, the results obtained for the certified material yield a 100% recovery of Co.

Mineralization of the samples

Mushroom samples (*Amanita calsarea* and *Boletus pinophilus*) were collected in Batak-Shiroka Poliana, Bulgaria, in 2015 and dried at 105 ^{0}C for 24 h.

Tobacco samples (*Burley*) were collected in Stara Zagora, Bulgaria in 2015. They were dried at 650 ºC in a fan oven and stored in dark polyethylene bottles.

The mineralization of the samples was carried out according to EPA Method 3052 [27]. 0.5 g of air - dry sample was weighed to the nearest 0.001 g in PTFE vessels. HNO_3, HF, HCl and H_2O_2 were added using a microwave system Multiwave 3000. The maximum power was 1400 W, and the maximum pressure in the Teflon vessels - 40 bar.

The concentrations of cobalt were determined in an air-acetylene flame by the Perkin–Elmer Aanalyst 800 model atomic absorption spectrometer.

Cobalt extraction

A 5 ml sample, several drops of 1 M NaOH (for adjustment of pH 9.0-10.0) and 5 ml of 0.02% dithizone in chloroform were consecutively added in a 100 ml separating funnel. The system was left to extract for 1 min. Adequate quantity of HCl (1:1) (for adjustment of pH 1.0-2.0) and 2 ml of 4 M thiocyanate solution were added to the organic phase. Re-extraction was carried out for 1 min. The organic phase was discharged, then 1 M NaOH (for pH 3.0-4.0), 0.6 ml of 1.5×10^{-2} M INT and 2 ml of a saturated solution of ascorbic acid (for masking of the interfering ions) were added to the aqueous phase. Distilled water was supplemented to a volume of 20 ml. Extraction with 3 ml 1,2-dichloroethane was carried out for 30 s. After phase separation the organic was filtered through a paper filter, placed in a cuvette, b = 1 cm, and measured at λ = 630 nm.

A blank, not containing a sample, was also analyzed for reliability of the experimental results.

The experimental data are shown in Tables 3 and 4. A good correlation of our results with data for the certified Virginia Tobacco Leaves (CTA – VTL – 2) samples was established.

Table 3. Co content in Virginia Tobacco-CTA-VTA-2 certified reference material (n = 3).

	Co CTA-VTA-2 $X \mp \sigma$ mg/kg	R %
Certified value	0.429 ± 1.4	100
AAS	0.420 ± 0.027	98
INT	0.415 ± 0.015	97

Table 4. Co content in mushroom samples (*Amanita calsarea* and *Boletus pinophilus*) (n = 15) and Burley tobacco (n = 15).

Sample	Co AAS X±σ mg/kg	Co INT X±σ mg/kg
Amanita calsarea	1.543 ± 0.158	1.532 ± 0.154
Boletus pinophilus	0.257 ± 0.042	0.246 ± 0.041
Burley Tobacco	1.967 ± 0.229	1.896 ± 0.251

CONCLUSIONS

The extraction-spectrophotometric method for cobalt determination in biological samples with iodnitrotetrazole chloride (INT) developed in the present study is characterized with high speed, high selectivity and satisfactory accuracy. According to these indices, this method excels some of the currently applied techniques for cobalt determination in biological samples.

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СПЕКТРОФОТОМЕТРИЧНО ИЗСЛЕДВАНЕ НА СИСТЕМИ ЗА ТЕЧНО-ТЕЧНА ЕКСТРАКЦИЯ, СЪДЪРЖАЩИ КОБАЛТ И ТЕТРАЗОЛИЕВИ СОЛИ. ПРИЛАГАНЕ НА РАЗРАБОТЕНИЯ МЕТОД ЗА ОПРЕДЕЛЯНЕ СЪДЪРЖАНИЕТО НА КОБАЛТ В БИОЛОГИЧНИ ПРОБИ (ГЪБИ И ТЮТЮН)

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(Резюме)

Разработена е методика за екстракционно-спектрофотометрично определяне на кобалт в проби чрез използване на йоднитротетразолов хлорид. Нашите изследвания показаха, че електронният спектър на кобалтовия йонен асоциат има един максимум при 630 nm. При тази дължина на вълната поглъщането е максимално. Съставът на тройния йонно-асоцииран комплекс е с отношение INT: [Co(SCN)$_4$] = 2:1, а поради добрата разтворимост на йонно-асоциирания комплекс на кобалт(II) и INT в 1,2-дихлороетан, максимално извличане в органичната фаза се постига при 30 s екстракция. Моларната абсорбируемост на изследвания йонен асоциат INT$_2$[Co(SCN)$_4$] е ε_{630} = 0,6×10^3 L/mol.cm. Чувствителността на метода по Сендел е 9,8×10^{-2} µg/cm^2. Установено е, че максимално извличане на кобалт(II) с INT се постига в pH интервала от 2 до 7. Също така е установено, че чрез вариране количеството на тиоцианатните йони, при концентрация ≥ 1 М във водната фаза, светлинната абсорбция на екстрактите е максимална. Светлинната абсорбция на йонния асоциат има максимална стойност при концентрация на INT ≥ 6×10^{-4} М. Определена е зависимостта между концентрацията на кобалт(II) и светлинната абсорбция, която е линейна в интервала от 6 µg до 125 µg Co(II) в 10 ml водна фаза. Концентрацията на Со в гъби Булка (*Amanita calsarea*) е 1,543 ± 0,158 mg/kg, в гъби Манатарка (*Boletus pinophilus*) - 0,257 ± 0,042 mg/kg и в проби от тютюн (*Burley*) - 1,967 ± 0,229 mg/kg. Разработеният екстракционно-спектрофотометричен метод за определяне на кобалт в биологични проби с йоднитротетразолов хлорид (INT) е прост, евтин, експресен, селективен и се характеризира със задоволителна точност.