Journal of Chemistry and Technologies

pISSN 2663-2934 (Print), ISSN 2663-2942 (Online).

journal homepage: http://chemistry.dnu.dp.ua

UDC 543.6

VOLTAMMETRIC DETERMINATION OF VITAMIN B12 USING SOME AZO DYES

Svitlana V. Tymoshuk[1], Orest S. Fedyshyn[1], Lesya O. Kobryn[2], Ihor O. Patsay[1], Lesya V. Oleksiv[1], Oleksandr S. Tymoshuk[1]

[1]*Ivan Franko National University of Lviv, Kyryla & Mephodia Street, 6 Lviv, 79005 Ukraine*
[2]*Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Pekarska Street, 50 Lviv 79000 Ukraine*

Received 23 July 2020; accepted 30 May 2021; available online 20 July 2021

Abstract

The article is devoted to the study of the voltammetric activity of Co(II) in the presense of azo dyes with further using of the results obtained in analytical practice. The azo compounds belong to the largest class of organic reagents which are used in analytical chemistry as metallochromic indicators and complexants. The voltammetric behaviour of Co(II) complexes with eriochrome red B, eriochrome black T, kalces and 1-[(5-(3-nitrobenzyl)-1,3-thiazol-2-yl)diazenyl]naphthalen-2-ol was investigated using cyclic linear sweep voltammetry. The optimal conditions for complex formation were established. New peaks caused by the reduction of the Co(II)–azodye complex compounds are observed on the voltammograms. Sensitive and highly reproducible methods for cobalt determination were developed using the linear dependence of peaks height on metal concentration with limit of detection of 10^{-7} mol·L^{-1}. The accuracy of the proposed method was assessed by the method of standard additions. The developed method was successfully applied for the determination of vitamin B12 in pharmaceutical formulations.

Keywords: voltammetry; azodye; cobalt; reduction; vitamin B12.

ВОЛЬТАМПЕРОМЕТРИЧНЕ ВИЗНАЧЕННЯ ВІТАМІНУ B12 З ВИКОРИСТАННЯМ АЗОБАРВНИКІВ

Світлана В. Тимошук[1], Орест С. Федишин[1], Леся О. Кобрин[2], Ігор О. Пацай[1], Леся В. Олексів[1], Олександр С. Тимошук[1]*

[1]*Львівський національний університет імені Івана Франка, вул. Кирила і Мефодія, 6, Львів, 79005, Україна*
2Львівський національний університет ветеринарної медицини та біотехнологій імені С.З. Гжицького, вул. Пекарська, 50, Львів 79005, Україна

Анотація

Стаття присвячена дослідженню вольтамперометричної активності Co(II) у присутності азобарвників з наступним використанням одержаних результатів в аналітичній практиці. Досліджено процес взаємодії кобальту з еріохром чорним Т, еріохром червоним В, кальцесом та 1-[(5-(3-нітробензил)-1,3-тіазол-2-іл)діазеніл]нафтален-2-олом методом вольтамперометрії з лінійною розгорткою потенціалу. Встановлено оптимальні умови комплексоутворення. Зафіксовано появу нових піків, що відповідають відновленню комплексних сполук Co(II) – азобарвник. На підставі лінійної залежності висоти піку від концентрації металу, яка охоплює широкий концентраційний інтервал, запропоновано достатньо чутливі, добре відтворювані методики визначення кобальту (межа виявлення ≈ 10^{-7} моль·л$^{-1}$). Правильність та відтворюваність результатів перевіряли методом «введено-знайдено». Методики апробовано при визначенні вітаміну В12.

Ключові слова: вольтамперометрія; азобарвник; кобальт; відновлення; вітамін В12.

**Corresponding author: e-mail: otymoshuk@ukr.net*

doi: 10.15421/jchemtech.v29i2.207847

ВОЛЬТАМПЕРОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ВИТАМИНА B12 С ИСПОЛЬЗОВАНИЕМ АЗОКРАСИТЕЛЕЙ

Светлана В. Тимошук[1], Орест С. Федишин[1], Леся О. Кобрын[2], Ігорь О. Пацай[1], Леся В. Олексив[1],
Александр С. Тимошук[1]

[1]Львовский национальный университет имени Ивана Франко, ул. Кирилла и Мефодия, 6, Львов, 79005, Украина

[2]Львовский национальный университет ветеринарної медицины и биотехнологий имени С.З. Гжыцкого,
ул. Пекарская, 50, Львов 79005, Украина

Аннотация

Статья посвящена исследованию вольтамперометрической активности Co (II) в присутствии азокрасителей с последующим использованием полученных результатов в аналитической практике. Исследован процесс взаимодействия кобальта с эриохромом черным T, эриохромом красным B, кальцесом и 1-[(5-(3-нитробензил)-1,3-тиазол-2-ил)диазенил]нафтален-2-олом методом вольамперометрии с линейной разверткой потенциала. Установлены оптимальные условия комплексообразования. Зафиксировано появление новых пиков, соответствующих восстановлению комплексных соединений Co (II) – азокраситель. На основании линейной зависимости высоты пика от концентрации металла, которая охватывает широкий концентрационный интервал, предложено достаточно чувствительные, хорошо воспроизводимые методики определения кобальта (предел обнаружения ≈ 10^{-7} моль·л$^{-1}$). Правильность и воспроизводимость результатов проверяли методом «введено-найдено». Методики апробированы при определении витамина B12.

Ключевые слова: вольтамперометрия; азокраситель; кобальт; восстановление; витамин B12.

Introduction

The azo compounds belong to the largest class of organic reagents which are used in analytical chemistry as metallochromic indicators and complexants. In addition, the azo compounds are widely used in food industry [1], as well as in medical practice [2–4]. These compounds participate in a number of biological reactions, characterized by antimicrobial and anticarcinogenic activity.

The presence of the azo group – N = N – promotes the formation of strong complex compounds with a large number of metal ions, what is the basis for their further quantitative determination [5–7]. Azo dyes are the analytical reagents for determination of Cu(II), Ni(II), Co(II), Co(III) [6–17], Zn(II), Cd(II), Mn(II), Fe(III) [14–21], Hg(II), Ag(I), Pd(II), Os(IV) [21–29], Th (III), and U(VI) [21; 30]. The azo group can be electrochemically reduced on the electrodes of different nature giving an opportunity to use the azo dyes not only for spectrophotometric determination of metals but also for the voltammetric methods of analysis [16; 26; 31].

Nowadays analytical chemistry is developing in direction of using already known organic reagents, as well as new ones, synthesized with previously known characteristics. Synthetic possibilities of organic chemistry allow modifying the structure of reagents through involving the functional groups that will provide selectivity of analytical reactions [32–34]. The transformation of the planar structure of azodye into a branched form allows creating a steric barrier for the ions of accompanying elements, which increaseas the selectivity of analytical methods. The reagent with such characteristicsis (branched form) is 1-(5-benzylthiazol-2-yl)azonaphthalen-2-ol (BnTAN) having a dihedral angle between the planes of phenil and thiazol equal to 56.7 °C [5; 34]. This reagent and its derivatives were effectively used for the determination of transition metals [5; 11–13; 15; 19; 22–25; 34].

The aim of this work is to develop the voltammetric method for cobalt determination in complex biological systems. Cobalt is very important element not only for industry but also for the biological systems, as well as for all living organisms. It is present in vitamin B12. The vitamin B12 is a cofactor in DNA synthesis, and in both fatty and aminoacids metabolism. It is used for prevention of anemia. However, excess of vitamin B12 is toxic and causes pulmonary disorders, dermatitis and nausea. Vitamin B12, unlike most vitamins produced by chemical synthesis, is obtained by biosynthesis during fermentation [35]. In this process the addition of the small amount of cobalt to the microbial environment is necessary to increase the yield of vitamin B12.

Methods of vitamin B12 determination are mainly based on two approaches: direct determination of cianocobalamin (liquid chromatography, mass spectrometry, electroluminescence and capillary electrophoresis) and determination of cobalt (radioimmunoassay and atomic absorption spectroscopy, voltammetry) [36–40]. Methods of the first group are characterized by high sensitivity of determination up to 80 ng·mL^{-1} (high performance liquid chromatography). However, these methods suffer from the high cost of equipment. The methods for the cobalt determination of the second group are less sensitive with a limit of quantitative determination of 1 μg·mL^{-1} (raman spectroscopy) and have a low selectivity, requiring extensive sample preparation.

As can be understood from the standard procedure, there are no specific methods for determining vitamin B12. The recommended procedures refer to microbiological methods. Such assays can be considered routine for vitamin B12 but require overnight incubation, can be subject to contamination and include considerable laboratory set-up and maintenance time. The sensitivity is high enabling the detection of low concentrations of vitamin B12 but other components (food, antibiotics) may interfere with the determination. In this work, the simple indirect method has been proposed for the vitamin B12 determination based on cobalt assay as a constituent of vitamin B12. The voltammetric determination of cobalt in vitamin B12 was carried out using both existing and newl synthesized organic reagents having azo group. The influence of metal ions and matrix components on the determination of vitamin B12 was examined. Under the found optimal conditions for the complexation of Co(II) with azo dyes, the proposed methods which are accurate and well reproducible, can be used to determine Co(II) and vitamin B12 in biological systems, as well as in pharmaceuticals.

Materials and Methods

Apparatus. Voltammetric measurements were carried out on digital device MTech OVA-410 equipped with personal computer and temperature-controlled three-electrode cell of 30 mL volume. A dropping mercury electrode, a saturated calomel electrode, and platinum wire electrode were used as indicator, reference and auxiliary electrodes, respectively. The parameters for potential scanning were as follows: waveform voltage – triangular; scan rate – 0.1 V/s; the potential was set from 0.0 to – 2.0 V. Characteristics of the capillary in a 0.16 mol·L^{-1} sodium chloride solution without polarization voltage were as follows: m = 0.706 mg/s, t = 7.5 s. The dissolved oxygen was removed by purified argon purging for 10 min. All measurements were carried out at room temperature. The solution pH was controlled potentiometrically using pH-meter pH-150M with combined glass electrode.

Reagents. All solutions were prepared from double distilled water. Stock solutions (10^{-2} M) of metal ions were prepared by dissolving the appropriate quantity of pure metal containing 99.999 % of the major substance in a mixture of conc. HCl and conc. HNO_3 (cp grade). The solutions of metal ions were standardized using complexometry.

Stock solutions (10^{-3} mol·L^{-1}) of eriochrome red B (ERB), eriochrome black T (EBT), and kalces (KLC) were prepared by dissolving the exact amount of reagents in aqua ethanol mixture (where ethanol content was 40 % for ERB, 50 % for EBT, and 10 % for KLC). These solutions were standardized spectrophotometrically.

A 10^{-3} mol·L^{-1} alcoholic solution of 1-[(5-(3-nitrobenzyl)-1,3-thiazol-2-yl)diazenyl]naphthalen-2-ol (NBnTAN) was prepared by the dissolution of exact previously purified amount of sample in pure ethanol. 1-[(5-(3-nitrobenzyl)-1,3-thiazol-2-yl)diazenyl]naphthaene-2-ol was purified by double recrystallization from acetone and ethanol followed by filtration and vacuum distillation.

The following supporting electrolytes were employed: acetate and ammonium buffer solutions, sodium hydroxide.

2.3 Procedure. Working solutions for voltammetric investigations were prepared by adding reagents to the volumetric flask in the following order: supporting electrolyte with ionic strength more than 0.2 (acetate or ammonium bufer solutions, or sodium chloride), solution of Co(II) with concentration (0.2 – 1)·10^{-5} mol L^{-1}, azo dye solution with concentration (2 – 8))·10^{-5} mol L^{-1}. Finally double distilled water was added up to the mark. The obtained solutions were stirred and transferred to the voltammetric cell. Before obtaining the voltammograms, the argon was purged into solutions for 10-15 min.

Preparation of samples. The sample preparation was performed as described in [37]. Pharmaceutical preparation containing about 250 μg of vitamin B12 was taken into porcelain crucible. The sample was burned to ash first on the flame, then in an oven at 500 °C for 30–40 min. After burning, the residue was dissolved in 1-2 mL of conc. HNO_3 and evaporated to dryness in a boiling water bath. Then 5 mL of mixture of conc. HCl and HNO_3 (3 : 1) was added to the obtained residue and heated to completely dissolve it. Then 2.5 mol·L^{-1} HCl solution was added and the solution was cooled to room temperature and transferred into a 25.0 mL volumetric flask.

Results and Discussion

Investigation of Co(II) interaction with azo dyes. The reduction of Co(II) ammonium complex occurs at potential –1.2 V in the ammonium buffer solution at pH > 8.0. The increase of concentration of Co(II) ions in solution leads to linear increase in the height of the peak corresponding to the reduction of Co(II) (E_p^{Co} = −1.2 V). This linear relationship corresponds to the equation (at pH 9.0): $I_p^{Co} = 0.16 + 2.25 \times 10^4 \times C(Co(II))$. The limit of quantification (LOQ) was equal to $8.33 \cdot 10^{-6}$ $mol \cdot L^{-1}$. However, the reduction peak of the Co (II) ammonia complex is close to the discharge potential of the background electrolyte. The magnitude of the potential of this peak substantially depends on the composition of the matrix of the samples under study. Co(II) reduction peak may disappear when Co(II) ions are in other non ammonia forms. Thus, this peak cannot be used for the analysis of real objects.

The process of the reduction of azo dyes is multistage and was described in [31]. The peaks observed in the presence of ligand alone are caused by the reduction of the azo group. The reduction mechanism involves the formation of hydrazo derivatives followed by the cleavage of the –N=N– bond and the final formation of amines, according to the following scheme:

$$-N{=}N- + 2H^+ + 2e \rightarrow -NH{-}NH-$$

$$-NH{-}NH- + 2H^+ + 2e \rightarrow 2(-NH_2)$$

In the presence of some metal ions, a new peak appears at more negative potentials, related to the reduction of the ligand involved in the complexation, allowing the detection of the metal ions [26; 31; 41].

The reduction of Co (II) ions in the presence of azo dyes (ERB, EBT, KLC, NBnTAN) has been investigated in wide range of pH (1.0 – 11.5). It has been established that there was no complex formation of Co(II) with these reagents in acidic medium up to pH 4.0.

When Co(II) ions were introduced into solutions containing azo dyes (ERB, EBT, KLC) at pH ≥ 4, new reduction peaks appeared at potentials −0.94 V (ERB), −0.9 V (EBT), and −0.82 V (KLC) (Table 1). The new peaks corresponded to the reduction of –N=N– group in ligand.

The new peaks are shifted towards more negative potentials compared to the reduction peaks of free azo dyes (Fig. 1). The authors [31] explained the potential shift by the involvement of the azo group in complexation with a consequent stabilization of the nitrogen electrons in the ring, what made them less available to the reduction. The reduction of complexes is an irreversible process, as evidenced by the absence of anodic peaks on polarograms.

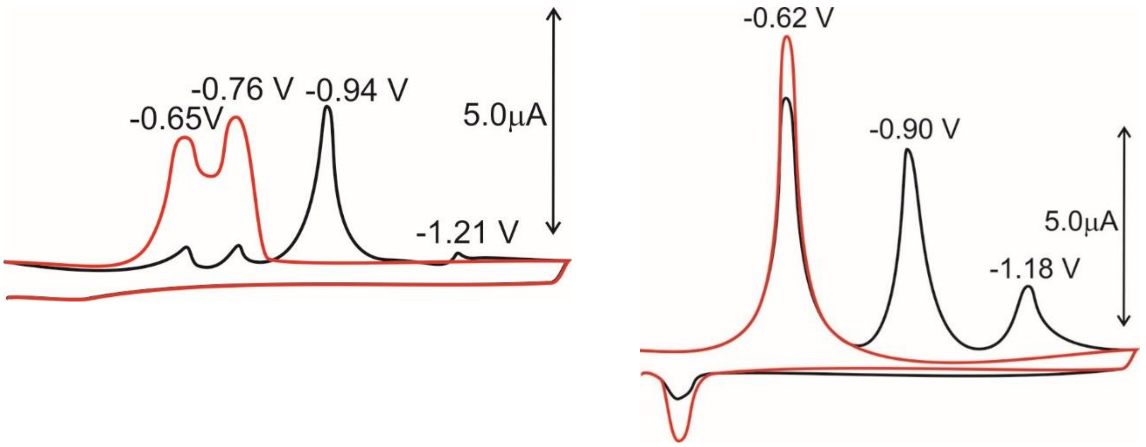
Table 1

Voltammetric characteristics of free azo dyes and its complexes with Co(II)

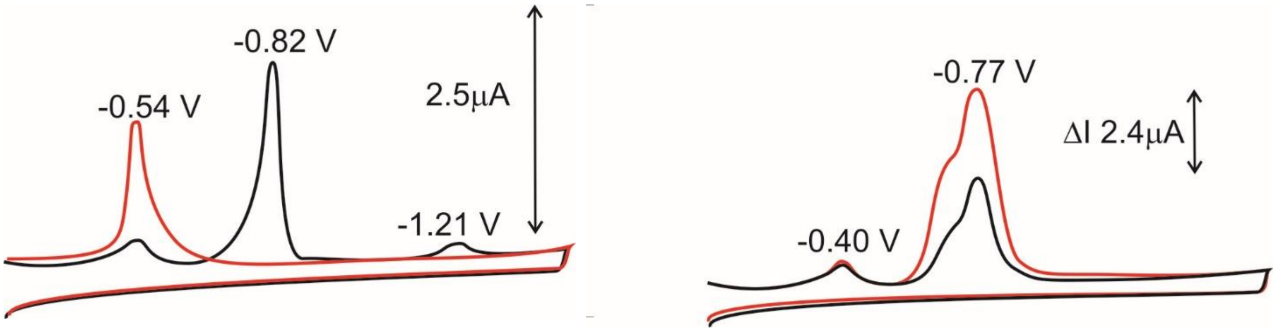
Dye	pH	Peaks	$-E^c_p$, V
EBT	4.0	P_1	0.57
		P_2	0.62
	10.0	P_2	0.63
EBT + Co(II)	4.0	P_1	0.56
		P_3	0.75
	10.0	P_2	0.60
		P_3	0.90
		P_4	1.18
ERB	4.0	P_1	0.69
	10.0	P_1	0.69
		P_2	0.75
ERB + Co(II)	4.0	P_1	0.71
		P_3	0.77
	10.0	P_1	0.67
		P_2	0.76
		P_3	0.94
		P_4	1.2
KLC	4.0	P_1	0.44
	10.0	P_1	0.56
KLC + Co(II)	4.0	P_1	0.46
		P_2	0.7
	10.0	P_1	0.54
		P_2	0.82
		P_3	1.2
NBnTAN	5.0	P_1	0.46
	11.0	P_1	0.77

The shape of voltammograms significantly depends on the acidity of the solution and the ratio of metal to dye in solution. When azo dye is in excess, reduction peaks of the dye and small peak of Co(II) complex reduction are observed. The peaks that appear on the voltammograms of complexes of the studied azo dyes with ions of Co(II), In(III), Re(III) Ga(III), Ni(II), Al(III) [31; 41] are close each to other, what indicates the reduction of ligands in complex compounds of cobalt(II) with the corresponding azo dyes. A small shift to the anodic region of the reduction potentials of complex compounds of Co(II) ions with azo dyes is due to the difference in the values of their stability constants. In addition to these peaks, there is also a peak at a potential of –1.2 V, which corresponds to the reduction of Co(II) ions on the background of ammonia buffer solution.

Voltammetric reduction of Co(II) in presence of NBnTAN differs from that in the presence of azo dyes described above. The reduction of NBnTAN on a mercury dropping electrode is accompanied by appearance of new peak at potential –0.77 V (Fig. 1). The presence of cobalt ions, in contrast to the previous cases, does not lead to the formation of new peak registered closely before reduction of the background electrolyte. The decrease of cathode current of NBnTAN reduction when Co (II) ion were present at pH > 5 was observed. This is the evidence of interaction between NBnTAN and Co (II) ions. The formation of complex compounds between NBnTAN and transition metal ions, including Co (II) ions in an alkaline medium [34] was confirmed by spectrophotometry [34]. The interaction of NBnTAN with Cu(II) and Pd(II) occurs in the acidic medium under similar conditions [24; 34]. Thus, the decrease in the peak height of the reagent reduction is the confirmation of the interaction of Co(II) ions with NBnTAN in an alkaline medium. The reduction peak of complex compound was not observed due to the overlapping with the reduction of the background electrolyte.



a) $C_{\mathrm{ERB}} = 2.0\cdot10^{-5}$ mol·L^{-1}, $C_{Co(II)} = 1.0\cdot10^{-5}$ mol·L^{-1} *b)* $C_{\mathrm{EBT}} = 8.0\cdot10^{-5}$ mol·L^{-1}, $C_{Co(II)} = 6.0\cdot10^{-5}$ mol·L^{-1}



c) $C_{\mathrm{KLC}} = 4.0\cdot10^{-5}$ mol L^{-1}, $C_{Co(II)} = 1.0\cdot10^{-5}$ mol L^{-1} *d)* $C_{NBnTAN} = 8.0\cdot10^{-5}$ mol L^{-1}, $C_{Co(II)} = 5.4\cdot10^{-6}$ mol L^{-1}

Fig.1 Voltammograms of ERB(*a*), EBT(*b*), KLC(*c*) in the presence of Co(II) ions, NBnTAN and NBnTAN + Co(II) (*d*).

Effect of ionic strength of solution. The solutions of NaCl, NH_4Cl/NH_4OH, $NaClO_4$ were used as media for the determination of optimal value of ionic strength. The best characteristics of electrochemical reduction were obtained in the presence of ammonium buffer solution for Co(II) in the presence of ERB, EBT, KLC, and in the medium of NaCl for the reduction of Co (II) in the presence of NBnTAN. The influence of ionic strength of the solution on voltammetric characteristics of the reduction process of studied complexes has been investigated. Increase of solution ionic strength leads to small decrease in the cathodic potential reaching the constant value at $\mu \geq 0.2$. This ionic strength of the solution was recommended for further analytical investigations.

Effect of pH. The influence of hydrogen-ions concentration on voltammetric characteristics of ions Co (II) reduction in the presence of azo dyes was investigated. The view of dependences of reduction current of complex compounds on pH is very similar. Reduction peaks of complex compounds appear under pH > 4.0 and reach the maximum value in ammonium buffer solution at pH > 8.0 (fig. 2). The solution pH 9.0 was chosen as optimal.

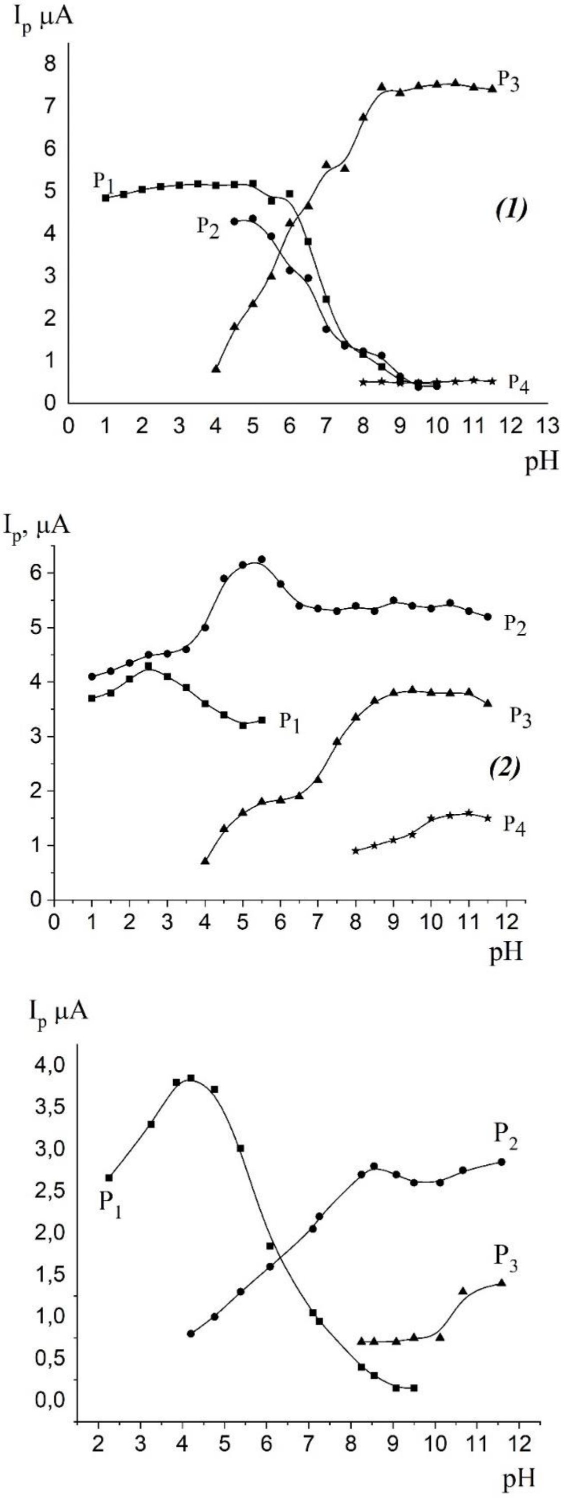


Fig. 2. Effect of pH on the peak current for Co(II) – ERB (1), Co(II) – EBT (2), Co(II) – KLC (3) $C_{azodye} = 4.0 \cdot 10^{-5}$ mol·L^{-1}, $C_{Co(II)} = 2.0 \cdot 10^{-5}$ mol·L^{-1}

It was established that the reduction peak of metal ions (E^c_p = –1.2 V) at Co (II) to azodye ratio 1 : 2 exists only at pH > 8.0, and its height slightly increases with decrease of medium acidity. The value of cathodic current of EBT reduction process in the presence of Co (II) is almost independent on medium acidity in the range pH 1.0 – 5.0 and further decrease of hydrogen ions concentration leads to the sharp decrease of height of corresponding peaks.

The presence of Co (II) ions has an influence on voltammetric characteristics of reduction process of EBT (P_1, P_2) under optimal conditions of determination, namely at pH 9.0 (see Table 1). Three peaks were registered on voltammograms, including reduction peak of EBT (P_2 at -0.6 V), reduction peak of complex compound (P_3 at -0.9 V) and reduction peak of metal (P_4 at -1.18 V). Peak P_1 disappeared at pH = 6.0.

The reduction processes of complexes of azo dyes solutions of ERB and KLC with Co (II) are very similar to those described above. The potentials of peaks (P_3, P_4) of investigated Co (II) complexes with ERB and KLC are in the same range as for the complex with EBT. We observed appearance of four peaks at pH 10 for ERB – Co (II) complex (-0.67, -0.76, -0.94, -1.2 V) and three peaks for KLC – Co (II) complex (-0.54, -0.82, -1.2 V) corresponding to reduction peaks of azo dyes (two peaks for ERT and one peak for KLC), complex ERT or KLC with Co (II) and reduction of metal Co (II) by itself (Table 1). The reduction peak of KLC disappeared at pH > 9.5.

The investigation of interaction of Co (II) with NBnTAN showed that decrease in height of reduction peak of NBnTAN depended on pH. In acidic medium at pH 5 the interaction did not occur what was confirmed by the constant value of cathode current of reduction peak of NBnTAN in the presence and absence of Co (II) ions respectively. With increase of solution pH, the height of reduction peak of NBnTAN in the presence of Co(II) increases and reaches the maximum value at pH 11. Thus the investigation of free NBnTAN and its complex with Co(II) was carried out at pH 11.

Table 2

The values of slope for dependence of E_p^c from pH;
$C_{Co(II)} = 2.0\times10^{-5}$ mol L^{-1}, $C_{azo\ dye} = 4.0\times10^{-5}$ mol L^{-1}.

Azodye	Peak	ΔE/ΔpH, mV/pH	pH
ERB	P_1	25	1.0 – 6.0
	P_2	34	4.5 – 8.5
	P_3	37	4.5 – 8.5
EBT	P_1	55	2.5 – 5.5
	P_2	65,7	2.5 – 6.0
	P_3	120; 50	4.0 –5.0; 5.0 – 8.0
KLC	P_1	90	2.0 – 7.0
	P_2	62; 25	4.0 –6.0; 8.5 – 11.5
NBnTAN	P_1	52	1.0 – 11.0

The potentials of reduction peaks of azo dyes and its complex compounds with Co(II) depend on medium acidity. With increase of solutions pH the reduction peak has been shifted towards negative values of potential (Table 2). The values of slope ΔE/ΔpH did not give us possibility to calculate the exact number of hydrogen ions taking part in electrochemical process, since studied processes are limited by adsorption on electrode. The reduction potential of Co(II) ions is almost independent on solution acidity.

The difference of reduction potentials of azo dyes and complex compounds (ΔE_p) is almost independent on acidity medium and concentration of metal ions at optimal conditions of determination (Table 3). We can assume that obtained complex compounds did not dissociate on electrode surface and ligand was reduced in bound form.

Table 3

The effect of pH on the difference of reduction potentials of azo dyes and complex compounds (ΔE_p); $C_{ERB} = C_{EBT} = 4.0\cdot10^{-5}$ mol·L^{-1}, $C_{KLC} = 8.0\cdot10^{-5}$ mol·L^{-1}, $C_{Co(II)} = 2.0\cdot10^{-5}$ mol·L^{-1}, v = 0.48 V/s.

pH	$-E_{p\ complex}^c$, V	$-E_{p\ azodye}^c$, V	$-\Delta E_p^c$, V
ERB			
8.0	0.92	0.66	0.26
9.0	0.94	0.66	0.28
10.5	0.93	0.66	0.27
EBT			
8.0	0.87	0.58	0.29
9.0	0.90	0.62	0.28
10.5	0.91	0.60	0.29
KLC			
9.0	0.84	0.55	0.29
10.5	0.82	0.53	0.29
11.0	0.83	0.56	0.27

Analytical characteristics of the voltammetric determination of Co(II) with azo dyes.

Effect of Co (II) concentration. The value of current of reduction of complex compounds and Co (II) ions increases with increase of metal concentration in solution. The analytical signal responsible for the azodye reduction process decreases (Fig. 3). Very similar dependences were observed for other azo dyes (ERB, EBT and KLC). The reduction peaks of ERB and KLC disappeares at the ratio C(Me) : C(azodye) = 1 : 2, which possibly characterizes the composition of complex.

The presence of Co(II) ions in the solutions of NBnTAN at optimal conditions of the determination leads to linear decrease in the reduction peak height of NBnTAN (Fig. 4).

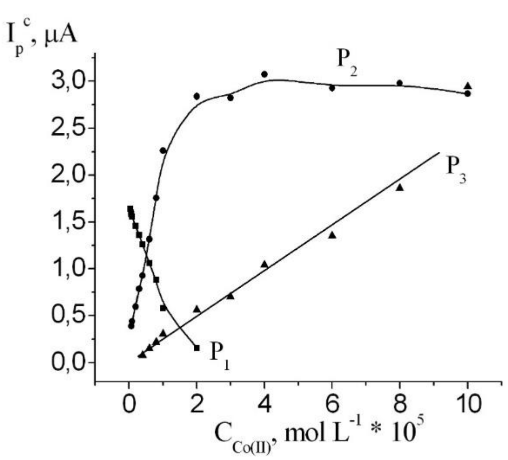


Fig. 3. Dependence of cathodic current of reduction of complex formed between Co(II) and KLC on metal concentration; C_{KLC}=4.0·10^{-5} mol·L^{-1}, pH 9.0.

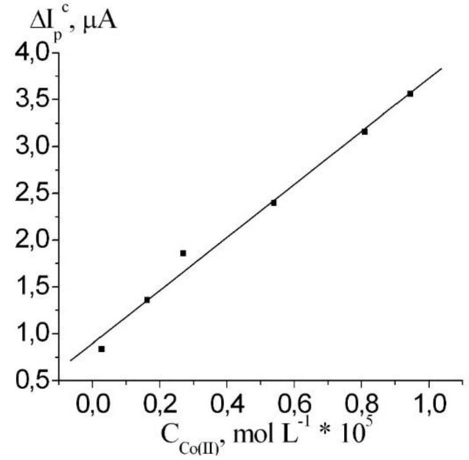


Fig. 4. Dependence of difference of value of cathodic current of NBnTAN reduction in the presence of Co (II) ions; C_{NBnTAN} = 4.0·10^{-5} mol L^{-1}, pH = 11.0.

The obtained dependence $\Delta I - f(C_{Co(II)})$ remains linear within two orders of concentrations.

The specific character of Co(II) ions reduction in the presence of azo dyes is a prerequisite for development of a new, sensitive method of its determination. Consequently, the linear dependence of complex reduction peaks from concentration of Co (II) in the voltammetric determination of Co (II) ions can be used. Decreasing the reduction of NBnTAN's peak height with using ERB, EBT, KLC and NBnTAN as reagents proves it too. Table 4 gives the metrological characteristics of procedures of volatammetric determination of Co (II) in model solutions.

Table 4

Metrological characteristics of the procedures for the voltammetric determination of Co(II) using ERB, EBT, NBnTAN, KLC, C_{azodye} = 4·10^{-5} M (R – correlation coeficient, SD – standard deviation)

Reagent	Calibration equation	R	SD	Range of linearity (mol L^{-1})	LOD (μmol L^{-1})
EBT	I_p^c=0.19+2.26·10^{5}C	0.996	0.013	1.0·10^{-6} – 4.0·10^{-5}	0.70
ERB	I_p^c=0.22+3.26·10^{5}C	0.997	0.029	2.0·10^{-6} – 1.0·10^{-5}	0.50
KLC	I_p^c=0.20+1.97·10^{5}C	0.996	0.018	6.0·10^{-6} – 4.0·10^{-5}	1.40
NBnTAN	ΔI_p^c=0.89+2.83·10^{5}C	0.995	0.012	3.0·10^{-7} – 1.0·10^{-5}	0.20

Table 5

Accuracy of the voltammetric determination of Co(II) with organic ligands in model solutions, n =3; P=0.95

Added Co(II), μmol·L^{-1}	*DYE*	Found Co(II) $\overline{X} \pm \frac{St_\alpha}{\sqrt{n}}$, μmol·L^{-1}	Sr, %
1.0	KLC	0.90± 0.10	4.4
	NBnTAN	1.10± 0.12	4.6
	EBT	1.1± 0.1	3.7
	ERB	1.1± 0.1	3.7
5.0	KLC	4.9 ± 0.2	1.6
	NBnTAN	5.1 ± 0.3	2.3
	EBT	4.8 ± 0.2	1.6
	ERB	4.9 ± 0.2	1.6
10.0	KLC	10.1 ± 0.2	0.8
	NBnTAN	9.8 ± 0.4	1.6
	EBT	10.3 ± 0.3	1.2
	ERB	10.2 ± 0.3	1.2

The accuracy of cobalt(II) voltammetric determination with azo dyes in model solutions was tested using the «added-found» method. Results of the analysis showed that the calculated amount of Co(II) in model solutions correlated with the experimental results and the uncertainty of the obtained data was acceptable (Table 5). The investigation was carried out three times under the optimal conditions.

Effect of foreign species. The effect of ions that can interfere with the Co(II) determination was studied by an addition of a known amount of interfering ion into the solution before the voltammetric investigation, and then the content of Co (II) was determined as described above. The tolerance limit was considered as a value causing a ± 5 % change in the current. Maximum possible concentration of concomitant elements for voltammetric determination of cobalt in presence of ERB, EBT of and of KLC is very similar. It was observed that the determination of $4 \cdot 10^{-6}$ mol·L^{-1} Co(II) was not affected by the presence of Al(III), Fe(III) in the ratio 1 : 10, Pd(II) 1 : 30, Cd(II) 1 : 25, Mg(II), Ca(II) 1 : 100, Zn(II) 1 : 60, Cu(II) 1 : 2. The determination of $4 \cdot 10^{-6}$ mol·L^{-1} Co(II) with NBnTAN was not altered by the presence of Cu(II) in the ratio 1 : 1, Mn(II) 1 : 25, Fe(III) 1 : 30, Zn(II) 1 : 2, Pb(II), Cd(II) 1 : 50, Mg(II), Ca(II), Ba(II), Sr(II) 1 > 200.

Application. The vitamin B12 is known as cobalamin. Structure of B12 contains the biochemically rare element cobalt positioned in the center of a corrin ring with general formula $C_{63}H_{88}CoN_{14}O_{14}P$. It is necessary ~ 0.04 mgs of cobalt per day in form of B12 for normal functioning of organism. It is known that molecule of cyanocobalamin contains approximately 4.36 % cobalt of whole molecular mass of vitamin.

The developed techniques of cobalt voltammetric determination have been applied to vitamin B12 assay in solution of vitamin B12 and preparation Neurobex ® NEO made in Indonesia. Preparation of vitamin B12 is a solution, thus ampoules were used for an analysis. Preparation of Neurobex is a mixture of vitamins (vitamins B1, B2, B6, B3, B5, B12 and vitamin C) in capsules. The content of capsules was used for analysis. The results of analysis are given in Table 6.

The atomic absorption spectroscopy was used as an alternative method for confirmation of accuracy and precision of the obtained results. Absorption was measured at λ = 240.7 nm. Obtained data are in a good agreement with the content of cobalt in the studied objects by voltammetry and atomic absorption spectroscopy methods.

Table 6

The results of Co(II) determination with azo dyes using voltammetry and atomic absorption spectroscopy, n =3; P=0.95

Dye	Preparation: Vitamin B12 (ampoule,solution) Corresponding amount of B12 – 0.50 mg/ml					
	Voltammetry			Atomic absorption spectroscopy		
	Amount of cobalt, μg/ml	calculated content of vitamin B12 mg/ml	S_r, %	Amount of cobalt, μg/ml	calculated content of vitamin B12 mg/ml	S_r, %
KLC	22.3±1.2	0.513±0,029	2.2	22.2±1.9	0.510±0.043	3.4
NBnTAN	21.8±2.2	0.500±0.050	4.0			
EBT	21.3±1.9	0.490±0.043	3.5			
ERB	22.0±1.2	0.507±0.029	2.0			
Dye	Preparation: Neurobex (capsule) Corresponding amount of B12 – 5.0 μg/capsule					
	Voltammetry			Atomic absorption spectroscopy		
	Amount of cobalt, μg/capsule	calculated content of vitamin B12 μg/capsule	S_r, %	Amount of cobalt, μg/ml	calculated content of vitamin B12 mg/ml	S_r, %
KLC	0.220±0.027	5.07±0.62	5.0	0.213±0.022	4.90±0.50	4.1
NBnTAN	0.210±0.025	4.83±0.57	4.8			
EBT	0.213±0.019	4.90±0.43	3.5			
ERB	0.222±0.019	5.10±0.43	3.4			

Conclusion

Simple and sensitive voltammetric methods for the determination of cobalt using organic reagents containing an azo group (ERB, EBT, KLC and NBnTAN) have been developed. It was found that the maximum yield of the reaction product is observed in the alkaline region at pH > 8. The formation of complex compounds is accompanied by the appearance of new peaks and a decrease of height of the reduction peaks of azo dyes on the voltammograms. The developed methods are relatively sensitive, the detection limits are in the range of 10^{-6}–10^{-7} mol·L^{-1}. The best sensitivity for the Co(II) determination of $2·10^{-7}$ mol·L^{-1} was obtained by using NBnTAN. Nevertheless, the selectivity is higher when using ERB. The studied effect of the accompanying elements showed the possibility of using the developed techniques for the analysis of complex objects. The proposed method can be applied for the determination of Co(II) in vitamin B12 in single and multi-component commercial preparation, natural, industrial and biological objects.

Acknowlegements

This study was carried out with the support of the Ministry of Education and Sciences of Ukraine (№ DR 0118U003611).

Bibliography

[1] Vishnikin A. B. Simultaneous spectrophotometric determination of food dyes in binary mixtures by H-point standard addition method / A. B. Vishnikin, L. P. Sidorova, A. O. Voloboy // Journal of Chemistry and Technologies. – 2019. – V.27, No 2. – P. 276–284.

[2] Chung King-Thom. Azo dyes and human health: A review / King-Thom Chung // Journal of Environmental Science and Health, Part C Environmental Carcinogenesis and Ecotoxicology Reviews. – 2016. – V. 34. No 2. – P. 233–261.

[3] Antimicrobial activities, absorption characteristics and tautomeric structures of o,o'-hydroxyazo dyes containing an acryloyloxy group and their chromium complexes / G. A. Avci, S. Ozkinali, A. Ozluk [et al.] //. Hacettepe J. Biol. Chem. – 2012. – V.40 – P. 119–126.

[4] Spectroscopic, thermal and antioxidant properties of novel mixed ligand-metal complexes obtained from saccharinate complexes and azodye ligand (mnppa) / N. Turan, R. Adiguzel, K. Buldurun, E. Bursal // International Journal of Pharmacology.– 2016. – V.12, No 2. – P. 92–100.

[5] On the structure of transition metals complexes with the new tridentate dye of thiazole series: Theoretical and experimental studies / M. Fizer, V. Sidey, A. Tupys [et al.] // Journal of Molecular Structure – 2017. – Vol. 1149. – P. 669–682.

[6] Pytlakowska K. Complex-forming organic ligands in cloud-point extraction of metal ions: a review / K. Pytlakowska, V. Kozik, M. Dabioch // Talanta. – 2013. – Vol. 100. – P. 202–228.

[7] Gaber M. Ni(II), Pd(II) and Pt(II) complexes of (1*H*-1,2,4-triazole-3-ylimino)methyl]naphthalene-2-ol. Structural, spectroscopic, biological, cytotoxicity, antioxidant and DNA binding / M. Gaber, H.A. El-Ghamry, S.K. Fathalla // Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. – 2015. – V. 139. – P.396–404.

[8] Niazi A. Simultaneous spectrophotometric determination of cobalt, copper and nickel using 1-(2-thiazolylazo)-2-naphthol by chemometrics methods / A. Niazi, A. Yazdanipour // Chinese Chem. Lett. – 2008. – Vol. 19. – P. 860–864.

[9] Gaber M. Spectrophotometric, conductometric and thermal studies of Co(II), Ni(II) and Cu(II) complexes with 2-(2-hydroxynaphthylazo)-4-hydroxy-6-methyl-1,3-pyrimidine / M. Gaber, I.A. Mansour, Y.S.Y. El-Sayed // Spectrochim. Acta A. – 2007. – Vol. 68. – P. 305–311.

[10] New thiophene-based azo ligands containing azo methine group in the main chain for the determination of copper(II) ion / H. Dinçalp, F. Toker, İ. [et al.] //Dyes and Pigments. – 2007. – V. 75, No 1. – P. 11–24.

[11] 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 / Y. Bazel, A. Tupys, Y. Ostapiuk [et al.] // Journal of Molecular Liquids.– 2017. – Vol. 242. – P. 471-477.

[12] Tupys A. Spectrophotometric Investigation of Cu(II) Ions Interaction with 1-(5-Benzylthiazol-2-yl)azonaphthalen-2-ol / A. Tupys, O. Tymoshuk, P. Rydchuk // Chemistry & Chemical Technology. – 2016. – Vol. 10, No. 1. – P. 19–25.

[13] A simple non-extractive green method for the spectrophotometric sequential injection determination of copper(II) with novel thiazolylazo dyes / Y. Bazel, A. Tupys, Y. Ostapiuk [et al.] // RSC Advances.– 2018. – Vol. 8. – P.15940–15950.

[14] Selective Photometric Determination of Nickel, Cobal, and Zinc in Drinking and Natural Waters Using 1-(2-pyridylazo)-2-naphthol and Surfactants (SAS) / A.V. Terletskaya, O.S. Ievleva, T.A. Bogoslovskaya, V.V. Goncharuk // J. Water Chem. Techno. – 2013. – Vol. 35, No.6. – P. 246–252.

[15] Tupys A.M. The Application of 1-(5-Benzylthiazol-2-yl)azonaphthalen-2-ol in Extraction-Photometric Analysis of the Main Soils Pollutants Content (Copper, Zinc, Cadmium and Lead) / A. M. Tupys, O. S. Tymoshuk, P. V. Rydchuk // Methods and objects of chemical analysis. – 2015. – Vol. 10, No. 2. – P. 80–88.

[16] Chavan S.S. Synthesis, structural characterization, thermal and electrochemical studies of Mn(II), Co(II), Ni(II) and Cu(II) complexes containing thiazolylazo ligands / S.S. Chavan, V.A. Sawant // Journal of Molecular Structure. – 2010. – Vol. 965. – P. 1–6.

[17] Shanthalakshmi K. Synthesis and spectrophotometric studies of some benzothiazolylazo dyes – determination of copper, zinc, cadmium, cobalt and nickel / K. Shanthalakshmi, S. L. Belagali // Bulg. Chem. Commun. – 2009. – Vol. 41, No.4. – P. 380–384.

[18] Chavan S.S. Zn(II) and Cd(II)-azido/ thiocyanato complexes with thiazolylazodye and triphenylphosphine: synthesis, characterization and fluorescence / S.S. Chavan, B.A. Yamgar, B.G. Bharate // J. Coord. Chem. – 2013. – Vol. 66, No.10. – P. 1837–1846.

[19] Tupys A. M. Extraction-photometric determination of cadmium(II) using 1-(5-benzylthiazol-2-

yl)azonaphthalen-2-ol / A.M. Tupys, O.S. Tymoshuk // Bulletin of Dnipropetrovsk University – Series Chemistry. – 2015. – Vol. 23, No. 1. – P. 50–58.

[20] Khan S. Rapid ionic liquid-based ultrasound assisted dual magnetic microextraction to preconcentrate and separate cadmium-4-(2-thiazolylazo)-resorcinol complex from environmental and biological samples / S. Khan, T.G. Kazi, M. Soylak // Spectrochim. Acta A. – 2014. – Vol. 123. – P. 194–199.

[21] Mahmoud W. H. Synthesis, spectral characterization, thermal, anticancer and antimicrobial studies of bidentate azodye metal complexes / W.H. Mahmoud, M.M. Omar, F.N. Sayed // Journal of Thermal Analysis and Calorimetry – 2016. – Vol. 124, No. 2. – P. 1071–1089.

[22] 1-[(5-Benzyl-1,3-thiazol-2-yl)diazenyl]naphthalene-2-ol: X-ray structure, spectroscopic characterization, dissociation studies and application in mercury(II) detection / A. Tupys, J. Kalembkiewicz, Y. Bazel [et al.] // J. Mol. Struct. – 2017. – Vol. 1127. – P. 722–733.

[23] Tupys A. 1-(5-Benzylthiazol-2-yl)azonaphthalen-2-ol — a new reagent for the determination of Pd(II) / A. Tupys, O. Tymoshuk // Acta Chimica Slovaca. – 2015. – Vol. 8, N 1. – P. 59–64.

[24] Spectroscopic and computational study of a new thiazolylazonaphthol dye1-[(5-(3-nitrobenzyl)-1,3-thiazol-2-yl)diazenyl]naphthalen-2-ol / O. Fedyshyn, Y. Bazel, M. Fizer [et al.] // J. Mol. Liquids. – 2020. – Vol. 304. – 112713.

[25] A New Method of Control Over the Content of Palladium in Intermetallic Alloys/ O. S. Tymoshuk, O. S. Fedyshyn, L. V. Oleksiv, P. V. Rydchuk, I. O. Patsai // Materials Science. – 2019. – Vol. 55. – P. 455–459.

[26] Levitskaya G. D. (2003). Oscillopolarography of palladium (II)-Tropeolin 0 complexes / G. D. Levitskaya, S. V.Timoshuk, V. M. Gritsai // Journal of Analytical Chemistry. – 2003. – Vol. 58, N 11. – P. 1065–1068.

[27] Naser N. A. Synthesis and Characterization of an Organic Reagent 4-(6-Bromo-2-Benzothiazolylazo) Pyrogallol and Its Analytical Application / N.A. Naser, K.H. Kahdim, D.N. Taha // J. Oleo Sci. – 2012. – Vol. 61. – P. 387–392.

[28] Mohammed H.J. Spectrophotometric, thermal and determination of trace amount of palladium (II) nickel (II) and silver (I) by using pyrazolone azo derivative / H.J. Mohammed, A.A. Syhood //. J Anal Pharm Res. – 2018. – Vol.7, No. 4. – P. 504–511.

[29] Osmium assay in fixatives and stained rat tissues by means of acid and o,o′-dihydroxo substituted monoazo dyes and some flavonoids / M. Rydchuk, G. Mykhalyna, O. Dobryanska [et al.] // Cent. Eur. J. Chem. – 2011. – Vol. 9, No. 5. – P. 886–895.

[30] Floatation-spectrophotometric Determination of Thorium, Using Complex Formation with Eriochrome Cyanine R / S. Shiri, A. Delpisheh, A. Haeri [et al.] // Anal Chem Insights. – 2011. – Vol. 6. – P. 1–6.

[31] Electrochemical behavior and sensitive methods of the voltammetric determination of food azo dyes amaranth and Allura Red AC on amalgam electrodes / S. Tvorynska, B. Josypčuk, J. Barek, L. Dubenska // Food Anal. Methods. – 2019. – Vol. 12. –P. 409–421.

[32] Al-Adilee K. J. Synthesis of some transition metal complexes with new heterocyclic thiazolyl azodye and their uses as sensitizers in photo reactions / K. J. Al-Adilee, A. K. Abass, A. M. Taher // J. Mol. Struct. – 2016. – Vol. 1108. – P. 378–397.

[33] Synthesis and spectroscopic properties of new azo dyes derived from 3-ethylthio-5-cyanomethyl-4-phenyl-1,2,4-triazole / Mariam Al-Sheikh, Hanadi Y. Medrasi, Kamal Usef Sadek, Ramadan Ahmed Mekheimer // Molecules. – 2014. – Vol.19. – P. 2993–3003.

[34] Тупис А. М. Спектрофотометрія сполук 1-(5-бензил-2-іл)азонафтален-2-олу з іонами перехідних металів та їх застосування в аналізі: Дис. ... канд. хім. наук: 02.00.02. – Ужгород, 2017. – 212 с.

[35] Herbert V. Vitamin B-12: plant sources, requirements, and assay // The American Journal of Clinical Nutrition. – 1988. – Vol.48, No. 3. –P. 852–858.

[36] Measurement of Vitamin B12 concentration. A Review on available methods / Ola Karmi, Ashraf Zayed, Suheir Baraghethi [et al.] // The IIOAB Journal. – 2011. – Vol. 2; N 2. – P. 23–32.

[37] A convenient colorimetric assay method for determination of vitamin B12 content in pharmaceutical preparation/ Firoj Ahmed, Rebecca Banoo, Ghazi Muhammed Sayedur Rahman, Md. Omar Fakur Khan// J. Med. Sci. – 2003. – Vol. 3, N 2. – P. 163–168.

[38] Simultaneous determination of cobalt and nickel in vitamin B12 samples using high-resolution continuum source atomic absorption spectrometry/ F. R. Adolfo, P. C. do Nascimento, D. Bohrer [et al.] // Talanta. – 2016. – Vol.147. – P. 241–245.

[39] Measuring and tracking vitamin B12: A review of current methods with a focus on optical spectroscopy / G. Tsiminis, E. P. Schartner, J. L. Brooks, M. R. Hutchinson // Applied spectroscopy reviews – 2017. – Vol. 52, N 5. – P. 439–455.

[40] Şükrü K. Determination of Vitamin B12 Using Differential Pulse Polarography / K. Şükrü, Ü. Ülkü, S. Güler //American Journal of Analytical Chemistry. – 2020. – Vol. 11, N 2. – P. 187–196.

[41] Voltammetric Reduction of In(III)-Eriochrome Red B Complexes / L. O. Dubenskaya, G. D. Levitskaya, , N. P. Poperechnaya [et al.] // Journal of Analytical Chemistry. – 2005. – Vol. 60, N 11. – P.1052–1055.

References

[1] Vishnikin, A. B., Sidorova L. P., Voloboy A. O. (2019). Simultaneous spectrophotometric determination of food dyes in binary mixtures by H-point standard addition method. *Journal of Chemistry and Technologies*, *27*(2), 276–284 https://doi.org/10.15421/081928

[2] Chung, King-Thom (2016). Azo dyes and human health: A review. *Journal of Environmental Science and Health, Part C Environmental Carcinogenesis and Ecotoxicology Reviews*, *34*(4), 233–261. https://doi.org/10.1080/10590501.2016.1236602

[3] Avci, G.A., Ozkinali, S., Ozluk, A., Avci, E., Kocaokutgen, H. (2012). Antimicrobial activities, absorption characteristics and tautomeric structures of o,o'-hydroxyazo dyes containing an acryloyloxy group and their chromium complexes. *Hacettepe J. Biol. Chem*., *40*(2), 119–126.

[4] Turan, N., Adiguzel, R., Buldurun, K., Bursal, E. (2016). Spectroscopic, thermal and antioxidant properties of novel mixed ligand-metal complexes obtained from saccharinate complexes and azodye ligand (mnppa). *International Journal of Pharmacology*, *12*(2), 92–100. https://doi.org/10.3923/ijp.2016.92.100

[5] Fizer, M., Sidey, V., Tupys, A., Ostapiuk, Y., Tymoshuk, O., Bazel, Y. (2017). On the structure of transition metals complexes with the new tridentate dye of thiazole series: Theoretical and experimental studies. *Journal of Molecular Structure,* 1149, 669–682. https://doi.org/10.1016/j.molstruc.2017.08.037

[6] Pytlakowska, K., Kozik, V., Dabioch, M. (2013). Complex-forming organic ligands in cloud-point extraction of metal ions: a review. *Talanta*, 100, 202–228. http://dx.doi.org/10.1016/j.talanta.2013.02.037

[7] Gaber, M., El-Ghamry, H.A., Fathalla, S.K. (2015). Ni(II), Pd(II) and Pt(II) complexes of (1*H*-1,2,4-triazole-3-ylimino)methyl]naphthalene-2-ol. Structural, spectroscopic, biological, cytotoxicity, antioxidant and DNA binding. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 139, 396–404. https://doi.org/10.1016/j.saa.2014.12.057

[8] Niazi, A., Yazdanipour, A. (2008). Simultaneous spectrophotometric determination of cobalt, copper and nickel using 1-(2-thiazolylazo)-2-naphthol by chemometrics methods. *Chinese Chem. Lett.,* 19, 860–864. https://doi.org/10.1016/j.cclet.2008.04.047

[9] Gaber, M., Mansour, I. A., El-Sayed, Y. S. Y. (2007). Spectrophotometric, conductometric and thermal studies of Co(II), Ni(II) and Cu(II) complexes with 2-(2-hydroxynaphthylazo)-4-hydroxy-6-methyl-1,3-pyrimidine. *Spectrochim. Acta A*, 68, 305–311. https://doi.org/10.1016/j.saa.2006.11.035

[10] Dinçalp, H., Toker, F., Durucasu, İ., Avcıbaşı, N., & Icli, S. (2007). New thiophene-based azo ligands containing azo methine group in the main chain for the determination of copper(II) ions. *Dyes and Pigments, 75*(1), 11–24. https://doi.org/10.1016/j.dyepig.2006.05.015

[11] Bazel, Y., Tupys, A., Ostapiuk, Y., Tymoshuk, O., Matiychuk, V. (2017). 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. *Journal of Molecular Liquids,* 242, 471–477. https://doi.org/10.1016/j.molliq.2017.07.047

[12] Tupys, A., Tymoshuk, O., Rydchuk, P. (2016). Spectrophotometric Investigation of Cu (II) Ions Interaction with 1-(5-Benzylthiazol-2-yl)azonaphthalen-2-ol. *Chemistry & Chemical Technology, 10*(1), 19–25. https://doi.org/10.23939/chcht10.01.019

[13] Bazel, Y., Tupys, A., Ostapiuk, Y., Tymoshuk, O., Imricha, J., Sandrejov, J. (2018). A simple non-extractive green method for the spectrophotometric sequential injection determination of copper(II) with novel thiazolylazo dyes. *The Royal Society of Chemistry Advances*, 8, 15940–15950. https://doi.org/10.1039/c8ra02039f.

[14] Terletskaya, A.V., Ievleva, O.S., Bogoslovskaya, T.A., Goncharuk, V.V. (2013). Selective Photometric Determination of Nickel, Cobal, and Zinc in Drinking and Natural Wat ers Using 1-(2-pyridylazo)-2-naphthol and Surfactants (SAS). *J. Water Chem. Techno*., *35*(6), 246–252. https://doi.org/10.3103/S1063455X13060027

[15] Tupys, A.M., Tymoshuk, O.S., Rydchuk, P.V. (2015). The Application of 1-(5-Benzylthiazol-2-yl)azonaphthalen-2-ol in Extraction-Photometric Analysis of the Main Soils Pollutants Content (Copper, Zinc, Cadmium and Lead). *Methods and objects of chemical analysis*, *10*(2), 80–88. https://doi.org/10.17721/moca.2015.80-88

[16] Chavan, S.S., Sawant, V.A. (2010). Synthesis, structural characterization, thermal and electrochemical studies of Mn(II), Co(II), Ni(II) and Cu(II) complexes containing thiazolylazo ligands. *Journal of Molecular Structure,* 965, 1–6. https://doi.org/10.1016/j.molstruc.2009.11.010

[17] Shanthalakshmi, K., Belagali, S. L. (2009). Synthesis and spectrophotometric studies of some benzothiazolylazo dyes – determination of copper, zinc, cadmium, cobalt and nickel. *Bulg. Chem. Commun., 41*(4), 380–384.

[18] Chavan, S.S., Yamgar, B.A., Bharate, B.G. (2013). Zn (II) and Cd(II)-azido/thiocyanato complexes with thiazolylazodye and triphenylphosphine: synthesis, characterization and fluorescence. *J. Coord. Chem.*, *66*(10), 1837–1846. http://dx.doi.org/10.1080/00958972.2013.791394

[19] Tupys, A.M., Tymoshuk, O.S. (2015). Extraction-photometric determination of cadmium(II) using 1-(5-benzylthiazol-2-yl)azonaphthalen-2-ol. *Bulletin of Dnipropetrovsk University*, *Series Chemistry*, *23*(1), 50–58. https://doi.org/10.15421/081507

[20] Khan, S., Kazi, T.G., Soylak, M. (2014). Rapid ionic liquid-based ultrasound assisted dual magnetic microextraction to preconcentrate and separate cadmium-4-(2-thiazolylazo)-resorcinol complex from environmental and biological samples. *Spectrochim. Acta A.,* 123, 194–199. http://dx.doi.org/10.1016/j.saa.2013.12.065

[21] Mahmoud, W.H., Omar, M.M., Sayed, F.N. (2016). Synthesis, spectral characterization, thermal, anticancer and antimicrobial studies of bidentate azodye metal complexes. *Journal of Thermal Analysis and Calorimetry*, *124*(2), 1071–1089 https://doi.org/10.1007/s10973-015-5172-1

[22] Tupys, A., Kalembkiewicz, J., Bazel, Y., Zapała, L., Dranka, M., Ostapiuk, Y., Tymoshuk, O., Woźnicka, E. (2017). 1-[(5-Benzyl-1,3-thiazol-2-yl)diazenyl]naphthalene-2-ol: X-ray structure, spectroscopic characterization, dissociation studies and application in mercury(II) detection. *J. Mol. Struct.,* 1127, 722–

733. http://dx.doi.org/10.1016/j.molstruc.2016.07.119

[23] Tupys, A., Tymoshuk, O. (2015). 1-(5-Benzylthiazol-2-yl)azonaphthalen-2-ol — a new reagent for the determination of Pd(II). *Acta Chimica Slovaca*, *8*(1), 59–64. https://doi.org/10.1515/acs-2015-0011

[24] Fedyshyn, O., Bazel, Y., Fizer, M., Sidey, V., Imrich, J., Vilkova, M., Barabash, O., Ostapiuk, Y., Tymoshuk, O. (2020). Spectroscopic and computational study of a new thiazolylazonaphthol dye1-[(5-(3-nitrobenzyl)-1,3-thiazol-2-yl)diazenyl]naphthalen-2-ol. *J. Mol. Liquids*, 304, 112713. https://doi.org/10.1016/j.molliq.2020.112713

[25] Tymoshuk, O. S., Fedyshyn, O. S., Oleksiv, L. V., Rydchuk, P. V., Patsai, I. O. (2019). A new method of control over the content of palladium in intermetallic alloys. *Materials Science*, 55, 455–459. https://doi.org/10.1007/s11003-019-00325-9

[26] Levitskaya, G. D., Timoshuk, S. V., Gritsai, V. M. (2003). [Oscillopolarography of palladium(II)-Tropeolin 0 complexes]. *Journal of Analytical Chemistry*, 58 (11), 1065-1068. https://doi.org/10.1023/A:1027385306485 (in Russian).

[27] Naser, N.A., Kahdim, K.H., Taha, D.N. (2012). Synthesis and Characterization of an Organic Reagent 4-(6-Bromo-2-Benzothiazolylazo) Pyrogallol and Its Analytical Application. *J. Oleo Sci.*, 61, 387–392. https://doi.org/10.5650/jos.61387

[28] Mohammed, H.J., Syhood, A.A. (2018). Spectrophotometric, thermal and determination of trace amount of palladium (II) nickel (II) and silver (I) by using pyrazolone azo derivative. *J Anal Pharm Res.*, *7*(4), 504–511. https://doi.org/10.15406/japlr.2018.07.00275

[29] Rydchuk, M., Mykhalyna, G., Dobryanska, O., Korkuna, O., Vrublevska, T. (2011). Osmium assay in fixatives and stained rat tissues by means of acid and o,o′-dihydroxo substituted monoazo dyes and some flavonoids. *Cent. Eur. J. Chem.*, *9*(5), 886–895. https://doi.org/10.2478/s11532-011-0070-2

[30] Shiri, S, Delpisheh, A, Haeri, A, Poornajaf, A, Khezeli, T, Badkiu, N. (2011). Floatation-spectrophotometric Determination of Thorium, Using Complex Formation with Eriochrome Cyanine R. *Anal Chem Insights*., 6, 1–6. https://doi.org/10.4137/ACI.S5949

[31] Tvorynska, S., Josypčuk, B., Barek, J., Dubenska, L. (2019). Electrochemical behavior and sensitive methods of the voltammetric determination of food azo dyes amaranth and Allura Red AC on amalgam electrodes. *Food Anal. Methods,* 12, 409–421. https://doi.org/10.1007/s12161-018-1372-1

[32] Al-Adilee, K.J., Abass, A.K., Taher, A.M. (2016). Synthesis of some transition metal complexes with new heterocyclic thiazolyl azodye and their uses as sensitizers in photo reactions. *J. Mol. Struct.,* 1108, 378–397. https://doi.org/10.1016/j.molstruc.2015.11.038

[33] Al-Sheikh, M., Medrasi, H. Y., Sadek, K. U., Mekheimer, R. A. (2014). Synthesis and Spectroscopic Properties of new azo dyes derived from 3-ethylthio-5-cyanomethyl-4-phenyl-1,2,4-triazole. *Molecules,* 19, 2993–3003 https://doi.org/10.3390/molecules19032993

[34] Tupys, A.M. [Spectrophotometry of 1- (5-benzyl-2-yl) azonaphthalen-2-ol compounds with transition metal ions and their use in analysis], Ushgorod, 2017. (in Ukrainen).

[35] Herbert, V. (1988). Vitamin B-12: plant sources, requirements, and assay. *The American Journal of Clinical Nutrition,* *48*(3), 852–858. https://doi.org/10.1093/ajcn/48.3.852

[36] Karmi, O., Zayed, A., Baraghethi, S., Qadi, M., Ghanem, R. (2011). Measurement of Vitamin B12 concentration. A Review on available methods. *The IIOAB Journal, 2*(2), 23–32.

[37] Firoj, Ahmed, Banoo, Rebecca, Ghazi, Muhammed Sayedur, Rahman, Md. Omar Fakur Khan (2003). A convenient colorimetric assay method for determination of vitamin B12 content in pharmaceutical preparation. *J. Med. Sci, 3*(2), 163–168. https://doi.org/10.3923/jms.2003.163.168

[38] Adolfo, F. R., do Nascimento, P. C., Bohrer, D., de Carvalho, L. M., Viana, C., Guarda, A., Mattiazzi, P. (2016). Simultaneous determination of cobalt and nickel in vitamin B12 samples using high-resolution continuum source atomic absorption spectrometry. *Talanta,* 147, 241–245. https://doi.org/10.1016/j.talanta.2015.09.073

[39] Tsiminis, G., Schartner, E. P., Brooks, J. L., Hutchinson, M. R. (2017). Measuring and tracking vitamin B12: A review of current methods with a focus on optical spectroscopy. *Applied spectroscopy reviews*, *52*(5), 439–455. https://doi.org/10.1080/05704928.2016.1229325

[40] Şükrü, K., Ülkü, Ü., Güler, S. (2020). Determination of Vitamin B12 Using Differential Pulse Polarography. *American Journal of Analytical Chemistry*, *11*(5), 187–196. https://doi.org/10.4236/ajac.2020.115014

[41] Dubenskaya, L. O., Levitskaya, G. D., Poperechnaya, N. P., Tymoshuk, S. V., & Kopot', O. V. (2005). Voltammetric Reduction of In(III)-Eriochrome Red B Complexes. *Journal of Analytical Chemistry, 60*(11), 1052–1055. https://doi.org/10.1007/s10809-005-0237-0