UDC 546.05+546.06 doi: 10.33609/2708-129X.87.10.2021.74-89

METHODS OF SYNTHESIS AND FEATURES OF USING SYSTEMS BASED ON MORIN-METAL COMPLEXES IN FLUORESCENT ANALYSIS METHODS

***Asieieva D.A.*[1,2]**

[1]*Université Toulouse III - Paul Sabatier, 118 route de Narbonne, Toulouse Cedex, 9, 31062, France*
[2]*Taras Shevchenko National University of Kyiv, Volodymyrska St, 60, Kyiv 01033, Ukraine*
email: dasha.aseeva@gmail.com

The review describes modern physicochemical systems based on complex compounds with organic ligands, which may have fluorescent properties when interacting with metal ions or proteins. Modern methods of synthesis of these compounds and their use in physical-chemical methods of analysis are given. Approaches to detecting the content of metals and proteins using the fluorescent properties of morin complex compounds are considered. Areas of use of the effects of amplification and quenching of fluorescence for the determination of organic compounds and metal ions, especially in the presence of DNA and RNA of different biological origin are described. The influence of surfactants on the fluorescence intensity of complexes with morin was analyzed separately.

Key words: morin, complex compounds, fluorescence, analysis, metal ions, protein.

INTRODUCTION. Physical-chemical methods using fluorescent materials make it possible to solve various scientific and applied problems in the field of chemistry, physics, biology, environmental monitoring and medical diagnostics due to high sensitivity, selectivity and expressiveness [1]. It is noteworthy that in many cases the use of micellar systems leads to an increase in the intensity of fluorescence and quantum yields by more than two orders of magnitude, and, accordingly, to a decrease in the detection limits of analytes. Surfactants have specific and sometimes unique properties. They are not only analytical reagents, but also able to effectively affect the physicochemical properties of other substances in solutions, such as proteins [2, 3], which are involved in building muscle tissue, as well as parts of the hair, nails and internal bodies. Therefore, qualitative and quantitative analysis of proteins is important in clinical trials and applications [4–6]. Therefore, the development of new physical-chemical methods and selection of conditions for the rapid determination of microquantities of protein is very relevant today. In addition, fluorescent properties can be

used in the development of labeling reagents for the further development of such methods as: high performance liquid chromatography, spectrophotometry and selective sensors [7–9]. For the development of these methods, the conditions for the synthesis of reagents and their organic component are of great importance, which in many cases is the key to the substance's acquisition of selective properties in reactions with organic compounds. Flavonoids are of particular interest, which are the most numerous class of natural phenolic compounds, characterized by structural diversity, high chemical activity and low toxicity. They have a wide range of biological activity, which is associated with many structures that lead to changes in the physicochemical properties of systems based on them [10,11].

Synthesis and use of organic reagents with fluorescent properties in physical-chemical methods of analysis. In recent years, the development of new organic fluorescent reagents, which are further used in various fields of analysis, is becoming more widespread. Thus, in [12] an organic reagent was obtained through the reaction of selenoic acid with three aromatic orthodiamines. Selenoic acid reacted with 2,3-diaminonaphthalene in an acidic solution to form 4,5-benzopiaselenol, which has fluorescent properties. It was found that this compound can then be extracted from the acidic phase with organic solvents and used to determine selenium with a detection limit of 0.002 μg. 2,3-diaminonaphthalene has been found to be significantly more sensitive than the previously recommended 3,3'-diaminobenzidine as a fluorescent reagent.

In [13], new bipyridyl receptors for ruthenium (II) imidazole were synthesized, which can recognize anions of chloride, bromide, dihydrogen phosphate, and ATP in mixed polar aqueous-organic solutions by fluorescence. Interestingly, the combined amidimidazole receptor Ruthenium (II) exhibits selectivity for the determination of anhydrous chloride in a solution of acetonitrile – water with a ratio of 90:10. Also, this receptor is selective for the determination of ATP in the acetonitrile – water (50:50) solvent. The aim of [14] was to develop a new fluorescent labeling reagent 9-anthryldiazomethane (ADAM), for carboxylic acids. 9-anthraldehyde hydrazone was first oxidized with N-chlorosuccinimide in an organic solvent such as ethyl acetate to obtain 9-anthryldiazomethane and then used directly as a reagent for the derivatization of carboxylic acids. Both the oxidation reaction and the derivatization reaction were performed at room temperature, and an aliquot of the derivative mixture was introduced directly into the chromatograph. Derivatives of 9-anthrylmethyl esters formed from ADAM and various carboxylic acids were separated on a reversed-phase column by fluorometric detection. This method can be used for the high performance liquid chromatographic determination of long and short chains of fatty acids, keto acids and hydroxy acids.

Also, in [15] the use of fluorescent reagents such as 4-bromomethyl-7-acetoxycomarin (Br-Mac) in high performance liquid chromatography is described. It was found that Br-Mac reacts with carboxylic acids to form esters, which are then separated by liquid phase chromatography. Next, the column eluate was mixed with an alkaline solution, where the labeled carboxylic acids were hydrolyzed to fluorescent coumarin derivatives, which were then passed through a fluorimeter. Thus, a fluorescent hydrolyzate equimolar to a carboxylic acid was determined, the fluorophore

of which is common to each carboxylic acid. There are only slight peak differences for different carboxylic acids. It is established that this method can detect low levels of femtomol in carboxylic acids. 6-oxy-(N-succinimyl acetate)-9-(2'-methoxycarbonyl) fluorescein (SAMF) is a new fluorescein-based fluorescent probe that was developed in [16] as a derivatizing reagent for the determination of aliphatic amines. Stable fluorescence intensity was observed at pH 4–9. Derivatization took place at room temperature for 6 minutes. Separation was performed on a C18 column, where SAMF derivatives with eight aliphatic amines were separated after 28 min with a mobile methanol-water phase (57:43) containing 10 mmol/l H_3Cit_3-NaOH buffer (pH 5.0). In fluorescent detection at $\lambda_{ex}/\lambda_{em}$ = 484/516 nm, the detection limit reached 2–320 fmol (signal-to-noise ratio = 3), which is better than the detection limits obtained in other analytical methods for the determination of aliphatic amines (Table 1). The proposed method has been successfully used to determine aliphatic amines in environmental samples and food products such as lake water, red wine, white wine and cheese.

In [17] morin was studied (Fig. 1) and its interaction with organotin compounds (chemical compounds with tin and carbon substitutes), which is accompanied by green fluorescence.

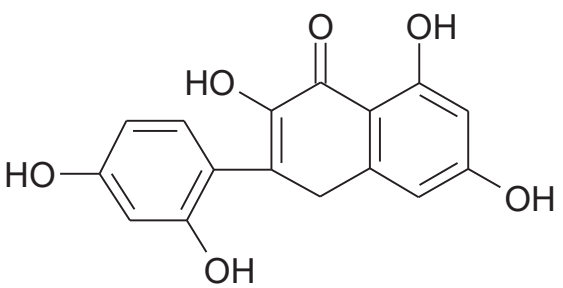

Fig. 1 Structural formula of morin (3,5,7,2',4'-pentahydroxyflavone).

The sensitivity of the reagent for the determination of dialkyltin compounds was especially noted. The excitation and luminescence wavelengths were 415 nm and 495 nm for alkyltin-morin complexes and approximately 405 nm and 520 nm for triphenyltin-morin complexes, respectively. The maximum fluorescence intensity was achieved with the ratio of reagents: from 3 to 9 mol of morin per 1 mol of dialkyl and triphenyltin and from 6 to 12 mol of morin to 1 mol for trialkyltin. The authors set the following limits for the detection of organotin compounds: for dialkyltins $1 \cdot 10^{-9}$ mol/l, for monoalkyltins $1 \cdot 10^{-7}$ mol/l, for trialkyltins $5 \cdot 10^{-7}$ mol/l and $5 \cdot 10^{-7}$ mol/l for triphenyltins (Table 1).

The authors [18] investigated the formation of fluorescent compounds of o-phthaldialdehyde with amino acids in an alkaline medium in the presence of a reducing agent. The excitation and emission wavelengths were 340 nm and 455 nm, respectively. The technique allows to perform fluometric analysis of amino acids to the nanomolar range. It was found that the sensitivity of the method using o-phthaldialdehyde is much higher than that of the method of determination of amino acids by ninhydrin, which was used previously (Table 1).

The phenomenon of fluorescence quenching of rhodamine B due to its interaction with hydroxyl radicals formed by the Fenton reagent was studied in [19]. The inhibitory effect of pentachlorophenol on this interaction has been established. The obtained data allowed the determination of pentachlorophenol with a detection limit of 3.0 ng/ml and a linear range of determination of 4.0–240 ng/ml. The method was used to determine pentachlorophenol in synthetic samples and natural water samples with satisfactory results. A rapid method for

the fluorescence determination of uranium using the interaction between a uranylbenzoic acid complex and Rhodamine B was developed. It was found that the fluorescence intensity depends on the concentration of benzoic acid, the concentration of rhodamine B, pH and the volume of the aqueous phase, and that the increase in fluorescence intensity is proportional to the increase in the concentration of uranium. The detection limit of uranium was $5 \cdot 10^{-8}$ mol/l [20] (Table 1).

A new fluorescent reagent 2-amino-5,7-dimethyl-1,8-naphthyridine was synthesized [21], which was further used to determine trace amounts of nitrites. The authors found that the fluorescence quenching of the reagent by nitrite ion has a linear relationship in the range of nitrite concentrations from $1 \cdot 10^{-7}$ to $2.5 \cdot 10^{-6}$ mol/l with a detection limit of $4.06 \cdot 10^{-8}$ mol/l (Table. 1).

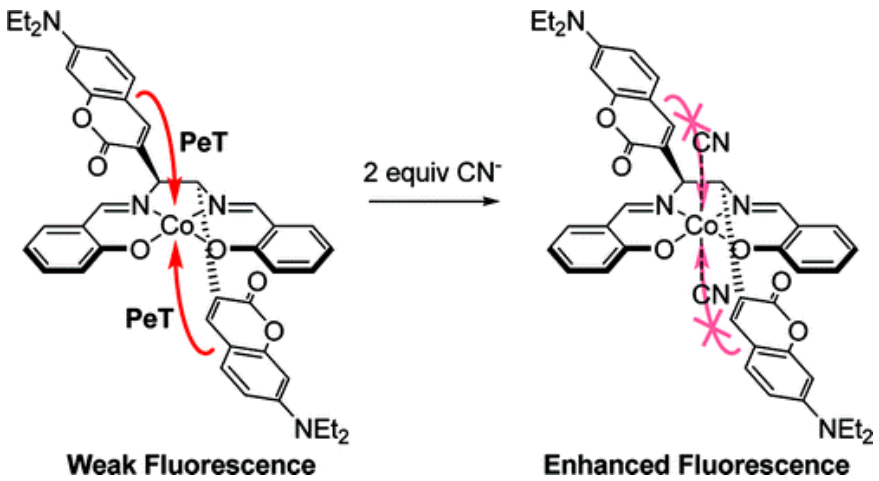

Fig. 2. Enhancement of fluorescent properties in a Co-salen complex in the presence of cyanide ions [22].

A sensor based on a Co (II) complex [22] was developed to determine cyanide anions, which are able to form a 1:2 complex in comparison with other anions. The complex was synthesized by adding 2,2-dihydroxyphenyl ethylenediamine to two equivalents of 7-di-ethylaminocoumarin-3-carboxaldehyde. The resulting compound was then mixed with cobalt (II) acetate in the presence of triethylamine (TEA) followed by recrystallization of the complex in methanol and dichloromethane. With increasing cyanide concentration, the authors observed an increase in the fluorescence intensity of the complex due to the cessation of the process of photoinduced electron transfer from coumarin fluorophore to cobalt (II) ion (Fig. 2).

Analysis of literature shows that increasing the fluorescent properties of complexes in the presence of anions is possible by stopping the process of photoinduced electron transfer from the organic fluorophore to metal ion, and that determination of organotin compounds is possible using complexes with morin. Other complexing agents are ineffective.

Table 1

Detection limits and linear ranges of fluometric methods using organic reagents.

Organic reagent	Analytes	Determination limit	Linear range of concentration change	Reference
2,3-diaminonaphtalene	Selenium	0,002 мкг	–	[12]
(3,5,7,2',4'- pentahydroxyflavone) Morin	Organotin compounds	$1{\cdot}10^{-7}$ - $1{\cdot}10^{-9}$ mol/l	–	[16]
6-oxy-(N- succinimilacetate)-9-(2'-methoxycarbonyl) fluorescein	Aliphatic amines	2–320 fmol	–	[17]
[9-(2- carboxyphenyl)-6-diethyl-amino-3- xanthenyliden]-diethyl-ammonium cloride) Rhodamine B	Pentachloro-phenol	3,0 ng/ml	4,0-240 ng/ml	[19]
Rhodamine B	Uranium	$5{\cdot}10^{-8}$ mol/l	–	[20]
2-amino-5,7-dimethyl-1,8-naphthyridine	Nitrites	$4{,}06{\cdot}10^{-8}$ mol/l	$1{\cdot}10^{-7}$ - $5{\cdot}10^{-6}$ mol/l	[21]

Approaches to detecting the content of metals and proteins using the fluorescent properties of morin complex compounds. Flavonoids are most often used in modern methods of analysis to determine metals. Morin, which belongs to this class of compounds (Fig. 1), can form stable complexes with metal cations, which in some cases have fluorescent properties in the presence of protein structures in solutions. Also, these complexes are quite stable when interacting with protein molecules in a wide pH range (table 2). In [23], the interaction of a Bi (III)-morin complex with DNA using methyl blue dye was studied using fluorescence, spectrophotometry and voltammetry. A Bi (III)-morin (2:1) complex was used in the work, the composition of which was calculated from the results of UV-Vis spectroscopy. It was found experimentally that the fluorescence signal of the Bi (III)-morin complex increases with the addition of DNA, while the fluorescence signal of pure morin decreases accordingly. Addition of methyl blue to the Bi (III)–morin–DNA complex reduces the emission signal and causes a hypochromic shift of 2 nm, which confirms the intercalation of the complex into the DNA molecule. The stability constant of the Bi (III)–morine complex with DNA, which is $2{,}8{\cdot}10^4$, was found in the work.

Table 2

Stability constants of metal complexes with morin [24].

Metal ion	Stability constant, *logβ*	pH
Cu (II)	4,94	5,8
Zn (II)	6,74	5,5
WO_4^{2-}	11,6	3,0
Pd(II)	4,55	4,0
$Ti(C_2O_4)_2^{2-}$	7,35	8,0
Ba (II)	4,55	4,2

During extraction into isopropyl alcohol from acid solutions at different pH values, the duration of fluorescence of morin complexes was established [25]. Complexes of Al (III), Ga (III) and In (III) change their composition in accordance with the change in the acidity of the medium and form complexes with molar ratios of 1:1 or 1:2 (metal:morin). The lifetime of fluorescence or the average lifetime of the molecule in the excited state also changed according to the change in the composition of the complex. Such properties have made it possible to develop optical sensors for detecting ions of these metals. The sensor was based on the formation of a complex between specific metal ions and the complexing ligand. Several chelate systems and several mechanisms of their immobilization are proposed. Morin and its complexes with AI^{3+}, Ga^{3+} and In^{3+} at a concentration of 5 μmol are markedly fluorescent when exposed to normal indoor lighting. Morin complexes with AI^{3+}, Ga^{3+} and In^{3+} were excited at a wavelength of 457.9 nm, the emission wavelength was 525 nm. These spectra overlap strongly, and therefore morin complexes are difficult or impossible to distinguish by conventional fluometric methods. Immobilization did not lead to significant changes in the duration of luminescence of metal-morin complexes. Thus, the sensor based on morin and the formation of its complexes with AI^{3+}, Ga^{3+} and In^{3+} was not suitable for multi-element determinations using spectrofluorometry [26].

The aim of [27] was to determine Aluminum using three different methods based on an Al (III)-morin complex. The methods of spectrophotometry, spectrofluorometry and differential pulse adsorption stripping voltammetry were compared under optimized experimental conditions. Fluorescence spectra were measured in acetoacetate at pH = 5, the concentration of morin was 10 μmol. The maximum excitation wavelength was set to λ_{ex} = 350 nm, and the emmition spectra were recorded in the range from 400 to 650 nm. The maximum fluorescence intensity was reached at λ_{em} = 505 nm. The authors found that the emission intensity is influenced by many factors, including the acidity of the solutions and the concentration of morin. A calibration graph for the determination of Al (III) from 0.1 to 1.0 μmol was also obtained. The method showed good reproducibility and a detection limit of 110 nmol.

In [28], complexes of Al (III) with morin and quercetin were studied by fluorescence. The stoichiometry of the complexes was evaluated by the Job method, the number of fluorescent forms in the solution was calculated by the TRES method. It was found that Al (III) with morin is able to form two complexes with stoichiometries of morin:Al (III) either 1:1 or 2:1 with lifetimes of 4.3 and 2.0 ns, respectively. Morin, which was immobilized on cellulose powder and attached to the end of bifurcated optical fiber, was used to determine Al^{3+} based on their fluorescent complex [29]. When immobilized morin was placed in a solution containing Al^{3+}, the authors observed the fluorescence of the Al (III)-morin complex. A linear dependence of the fluorescence intensity on the wavelength in the range of Al (III) concentrations from $1{\cdot}10^{-6}$ to $1{\cdot}10^{-4}$ mol/l with the detection limit of $1{\cdot}10^{-6}$ mol/l was established. The study of the effect of pH on the fluorescence of the complex showed that at a low acidity of solutions, the stability constant of the complex decreases due to the destruction of the complex due to Al^{3+} protonation, and that at high pH, most of aluminum is in the form of hydroxides. Based on this, the most optimal

pH value of 4.8 was established, at which all measurements were performed. The stability constant of the Al (III)–immobilized morin complex of $1.7 \cdot 10^4$ was determined.

The fluorescent activity of the Lanthanum (III)–quercetin–nucleic acid ternary complex was established in [30]. The authors chose natural calf thymus DNA and thermally denatured yeast DNA and RNA as nucleic acids. The fluorescence intensity of the complexes increased with the formation of ternary complexes in the pH range of 7.8-8.3, NH_3 - NH_4Cl was used as a buffer mixture. The maximum fluorescence intensity of the ternary complexes was observed at an emission wavelength of 470 nm and at an excitation wavelength of 280 nm. Based on these observations, a technique for the determination of nucleic acids was developed. The concentrations of La (III) ($2.2 \cdot 10^{-5}$ mol/l) and quercetin ($5.0 \cdot 10^{-5}$ mol/l) were selected to establish the optimal conditions. The authors obtained calibration graphs with linear ranges of 0.5-3.0 μg/ml for calf thymus DNA and 0.5-4.0 μg/ml for yeast DNA and RNA. The limits of detection were calculated by the 3σ test and were 0.072 μg/ml, 0.142 μg/ml and 0.307 μg/ml for calf thymus DNA, yeast DNA and RNA, respectively.

It is also possible to determine the content of nitric oxide in aqueous and methanolic solutions based on fluorescence using a copper complex with tridentate N-donor ligand, in which with increasing amount of nitric oxide there is an increase in the fluorescence intensity of copper with fluorescent ligand in degassed methanol and aqueous solution (pH=7.2) [31]. Thus, this complex can function as a sensor of nitric oxide based on fluorescence. It is noteworthy that it is possible to determine nanomolar amounts of nitric oxide.

The effect of room temperature ionic liquid on the formation of the fluorescent ternary oxalate-sodium morine-5-Aluminum sulfonate complex was studied [32]. In the presence of 1-butyl-3-methylimidazole hexafluorophosphate (BMIM-PF6) the formation of a complex with better fluorescent characteristics is achieved and as a result a sensitive method for the determination of oxalate ions has been developed. The maximum excitation wavelength was set λ_{ex} = 420 nm, and emission λ_{em} = 513 nm. The detection limit of oxalate is 0.57 ng/ml. The method showed satisfactory results in determining the content of oxalate in plant tissue (spinach leaves).

Using the effects of amplification and quenching of fluorescence to determine organic compounds and metal ions. In [33], the enhancement of fluorescence of a Eu^{3+} tetracycline complex due to DNA or RNA was studied. It was found that double-stranded and single-stranded DNA can strongly enhance the fluorescence of the Eu^{3+} tetracycline complex, in contrast to RNA, which showed a very small amplification effect, based on which a method of selective determination of DNA in the presence of RNA was developed. The most optimal pH conditions, when the maximum fluorescence intensity was reached at an acidity of solutions of 8.0–9.7. The excitation wavelength at which the complex was excited was 398 nm, and the fluorescence wavelength was 615 nm. Calibration graphs with linearity ranging from 0.02 to 1.0 μg for single-stranded and double-stranded DNA were obtained. The relative standard deviation (at n = 7) was in the range of 3.0%.

In [34], a new spectrofluometric method for the determination of lysozyme in the formation of its triple complex with Eu^{3+}-metacycline was developed. Due to the intramolecular

energy transfer from the ligands to the central Eu^{3+} atom, the fluorescence intensity increases threefold at an emission wavelength of 612 nm. The excitation wavelength is 285 nm. Optimal conditions were also established: pH 9.6, metacycline concentration $2.0 \cdot 10^{-5}$ mol/l and Eu^{3+} concentration $2.4 \cdot 10^{-5}$ mol/l The increase in fluorescence intensity is proportional to the lyozyme concentration, the linear range is from 0 to $3.5 \cdot 10^{-5}$ mol/l with a detection limit of $4.74 \cdot 10^{-7}$ mol/l. The mechanism by which an increase in fluorescence between the Eu^{3+} metacycline complex and lysozyme occurred was also investigated. The developed method is simple, sensitive and has been successfully used to determine lysozyme in urine.

In [35], another approach was used to determine cysteine and glutathione with quenching of the fluorescence of the complex. It was found that the addition of thiol compounds to the fluorescent system Zn (II)-8-hydroxyquinoline-5-sulfonic acid Zn (II)-HQS in a buffer mixture of H_3BO_3-$Na_2B_4O_7$ (pH 8.50) leads to quenching of the fluorescence of the complex. Based on these studies, a linear dependency was obtained between the amount of cysteine or glutathione and the corresponding decrease in the relative fluorescence intensity of the Zn (II)-HQS system. The complexes were excited at a wavelength of 365 nm, and the emission wavelength was 512 nm. For optimal conditions, HQS and Zn (II) concentrations of $4.44 \cdot 10^{-6}$ mol/l and $4.59 \cdot 10^{-6}$ mol/l were taken. In the analysis of cysteine in the protein hydrolyzate and reduced glutathione in blood serum, the detection limits were 17 ng/ml and 0.6 μg/ml, respectively. The removal degree was 95.6-104.5%.

The effect of protein on the fluorescence of zinc complexes with morin and fluorescein was studied [36]. The introduction of protein into the Zn-morin complex causes the quenching of fluorescence, which is proportional to the amount of protein. Under optimal conditions, the limits of detection for the determination of bovine serum albumin and human serum albumin are 0.22 g/ml and 0.18 g/ml, respectively.

Based on the fluorescence of DNA-platinum complexes, new methods for the determination of platinum have been developed [37]. The authors found that cis- or trans-bidentate complexes are formed between DNA and platinum. DNA with ethidium bromide forms fluorescent complexes, and the addition of platinum inhibits the intercalation of ethidium bromide, and as a result there is a linear decrease in fluorescence intensity. The method was tested in different ionic media and in a wide range of ionic strength. The detection limit of platinum is $5 \cdot 10^{-8}$ g/ml.

The aim of the study [38] was to develop a sensor for mercury ions based on the fluorescence of iodide anion with the complex T-Hg^{II}-T (T = thymine). The authors synthesized a fluorescent anthracene-thymine (An-T) dyad, which forms with the Mercury ion an An-T-Hg^{II}-T-An complex, and it was found that the addition of mercury reduces the fluorescence intensity. In this case, the dyad A-T is a sensor for mercury (II) ions in aqueous media based on fluorescence quenching. However, with the addition of iodide, the fluorescence of the An-T-Hg^{II}-T-An complex is restored due to the binding of mercury to the iodide ion. The detection limit for iodine is 126 nmol. The sensor has shown high selectivity over other common anions and can be used to detect iodide in drinking water and biological fluids such as urine.

Influence of surfactants on the fluorescence intensity of complexes with morin. The use of micellar systems in spectrophotometric methods of analysis is the most popular and oldest field of application of surfactants in analytical chemistry. In recent years, surfactants have been successfully used in fluorometric determinations. In many cases, there is a multiple increase in fluorescence intensity, which leads to increased interest in these systems due to high sensitivity and selectivity.

In [39] the effect of cationic surfactants on the fluorescence of the zirconium (IV)-morine system is described. It was found that in a sulfuric acid medium the fluorescence intensity of this complex can be greatly enhanced by the cationic surfactant cetyltrimethylammonium bromide (CTAB). However, the addition of a nonionic surfactant TritonX-100 only slightly enhanced fluorescence, and the introduction of anionic surfactants such as sodium lauryl sulfate (SLS) and sodium dodecyl sulfonate (SDS) did not increase the fluorescence intensity. It was found that the determinant factor is the change of the composition of the analyzed complex in the presence of CTAB. When the concentration of CTAB reaches the critical concentration of micelle formation, a complex is formed with the ratio Zr (IV):morin = 1:3. As the concentration of CTAB increases, a mixed complex with the composition Zr (IV): morin:SO_4^{2-} = 1:1:2 is formed, and the fluorescence intensity sharply increases. It was found that the formation of mixed-ligand complexes of zirconium with several anions in the presence of cationic surfactants increase the fluorescence intensity of the system.

The effect of different surfactants on the systems Hf-quercetin, Zr-quercetin, Sn-morin, Mg-oxine-5-sulfonic acid (HIQS), Zn-HzQS, Cd-HZQS and Tb-EDTA-sulfosalicylic acid (SSA) was studied in [40]. Increase in fluorescence occurs due to the formation of complexes such as ionic associates with a rigid structure, which leads to a significant increase in fluorescence intensity. Not only traditional nonionic and cationic surfactants were used, but also zwitterionic and anionic one. It is established that the fluorescence intensity of each investigated complex strongly increases in the presence of the corresponding surfactant. The authors also determined the optimal conditions for the formation of ternary complexes, their structure and quantum yield. It was found that the fluorescence intensity of the complexes changes little when surfactants are added at concentrations lower than the critical micelle concentration (CMC). With a further increase in the surfactant content, the fluorescence intensity begins to increase sharply. Thus, the addition of anionic sodium dodecyl sulfonate (SDS) to the Sn-morin complex increases the fluorescent properties of the system by a factor of 4, and a ternary complex with Sn:morin:SDS = 1:2:2. stoichiometry is formed.

In [41], a simple and sensitive spectrofluorimetric method for the determination of Al (III) based on the formation of the ternary complex Al (III) - morin - Triton X-100 is described. The effects of other nonionic surfactants, such as Tween 80, Tween 20 and octyl glucoside (OG), have also been studied. The addition of Triton X-100 increases the sensitivity fivefold in the fluorometric determination of Al (III) using morin. The complex was excited at a wavelength of 410 nm, and the fluorescence signal was measured at a wavelength of 495 nm. The maximum fluorescence signal was observed at pH 4.0 (acetate buffer), with 0.6% TX-100 and at a morin concentration of $1.35 \cdot 10^{-3}$ mol/l. The

authors also obtained a calibration graph that is linear up to 7 mg/l, and the detection limit is 0.022 mg/l. The use of Triton X-100 eliminates the need to use additional extraction steps for the sensitive and selective determination of Al (III).

The effect of the anionic surfactant sodium dodecylbenzenesulfonate (SDBS) on the fluorescence intensity of the Al (III) – morin complex and the mechanism of their interaction were studied in [42]. It was found that the luminescence increases and, on this basis, a fluometric method for the determination of proteins was developed. The highest fluorescence intensity was achieved at pH 6.84, and it was also shown that the fluorescence intensity is influenced by the type of buffer mixture, among which the most effective was HMTA – HCl. The maximum fluorescence intensity was reached at concentrations of morin and Al (III) of $1.0 \cdot 10^{-6}$ mol/l and $1.0 \cdot 10^{-5}$ mol/l, respectively. Regarding the anionic surfactant SDBS, its concentration was $2.0 \cdot 10^{-4}$ mol/l, which is lower than the critical micelle concentration, which is $3.3 \cdot 10^{-4}$ mol/l for SDBS. Under optimal conditions, the increased fluorescence intensity was proportional to the protein concentration in the range of $1.0 \cdot 10^{-8}$–$1.3 \cdot 10^{-5}$ g/ml for bovine serum albumin (BSA), $4.0 \cdot 10^{-8}$–$1{,}2 \cdot 10^{-5}$ g/ml for egg albumin (EA) and $5.0 \cdot 10^{-8}$–$1.2 \cdot 10^{-5}$ g/ml for human serum albumin (HSA). The limits of their detection were $5.0 \cdot 10^{-9}$, $1.8 \cdot 10^{-8}$ and $1.6 \cdot 10^{-8}$ g/ml, respectively. Thus, the authors obtained a highly sensitive, stable and rapid method for the determination of proteins.

An increased fluorescence intensity of the aluminum (III)-morin complex was observed in [43] in the presence of the nonionic surfactant Tween-20. The fluorescence of the complex was measured at an excitation wavelength of 425 nm and an emission wavelength of 495 nm. The authors also established optimal conditions: pH = 4.5, the concentration of morin 20 mmol, and 0.8% Tween-20. A linear calibration graph from 50 to 100 μg/l was also obtained, and the detection limit was 3 μg/l.

In [44], a highly sensitive method of fluorometric determination of Fe (III) by reaction with 5-(4-methoxyphenylazo)-8-(4-toluenesulfonamido) quinoline in the presence of the cationic surfactant cetyltrimethylammonium bromide (CTAB) is described. As a result, a linear fluorescence intensity (λ_{ex} = 317 nm, λ_{em} = 534 nm) of up to 3 mmol (170 ng/ml) of Iron (III) in an aqueous solution was observed. A similar experiment was also performed in the presence of the anionic surfactant sodium dodecyl sulfate. The introduction of anionic surfactants led to a decrease in fluorescence intensity. In contrast to this, the introduction of the nonionic surfactants Tween-80 and TritonX-100 into the studied system as well as cationic surfactants (CTAB) increased the fluorescence intensity but gave larger background signals. The most optimal concentration of CTAB was determined, which was $1.7 \cdot 10^{-3}$ mol/l, which is higher than the critical concentration of micelle formation (CMC (CTAB) = $1.3 \cdot 10^{-3}$ mol/l). This method can also be used to determine the trace amounts of Fe (III) and Fe (II) without the need for prior concentration.

In [45], the authors determined how the addition of nonionic surfactants affects the fluorescence intensity of Al (III)-morin complexes in order to improve the analytical characteristics of the complex. Morin is one of the reagents most often used for the qualitative and quantitative determination of Al (III). The authors found that the addition of cationic surfactants such as cetyltrimethylammonium

bromide (CTAB) and nonionic surfactants such as: polyoxyethylene noniphenols, polyoxyethylene higher alcohols, fatty carboxylic acid esters and alkanolamides do not increase the fluorescence intensity, but on the contrary, cause its significant decrease contrary to the expectations of the authors. Only in some cases there were an initial increase in luminescence intensity at high surfactant concentrations and subsequent quenching with increasing content of nonionic surfactant in the system. In contrast, the authors found that the addition of GenapolPF-20 (ethylene oxide – condensate of epoxypropane) to the metal-morin complex causes an increase in fluorescence intensity, so the sensitivity of the determination can be increased tenfold, as well as the selectivity of the method compared to other methods. The optimal conditions for increasing the fluorescence intensity were: the concentration of nonionic surfactant 3%, morin 0.005%, pH 3.8 in an acetate buffer at a temperature of 25 °C. The excitation and emission wavelength maxima were λ_{ex} = 430 nm and λ_{em} = 495 nm, respectively. The maximum fluorescence intensity was observed after 1.5 hours and remained stable for another 5 hours, the detection limit was 0.2 μg/l. The introduction of surfactants did not lead to either bathochromic or hypsochromic shift, which indicates a slight effect of surfactants on the ground and excited states of the complex. It was also shown that other derivatives of polyoxyethyl compounds resulted in increased sensitivity in the same order, but with a different surfactant concentration.

In [46], the fluorometric determination of samarium and gadolinium by increasing the fluorescence of the samarium-tenoyltrifluoroacetone-gadolinium 1,10-phenanthrolinate complex (Sm (III) – TTA – Phen – Gd (III)) was investigated, which increases the fluorescence intensity almost twofold. To increase the stability of the Sm (III) – TTA – Phen – Gd (III) system, surfactants were added, and their effects were investigated. Thus, the cationic surfactant CTAB and nonionic surfactant Tween-80 caused a decrease in fluorescence intensity. In contrast, the nonionic surfactants TX-100 and PVA caused a sharp increase in the fluorescence intensity of the complex.

Therefore, TX-100 was used for further experiments. An increase in the fluorescence intensity at the concentration of TX-100 at the level of the critical micelle concentration was shown. The most optimal concentration range of TX-100 was 0.018–0.064% (fivefold increase in emissions). A further increase in the concentration of TX-100 led to a decrease in emission intensity. It is noteworthy that the excess of TX-100 caused a bathochromic shift of the maximum excitation wavelength. The maximum fluorescence intensity was obtained in a pH range of 5.3–6.0 at a wavelength of excitation and emission of 349 nm and 648 nm, respectively. Thus, the system Sm (III) – TTA – Phen – Gd (III) – TritonX-100 can be used to determine trace amounts of samarium in lanthanide oxides.

The aim of [47] was to determine tetracyclines (TC) in aqueous solutions based on the formation of fluorescent chelates with europium using EDTA as a coligand and cetyltrimethylammonium chloride (CTAC) as a surfactant. The method involves the formation of a chelate, where the lanthanide ion will be associated with the β-diketone group. Contrary to the authors' expectations, the addition of the nonionic surfactant TX-100 almost does not change the luminescence intensity in the pH range 5-9, and the addition

of EDTA has a negative effect and sharply reduces the fluorescence intensity. At the same time, with the addition of CTAC, the emission intensity increased and reached a maximum at pH 9. The Eu – TC – CTAC system has a sensitivity that is 6 times higher than that of the Eu – TC – TX-100 system, and the detection limits were $2{,}5\cdot10^{-10}$, $5\cdot10^{-10}$, $1{,}5\cdot10^{-9}$ and $2\cdot10^{-9}$ mol/l for TC, oxytetracycline, chlortetracycline and doxycycline, respectively.

In [48], the authors found how the molecular structure of the anionic surfactant affects the fluorescence of bovine serum albumin (BSA). Sodium alkyl sulfates (C_nSO_3, n = 8, 10, and 12) and sodium alkyl carboxylates (C_nCOONa, n = 9 and 11) were used as anionic surfactants. It was established that with the increase of the hydrophobic chain the ability to quench the fluorescence of BSA increases, and the hypsochromic shift increases. It is noteworthy that the replacement of acidic groups of anionic surfactants does not show a significant effect on the fluorescence of the albumin complex.

The authors of [49] studied the effect of surfactants on the fluorescence of the beryllium-morin system. It was found that the addition of the nonionic surfactant TX-100 significantly increases the fluorescence intensity of the complex, as opposed to the anionic surfactant sodium lauryl sulfate (SLS), zwitterionic surfactant dodecyldimethylaminoacetic acid (DDMAA), and cationic surfactant cetyltrimethylammonium bromide (CTAB), which cause only a slight increase of fluorescence. It was also found that when adding all surfactants, except for anionic SLS, a bathochromic shift occurs (up to 25 nm). The addition of Triton X-100 makes it possible to determine the nanoquantities of beryllium in weakly acidic solutions (pH 5.8–6.2, hexamine buffer solution), detection limit 0.06 ng/ml. The relative standard deviation is 2.2% for beryllium at a concentration of 0.5 ng/ml and 0.7% for 5.0 ng/ml. The method is used to determine beryllium in water quality control samples and therefore the effect of 25 ions that can affect the fluorescence of the Be-morin-TX-100 complex, among which Zn^{2+} and F^- interfere the most, was also studied.

In [50], a method of determination of Al (III) in a luminescent complex with lumogallion is presented. The addition of the nonionic surfactant Triton X-100 increased the fluorescence intensity of the Al (III)-morin complex by a factor of 5–6. Optimal conditions were also determined: pH 4.7, the concentrations of lumogallion and TX-100 were 1 μg/l and 0.5%, respectively. The detection limit of Al (III) is 0.2 μg/l. The sensitivity of the method does not depend on the salt concentration in water and can be used to determine aluminum in water.

Therefore, to date, the study of the effect of surfactants on the fluorescent properties of organic reagents and their complexes continues, because the increase or decrease in fluorescent properties is not systematic and is not always explainable.

CONCLUSIONS. To date, the complexes of morin with metals are actively studied and tend to be widely used in such physicochemical methods of analysis as: high performance liquid chromatography, spectrophotometry and fluorometry. It has been proved that in complex compounds of cobalt and salen, the increase of the fluorescent properties of complexes in the presence of anions is possible by stopping the process of photoinduced electron transfer from organic fluorophore to metal ion, and the determination of organotin

compounds is possible using complexes with morin. Other complexing agents are less effective and ineffective. The most studied is the complex Al (III) - morin in comparison with complexes of morin with other metals, such as In (III), Bi (III) and Ga (III). These complexes can be used to determine metals or to detect DNA of different biological origin or for the quantitative analysis of protein. All complexes with morin are stable in a wide pH range (3-8) and have a high fluorescence intensity. The fluorescence intensity can be increased by adding surfactants. For protein systems of morin with some metals (zinc and mercury), an opposite phenomenon is observed – fluorescence quenching, which can be used for the quantitative determination of proteins and metals.

ACKNOWLEDGMENT.
The work was done under financial support of Ministry of Education and Science of Ukraine.

В огляді описано сучасні фізико-хімічні системи на основі комплексних сполук з органічними лігандами, які можуть мати флуоресцентні властивості при взаємодії з іонами металів або білками. Наведено сучасні методи синтезу цих сполук та використання їх у фізико-хімічних методах аналізу. Розглянуто підходи до визначення вмісту металів і білків за флуоресцентними властивостями комплексних сполук морину. Описано сфери використання ефектів ампліфікації та гасіння флуоресценції для визначення органічних сполук та іонів металів, особливо за наявності ДНК та РНК різного біологічного походження. Окремо проаналізовано вплив поверхнево-активних речовин на інтенсивність флуоресценції комплексів із морином.

Ключові слова: морін, комплексні сполуки, флуоресценція, аналіз, іони металів, білок.

МЕТОДИ СИНТЕЗУ ТА ОСОБЛИВОСТІ ВИКОРИСТАННЯ СИСТЕМ НА ОСНОВІ МЕТАЛОКОМПЛЕКСІВ МОРІН У МЕТОДАХ ФЛУОРЕСЦЕНТНОГО АНАЛІЗУ

Асєєва Д. А.[1,2]

[1]*Університет Тулуза III - Поль Сабатьє, 118 траса Нарбон, Тулуза Cedex, 9, 31062, Франція*
[2]*Київський національний університет імені Тараса Шевченка, вул. Володимирська, 60, м. Київ 01033, Україна*
email: dasha.aseeva@gmail.com

REFERENCES

1. Rettig W., Strehmel B., Schrader S., & Seifert H. (Eds.). *Applied fluorescence in chemistry, biology and medicine*. Springer Science & Business Media. 2012.
2. Kulmyrzaev A. A., Karoui R., De Baerdemaeker J., & Dufour E. Infrared and fluorescence spectroscopic techniques for the determination of nutritional constituents in foods. *International Journal of Food Properties*. 2007. **10** (2): 299–320.
3. Kato A., & Nakai S. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. *Biochimica et biophysica acta (BBA)-Protein structure*. 1980. **624**(1): 13–20.

4. Jin H. G. Z. X. O. Application of Chemiluminescent Image to Biological Molecules Detection. *World Sci-tech R & D*, 04. 2004.
5. Paramita V. D., & Kasapis S. The role of structural relaxation in governing the mobility of linoleic acid in condensed whey protein matrices. *Food Hydrocolloids*. 2018. **76**. 184–193.
6. Tran T. M., Dai T. X. T., Tran D. B., Nguyen Q. C. T., & Nguyen D. H. Y. A simple spectrophotometric method for quantifying total lipids in plants and animals. *Can Tho University Journal of Science*. 2019. **11**(2): 106–110.
7. Gazioglu I., Zengin O. S., Tartaglia A., Locatelli M., Furton K. G., & Kabir A. Determination of polycyclic aromatic hydrocarbons in nutritional supplements by fabric phase sorptive extraction (FPSE) with high-performance liquid chromatography (HPLC) with fluorescence detection. *Analytical Letters*. 2020. **54**(10): 1683–1696.
8. Abd Ali L. I., Qader A. F., Salih M. I., & Aboul-Enein H. Y. Sensitive spectrofluorometric method for the determination of ascorbic acid in pharmaceutical nutritional supplements using acriflavine as a fluorescence reagent. *Luminescence*. 2019. **34**(2): 168–174.
9. Muñoz-Huerta R. F., Guevara-Gonzalez R. G., Contreras-Medina L. M., Torres-Pacheco I., Prado-Olivarez J., & Ocampo-Velazquez R. V. A review of methods for sensing the nitrogen status in plants: advantages, disadvantages and recent advances. *Sensors*. 2013. **13**(8): 10823–10843.
10. Panche A. N., Diwan A. D., & Chandra S. R. Flavonoids: an overview. *Journal of nutritional science*, *5*. 2016.
11. Harborne J. B., Marby H., & Marby T. J. *The flavonoids*. Springer. 2013.
12. Parker C. A., & Harvey L. G. Luminescence of some piazselenols. A new fluorimetric reagent for selenium. *Analyst*. 1962. **87**(1036): 558–565.
13. Vickers M. S., Martindale K. S., & Beer P. D. Imidazolium functionalised acyclic ruthenium (II) bipyridyl receptors for anion recognition and luminescent sensing. *Journal of Materials Chemistry*. 2005. **15**(27–28): 2784–2790.
14. Yoshida T., Uetake A., Yamaguchi H., Nimura N., & Kinoshita T. New preparation method for 9-anthryldiazomethane (ADAM) as a fluorescent labeling reagent for fatty acids and derivatives. *Analytical biochemistry*. 1988. **173**(1): 70–74.
15. Tsuchiya H., Hayashi T., Naruse H., & Takagi N. High-performance liquid chromatography of carboxylic acids using 4-bromomethyl-7-acetoxycoumarin as fluorescence reagent. *Journal of Chromatography A*. 1982. **234**(1): 121–130.
16. Arakawa Y., Wada O., & Manabe M. Extraction and fluorometric determination of organotin compounds with Morin. *Analytical Chemistry*. 1983. **55**(12): 1901–1904.
17. .Cao L. W., Wang H., Li J. S., & Zhang H. S. 6-Oxy-(N-succinimidyl acetate)-9-(2'-methoxycarbonyl) fluorescein as a new fluorescent labeling reagent for aliphatic amines in environmental and food samples using high-performance liquid chromatography. *Journal of Chromatography A*. 2005. **1063**(1-2): 143–151.
18. Roth M. Fluorescence reaction for amino acids. *Analytical Chemistry*. 1971. **43**(7): 880–882.
19. Fan J., Guo H. Q., & Feng S. L. Spectrofluorimetric determination of pentachlorophenol based on its inhibitory effect on the redox reaction between hydroxyl radicals and fluorescent dye rhodamine B. 2007.
20. Andersen N. R., & Hercules D. M. Fluorometric Determination of Uranium with Rhodamine B. *Analytical Chemistry*. 1964. **36**(11): 2138–2141.
21. Chen T., Tong A., & Zhou, Y. 2-Amino-5, 7-dimethyl-1, 8-naphthyridine as a fluorescent reagent for the determination of nitrite. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2007. **66**(3): 586–589.
22. Lee J. H., Jeong A. R., Shin I. S., Kim H. J., & Hong J. I. Fluorescence turn-on sensor for cya-

nide based on a cobalt (II) – coumarinylsalen complex. *Organic letters*. 2010. **12**(4): 764–767.
23. Ensafi A. A., Hajian R., & Ebrahimi S. Study on the interaction between morin-Bi (III) complex and DNA with the use of methylene blue dye as a fluorophor probe. *Journal of the Brazilian Chemical Society*. 2009. **20**(2): 266–276.
24. Malešev D., & Kuntić V. Investigation of metal-flavonoid chelates and the determination of flavonoids via metal-flavonoid complexing reactions. *Journal of the Serbian chemical society*. 2007. **72**(10): 921–939.
25. Sawada T., Shibamoto T., & Kamada H. Fluorescence Lifetime Measurements of Morin–Metal Ion Complexes. *Bulletin of the Chemical Society of Japan*. 1978. **51**(6): 1736–1738.
26. Carroll M. K., Bright F. V., & Hieftje G. M. Fiber-optic time-resolved fluorescence sensor for the simultaneous determination of aluminum (3+) and gallium (3+) or indium (3+). *Analytical Chemistry*. 1989. **61**(15): 1768–1772.
27. Domínguez-Renedo O., Navarro-Cuñado A. M., Ventas-Romay E., & Alonso-Lomillo M. A. Determination of aluminium using different techniques based on the Al (III)-morin complex. *Talanta*. 2019. **196**: 131–136.
28. Gutierrez A. C., & Gehlen M. H. Time resolved fluorescence spectroscopy of quercetin and morin complexes with Al^{3+}. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2002. **58**(1): 83–89.
29. Saari L. A., & Seitz W. R. Immobilized morin as fluorescence sensor for determination of aluminum (III). *Analytical Chemistry*. 1983. **55**(4): 667–670.
30. Zhou J., Gong G. Q., Zhang Y. N., Qu J. Q., Wang L. F., & Xu J. W. Quercetin–La (III) complex for the fluorimetric determination of nucleic acids. *Analytica chimica acta*. 1999. **381**(1): 17–22.
31. Mondal B., Kumar P., Ghosh P., & Kalita A. Fluorescence-based detection of nitric oxide in aqueous and methanol media using a copper (II) complex. *Chemical Communications*. 2011. **47**(10): 2964–2966.
32. Nizar S. A., & Suah F. B. M. Effect of room temperature ionic liquid on the formation of the complex oxalate-sodium morin-5-sulfonate-aluminium (III): Application to the fluorescence determination of oxalate ion. *Journal of fluorescence*. 2016. **26**(4): 1167–1171.
33. Ci Y. X., Li Y. Z., & Liu X. J. Selective determination of DNA by its enhancement effect on the fluorescence of the Eu3+-tetracycline complex. *Analytical Chemistry*. 1995. **67**(11): 1785–1788.
34. Chongqiu J., & Li L. Lysozyme enhanced europium–metacycline complex fluorescence: a new spectrofluorimetric method for the determination of lysozyme. *Analytica chimica acta*. 2004. **511**(1): 11–16.
35. Wang H., Wang W. S., & Zhang H. S. A spectrofluorimetric method for cysteine and glutathione using the fluorescence system of Zn (II)–8-hydroxyquinoline-5-sulphonic acid complex. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2001. **57**(12): 2403–2407.
36. Huang W., Cao N., & Wang F. Determination of Serum Albumin by its quenching effect on the fluorescence of Zn 2-Morin complex. 2016.
37. Butour J. L., & Macquet J. P. Platinum determination in DNA-platinum complexes by fluorescence spectrophotometry. *Analytical biochemistry*. 1978. **89**(1): 22–30.
38. Ma B., Zeng F., Zheng F., & Wu S. A. Fluorescence Turn – on Sensor for Iodide Based on a Thymine – HgII – Thymine Complex. *Chemistry – A European Journal*. 2011. **17**(52): 14844–14850.
39. Fan Y. X., & Zheng Y. X. Effect of cationic micelles on the fluorescence of the zirconium – morin complex. *Analytica chimica acta*. 1993. **281**(2): 353–360.
40. Hui-Ming S., Wan-Cang C., & Ru-Ji W. Sensitization of surfactant on the fluorescent

reactions of metals. *Acta Chimica Sinica English Edition*. 1984. **2**(2): 133–143.
41. Suah F. B. M., Ahmad M., & Mehamod F. S. Effect of non-ionic surfactants to the Al (III)-morin complex and its application in determination of Al (III) ions: A preliminary study [Kesan surfaktan tak-ionik kepada kompleks Al (III)-morin dan aplikasinya dalam penentuan ion Al (III): Satu kajian awal]. *Malaysian Journal of Analytical Sciences*. 2017.
42. Wang F., Yang J., Wu X., Sun C., Liu S., Wang F., & Jia Z. Fluorescence enhancement effect of the morin–Al3+–sodium dodecyl benzene sulphonate – protein system and the determination of proteins. *Luminescence: The journal of biological and chemical luminescence*. 2006. **21**(1): 49–55.
43. Al-Kindy S. M. Z., Suliman F. O., & Salama S. B. A sequential injection method for the determination of aluminum in drinking water using fluorescence enhancement of the aluminum – morin complex in micellar media. *Microchemical journal*. 2003. **74**(2): 173–179.
44. Zeng Z., & Jewsbury R. A. Fluorimetric determination of iron using 5-(4-methoxyphenylazo)-8-(4-toluenesulfonamido) quinoline. *Analyst*. 2000. **125**(9): 1661–1665.
45. Escriche J. M., Cirugeda M. D. L. G., & Hernandez F. H. Increase in the sensitivity of the fluorescent reaction of the complexing of aluminium with morin using surfactant agents. *Analyst*. 1983. **108**(1292): 1386–1391.
46. Ci Y., & Lan Z. Fluorometric determination of samarium and gadolinium by enhancement of fluorescence of samarium-thenoyltrifluoroacetone-1, 10-phenanthroline ternary complex by gadolinium. *Analytical Chemistry*. 1989. **61**(10): 1063–1069.
47. Arnaud N., & Georges J. Sensitive detection of tetracyclines using europium-sensitized fluorescence with EDTA as co-ligand and cetyltrimethylammonium chloride as surfactant. *Analyst*. 2001. **126**(5): 694–697.
48. Lu R. C., Cao A. N., Lai L. H., & Xiao J. X.. Effect of anionic surfactant molecular structure on bovine serum albumin (BSA) fluorescence. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2006. **278**(1–3): 67–73.
49. Wei, F., Ten, E., & Wu, Z.. Enhancement of the fluorescence of the beryllium-morin complex by non-ionic surfactants. *Talanta*. 1990. **37**(9): 947–950.
50. Howard, A. G., Coxhead A. J., Potter I. A., & Watt A. P. Determination of dissolved aluminium by the micelle-enhanced fluorescence of its lumogallion complex. *Analyst*. 1986. **111**(12): 1379–1382.

Стаття надійшла 09.11.2021.