LIQUID-LIQUID EXTRACTION AND SPECTROPHOTOMETRIC CHARACTERIZATION OF A NEW TERNARY ION-ASSOCIATION COMPLEX OF IRON(III)

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ABSTRACT

Complex formation and liquid-liquid extraction in a system containing iron(III), 4-nitrocatechol (4NC), 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), water, and chloroform were studied. The effect of some experimental parameters (pH, shaking time, concentration of reagents) was investigated, and the optimum conditions for Fe(III) extraction as an ion-association complex, $(TT^+)_3[Fe^{3+}(4NC)_3]^{3-}$, were found. The following key constants were calculated: constant of distribution (Log K_D=1.49±0.02), constant of association (Log β=11.3±0.1), and constant of extraction (Log K_{ex}=12.8±0.1). Some additional characteristics concerning the application of 4NC and TTC for extractive-spectrophotometric determination of Fe(III) were estimated as well: absorption maximum (λ=435 nm), apparent molar absorptivity (ε=2.7×10^4 l mol^{-1} cm^{-1}), Sandell's sensitivity (SS=2.0 ng cm^{-2}), recovery factor (R %=94.3±0.2), limit of detection (LOD=0.11 μg cm^{-3}), and limit of quantification (LOQ=0.37 mg cm^{-3}). Beer's law was obeyed for Fe(III) concentrations up to 2.0 μg ml^{-1} with a correlation coefficient of 0.9990.

Keywords: iron(III), 4-nitrobenzene-1,2-diol, tetrazolium salt, ternary complex, solvent extraction.

INTRODUCTION

Iron is the most abundant element in our planet (and the second in abundance metal in the Earth's crust) which invariably presents in all natural products. Being the most important metal for a long period of the mankind history, it continues to be the backbone of the modern economy [1, 2]. The biological role of iron includes oxygen transport, electron transport, DNA synthesis and photosynthesis. Despite the fact that its uptake, transport and storage in higher organisms is well regulated [3], iron deficiency can develop due to uncontrolled loss and/or inadequate diet. On the other hand, iron overload can cause severe gastrointestinal problems [4] and irreversible cell and organ damage by catalyzing free radical formation which injures cellular membranes, nucleic acids, and proteins [5].

It is known that iron ions form coloured complexes with various catecholic ligands [6-9]. The complexation behaviour of Fe(III) towards the well-known analytical reagent 4-nitrobenzene-1,2-diol (4-nitrocatechol, 4NC) [10, 11] has been studied for designing new chelators for treating iron overload [12, 13], nonheme iron-containing enzymes [14-16], surface chemistry [17], chemistry of the marine environment [18], comparative studies and stability assessment [19]. The obtained results have shown that three different complexes are formed in aqueous solutions depending on pH and concentration of 4NC: $[Fe(4NC)]^+$, $[Fe(4NC)_2]^-$, and $[Fe(4NC)_3]^{3-}$ [12, 19]. Having in mind the ion-association properties of some bulky cations deriving from tetrazolium salts [20], we decided to investigate in details the complex formation and liquid-liquid extraction in a system containing Fe(III), 4NC, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), water and chloroform. The study presented here is a first step of a series of investigations on the extraction-spectrophotometric behaviour of Fe-4NC-containing ternary species. The final aim is to develop

sensitive, selective and low-cost methods for iron determination and speciation.

EXPERIMENTAL

Reagents and apparatus

Iron(III) standard solution (1 mg ml^{-1}) was prepared by the procedure [8]: Weigh out 8.6350 g of ferric alum, FeNH$_4$(SO$_4$)$_2$.12H$_2$O, dissolve it in water containing 5 ml of conc. H$_2$SO$_4$, and dilute to volume with water in a 1-1itre standard flask. Working solutions (50 µg ml^{-1}) were obtained by suitable dilution of the stock solution with 0.01 mol l^{-1} H$_2$SO$_4$. Aqueous solutions of the reagents were used: 4NC from Fluka (2x10^{-3}mol l^{-1}) and TTC from LobaFeinchemie GMBH (4x10^{-3}mol l^{-1}). Redistilled chloroform was used. The acidity of the aqueous medium was set by the addition of buffer solution, prepared by mixing 2 mol l^{-1} aqueous solutions of CH$_3$COOH and NH$_4$OH. The resulting pH was checked by HI 83140 pH meter (Italy). A Camspec M508 spectrophotometer (United Kingdom), equipped with 10 mm path-length cells, was used for absorbance measuring.

Procedure for establishing the optimum conditions

Aliquots of Fe(III) solution, 4NC solution (up to 2.0 ml), TTC solution (up to 2.0 ml) and buffer solution (3 ml; pH ranging from 4.1 to 6.1) were introduced into 125-ml separatory funnels. The resulting solutions were diluted with distilled water to a total volume of 10 ml. Then 10 ml of chloroform were added and the funnels were shaken for a fixed time (up to 5.0 min). A portion of each organic extract was transferred through a filter
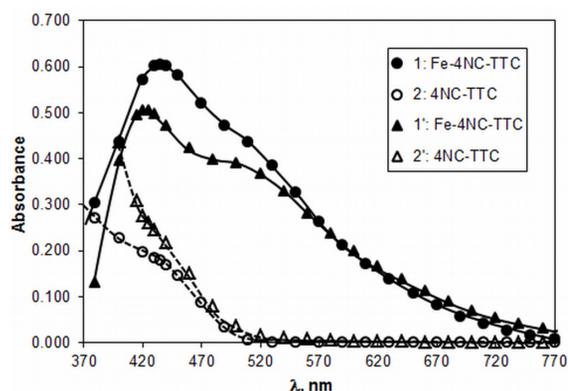
paper into a cell and the absorbance was read against a blank.

Procedure for determining the constant of distribution

The distribution constant K$_D$ was found from the ratio K$_D$ = A$_1$/(A$_3$-A$_1$) where A$_1$ is the light absorbance obtained after a single extraction (at the optimum operating conditions; see Table 1) and A$_3$ is the absorbance obtained after a triple extraction under the same conditions. The final volume of the solutions in both cases was 25 ml [21].

RESULTS AND DISCUSSION

Preliminary observations

Our preliminary investigations showed that the binary Fe(III)-4NC species are not chloroform-extractable. However, Fe(III) is among the interfering ions in the liquid-liquid extraction spectrophotometric determination of various metals (V, Mo, W, etc.) with 4NC and tetrazolium salts [22 - 30]. The observed in our laboratory positive errors under definite extraction-spectrophotometric conditions can be considered an evidence for the formation of chloroform-extractable ternary complexes between the Fe(III)-4NC anionic chelate(s) and the corresponding tetrazolium cations.

Absorption spectra

Spectra of the ternary Fe-4NC-TTC complex in water and chloroform are shown in Fig. 1. Two maxima are clearly visible in aqueous medium (curve 1'): at 420-425 nm and 505-510 nm. After extraction, the first one
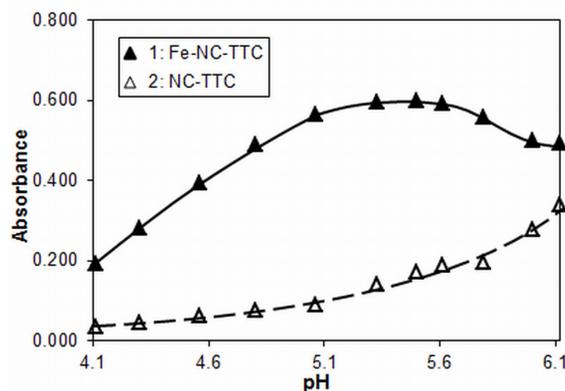

Fig. 1. Absorption spectra of the ternary Fe-4NC-TTC complex and the blank (4NC-TTC) in chloroform (curves 1 and 2) and water (curves 1' and 2'). C$_{Fe(III)}$ = 2.23×10^{-5}mol l^{-1}, C$_{4NC}$ = 2.0×10^{-4}mol l^{-1}, C$_{TTC}$ = 7.2×10^{-4}mol l^{-1}, pH=5.5.

Fig. 2. Absorbance of Fe-4NC-TTC extracts against blank (curve 1) and blank against chloroform (curve 2) as a function of pH, C$_{Fe(III)}$ = 2.23×10^{-5}mol l^{-1}, C$_{4NC}$ = 2.0×10^{-4}mol l^{-1}, C$_{TTC}$ = 7.2×10^{-4}mol l^{-1}, λ=435 nm.
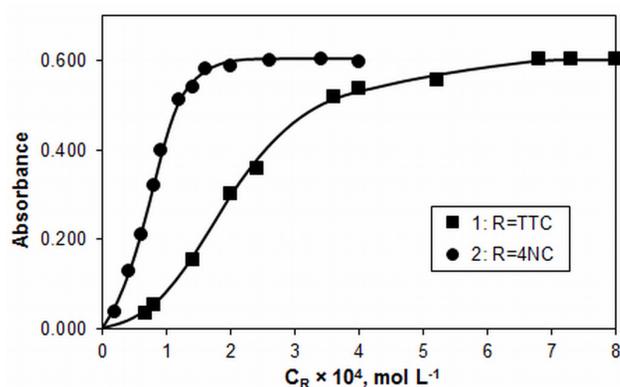
Fig. 3.Absorbance of the extracted ternary complex vs. concentration of the TTC (curve 1) and 4NC (curve 2) plots. 1-$C_{Fe(III)}$ = 2.23×10^{-5}mol l^{-1}, C_{4NC} = 2.0×10^{-4}mol l^{-1}, λ = 435 nm.
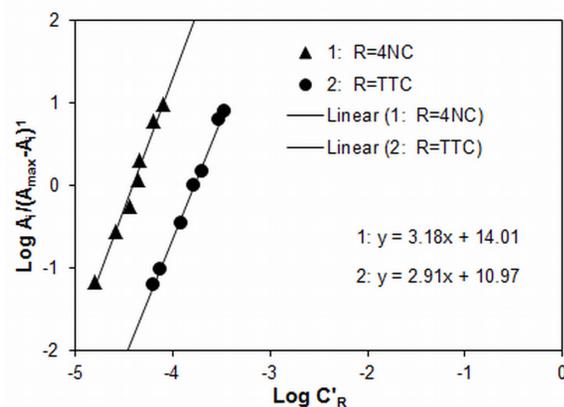

Fig. 4. Determination of the 4NC-to-Fe (straight line 1) andthe TTC-to-Fe (straight line 2) molar ratios by the mobile equilibrium method. 1- $C_{Fe(III)}$ = 2.23×10^{-5}mol l^{-1}, C_{TTC} = 7.2×10^{-4}mol l^{-1}, λ =435 nm.

is shifted to 435 nm and becomes more intensive (curve 1); at the same time, the blank gets lower (compare curves 2 and 2'). All further spectrophotometric measurements were carried out at $λ_{max}$ =435 nm. However, if necessary, measurements could be performed at higher wavelength where the absorbance of blank is practically zero (e.g. 520 nm).

Effect of pH

The effect of pH on the extraction of Fe(III) is shown in Fig. 2. It could be seen that the optimum pH interval is 5.3 - 5.6 (curve 1). The shape of the obtained curve is governed by the following two factors: 1) protonated 4NC (H_2L, HL^-) species [11, 12, 31] and cationic FeL^+ species [12, 19] predominate at pH values lower than pH_{opt}; 2) hydrolysed Fe(III) species exert noticeable effects on the complex formation at pH values higher than pH_{opt}[9, 32]. It should be mentioned that the absorption

of the blank increases rapidly with the increasing of pH (curve 2). This fact could be attributed to the increase of the HL^-/H_2L ratio (which results in the extraction of a coloured salt-like compound between HL^- and TT^+ [28]).

Effect of reagents concentration

The effect of 4NC and TTC concentrations on the absorbance is shown in Fig. 3. It could be accepted that 9-fold 4NC excess and 32-fold TTC excess are sufficient for maximum Fe(III) extraction. The optimum reagents concentrations are shown in Table 1.

Effect of shaking time

The extraction equilibrium was reached for ca. 1 min. A longer shaking time (up to 5 min) had no effect on the absorbance. In order to avoid accidental errors, caused by the combination of short shaking times and

Table 1.Optimum extraction-spectrophotometric conditions and some analytical characteristics of the system of Fe(III), 4NC, TTC, water and chloroform.

Optimum extraction – spectrophotometric conditions	Analytical characteristics
$λ_{max}$ 435 nm	Molar absorptivity: ε_{435}=2.7×10^4 l mol^{-1} cm^{-1}
pH: 5.5 (acetate buffer)	Sandell's sensitivity: 2.0 ng cm^{-2}
C_{4NC}: 2.0×10^{-4}mol l^{-1}	Range of linearity of the analytical curve: up to 2.0 µg ml^{-1}
C_{TTC}: 7.2×10^{-4}mol l^{-1}	Limit of detection: 0.11 µg ml^{-1}
Shaking time: 2 min	Limit of quantification: 0.37 µg ml^{-1} Recovery factor: R=(94.3±0.2)%

Table 2. Calculated values of Log β, Log K_D and Log K_{ex}

Equilibrium	Equilibrium constant	Value
		Log β=11.3±0.1[a]
Eq. 1	β=[(TT$^+$)$_3$[Fe(4NC)$_3$]] / [TT$^+$]^3× [[Fe(4NC)$_3$]$^{3-}$]	Log β=11.3±0.1[b]
		Log β=11.0±0.2[c]
Eq. 2	K_D=[(TT$^+$)$_3$[Fe(4NC)$_3$]]$_{org}$ / [(TT$^+$)$_3$[Fe(4NC)$_3$]]$_{aq}$	Log K_D=1.49±0.02
Eq. 3	K_{ex}=[(TT$^+$)$_3$[Fe(4NC)$_3$]]$_{org}$ / [TT$^+$]$^3_{aq}$ × [[Fe(4NC)$_3$]$^{3-}$]$_{aq}$	Log K_{ex}=12.8±0.1[d]

a – Calculated by the Holme-Langmyhr method (N = 5, P = 95 %)
b – Calculated by the Harvey-Manning method (N = 3, P = 95 %)
c – Calculated by the mobile equilibrium method (N = 7, P = 95 %)
d – Calculated by the formula $K_{ex}=K_D×β$, where β is determined by the Holme-Langmyhr method and Harvey-Manning method.

different shaking rates, 2 min shaking time was used in further experiments.

Composition of the complex and suggested formula

The molar 4NC-to-Fe(III) and TTC-to-Fe(III) ratios were determined on the basis of the saturation curves presented in Fig. 3. Two different methods were used: the mobile equilibrium method [33] (Fig. 4) and the straight-line method of Asmus [34].

The results of both methods showed that the extracted ternary complex has a composition of 1:3:3 (Fe:4NC:TTC). Having in mind the possibility of formation of a tris-4-nitrocatecholato anionic complex in aqueous medium [Fe(4NC)$_3$]$^{3-}$[12, 19] and the ion-association properties of 2,3,5-triphenyl-2H-tetrazolium cation (TT$^+$)[11], we suggest the following formula of the extracted ternary species: (TT$^+$)$_3$[Fe^{3+}(4NC)$_3$]$^{3-}$. In this formula 4NC is in its doubly deprotonated form (4NC^{2-}) and the iron ion save its initial +3 oxidation state.

According to the literature [12], [Fe^{3+}(4NC)$_3$]$^{3-}$ species predominates over [Fe^{3+}(4NC)$_2$]$^-$ species in aqueous medium at pH>6.7.

The formation of (TT$^+$)$_3$[Fe^{3+}(4NC)$_3$]$^{3-}$ at lower pH (∼ 5.5) could be explained with displacement of the equilibrium [FeL$_2$]$^-$ + HL$^-$ ⟷ [FeL$_3$]$^{3-}$ + H$^+$to the right due to the formation of an extractable ternary complex, (TT$^+$)$_3$[Fe(4NC)$_3$], which leaves the aqueous phase.

Equilibrium constants and recovery

Several equilibrium processes should be taken into account for the system of [Fe(4NC)$_3$]$^{3-}$, TT$^+$, water and chloroform.
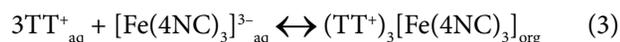
(i) Formation of an ion-association complex in the aqueous phase:

$$3TT^+ + [Fe(4NC)_3]^{3-} \longleftrightarrow (TT^+)_3[Fe(4NC)_3] \qquad (1)$$
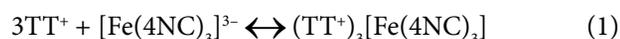
(ii) Distribution of the complex between the aqueous and the organic phase:

$$(TT^+)_3[Fe(4NC)_3]_{aq} \longleftrightarrow (TT^+)_3[Fe(4NC)_3]_{org} \qquad (2)$$

(iii) Extraction from water into chloroform:

$$3TT^+_{aq} + [Fe(4NC)_3]^{3-}_{aq} \longleftrightarrow (TT^+)_3[Fe(4NC)_3]_{org} \qquad (3)$$

The equilibrium constants describing these equations and the obtained values are shown in Table 2. The association constant β was determined by several independent methods: Holme-Langmyhr method [35], Harvey-Manning method [36] and mobile equilibrium method [33] (Fig. 4, straight line 2). The distribution constant K_D was calculated from the absorption values obtained after single and triple extraction as described above. The extraction constant (K_{ex}) and recovery fac-

tor (R %, see Table 1) were calculated by the formulae $K_{ex}=K_D\times\beta$ and $R=K_D\times100/(K_D+1)$, respectively. All experiments were performed at room temperature of $\sim 22°C$ and the calculations were carried out at a probability of 95 %.

Calibration curve and analytical characteristics

The dependence between the Fe(III) concentration in aqueous medium and the light absorbance of the chloroform extract was studied at the optimum conditions (Table 1). A good linearity was observed up to 2.0 μg ml^{-1}Fe(III) (r=0.9990). The obtained straight line equation was y=0.4709x+0.0169. Other important analytical characteristics of the studied in the present work system are included in Table 1. It should be mentioned that the molar absorptivity, 2.7×10^4 l mol^{-1} cm^{-1}, is higher than the molar absorptivities achieved in the iron-containing extraction-chromogenic systems [9, 37] based on complex formation with 2,3-dihydroxynaphtalene – cetyltrimethyl ammonium bromide [9] and TTC reduction in alkaline medium [37] (*ca.* 2.7 times and 12 times, respectively).

CONCLUSIONS

Iron(III) forms a ternary complex with 4-nitrocatechol and 2,3,5-triphenyltetrazolium chloride. The complex consists of an intensively coloured anion, [Fe(4NC)$_3$]$^{3-}$,and bulky hydrophobic cations, TT$^+$, which ensure a complete transfer of the ternary compound into organic phase. The calculated equilibrium constants and analytical characteristics (constant of extraction, constant of association, constant of distribution, recovery factor, molar absorptivity, Sandell's sensitivity, limit of detection and limit of quantification) show that the studied in the present work extraction-chromogenic system could compete successfully with similar systems used for determination of Fe.

REFERENCES

1. K.K. Chatterjee, Uses of Metals and Metallic Minerals, New Delhi, New Age International (P) Ltd. Publishers, 2007.

2. C. Bolm, A new iron age, Nat. Chem., 1, 2009, 420-420.

3. B. Borch-Iohnsen, Perspective. Determination of iron status: brief review of physiological effects on iron measures, Analyst, 120, 1995, 891-893.

4. J. Miller, Iron Toxicity: Onset and Emergency Treatment, Emsworld,2006, http://www.emsworld.com/article/10322675/iron-toxicity-onset-and-emergency-treatment

5. N.Hubert, M.W. Hentze, Previously uncharacterized isoforms of divalent metal transporter (DMT)-1: Implications for regulation and cellular function. Proc. Nat. Acad. Sci., 99, 2002, 12345-12350.

6. L. Sommer, M. Bartusek, Complexation of phenolic hydroxyl and its analytical consequences, Folia Fac. Sci. Nat. UJEP Brno Chem., 7, 1966, 46-63.

7. T. Kawabata, V. Schepkin, N. Haramaki, R. S. Phadke, L. Packer, Iron coordination by catechol derivative antioxidants, Biochem. Pharmacol., 51, 1996, 1569-1577.

8. Z. Marczenko, M. Balcerzak, Separation, preconcentration and spectrophotometry in inorganic analysis, Analytical Spectroscopy Library, Vol. 10, Amsterdam - Lausanne - New York - Oxford - Shannon - Tokyo, Elsevier, 2000.

9. P.K. Tarafder, R.K. Mondal, Micelle Mediated Extraction of Iron and Its Determination in Geological, Geochemical, Hydrogeochemical, Biogeochemical and Process Solutions, Am. J. Anal. Chem., 3, 2012, 339-346.

10. L. Sommer, G. Ackermann, D. T. Burns, S. B. Savvin, Present and future status of organic analytical reagents-Part I: General remarks, Pure Appl. Chem., 62, 1990, 2147-2166.

11. K.B. Gavazov, Nitroderivatives of catechol: from synthesis to application, Acta Chim. Slov., 59, 2012, 1-17.

12. V. M. Nurchi, T. Pivetta, J. I. Lachowicz, G. Crisponi, Effect of substituents on complex stability aimed at designing new iron(III) and aluminum(III) chelators, J. Inorg. Biochem., 103, 2009, 227-236.

13. G. Crisponi, M. Remelli, Iron chelating agents for the treatment of iron overload, Coord. Chem. Rev., 252, 2008, 1225-1240.

14. E. Skrzypczak-Jankun, O.Y. Borbulevych, J. Jankun, Soybean lipoxygenase-3 in complex with 4-nitro-

catechol, Acta Cryst. Sec. D, 60, 2004, 613-615.

15. L. J. M. Spaapen, J. Verhagen, G. A. Veldink, J. F. G. Vliegenthart, Properties of a complex of Fe(III)-soybean lipoxygenase-1 and 4-nitrocatechol, Biochim. Biophys. Acta, 617, 1980, 132-140.

16. C.A. Tyson, 4-Nitrocatechol as a colorimetric probe for non-heme iron dioxygenases, J. Biological Chem., 250, 1975, 1765-1770.

17. D. Vasudevan, A.T. Stone, Adsorption of 4-Nitrocatechol, 4-Nitro-2-Aminophenol, and 4-Nitro-1,2-Phenylenediamine at the Metal (Hydr)Oxide/Water Interface: Effect of Metal (Hydr)Oxide Properties, J. Coll. Interf. Sci., 202, 1998, 1-19.

18. G. W. Luther III, T. F. Rozan, A. Witter, B. Lewis, Metal–organic complexation in the marine environment, Geoch. Trans., 2, 2001, 65.

19. P. Hakkinen, Studies on the formation of manganese (II), iron (III), cobalt (II), and nickel (II) complexes of 1, 2 dihydroxy-4-nitrobenzene in aqueous solution, Finn. Chem. Lett., 3, 1984, 59-62.

20. K.B. Gavazov, A.N. Dimitrov, V.D. Lekova, The use of tetrazolium salts in inorganic analysis, Uspekhi Khim., 76, 2007, 187-198.

21. K. Gavazov, V. Lekova, A. Dimitrov, Extraction studies on the system vanadium(V) – 4-(2-thiazolylazo)-resorcinol – neotetrazolium chloride – water – chloroform, J. Univ. Chem. Technol. Met. (Sofia), 41, 2, 2006, 217-220.

22. Z. Simeonova, K. Gavazov, A. Alexandrov, Ternary complex of vanadium(V) with 4-nitrocatechol and tetrazolium salt and its application to extraction-spectrophotometric determination of vanadium(V) in steels, Anal. Lab., 7, 1998, 184-188.

23. A. Dimitrov, V. Lekova, K. Gavazov, B. Boyanov, Investigation of the extraction equilibrium of ion-association complexes of molybdenum(VI) with some polyphenols and thiazolyl blue. Extraction-spectrophotometric determination of molybdenum, Cent. Eur. J. Chem., 3, 2005, 747-755.

24. V. Lekova, K. Gavazov, A. Dimitrov, A ternary complex of tungsten(VI) with 4-nitrocatechol and thiazolyl blue and its application for extraction-spectrophotometric determination of tungsten, Chem. Papers, 60, 2006, 283-287.

25. A. Dimitrov, V. Lekova, K. Gavazov, B. Boyanov, Ternary complex of molybdenum(VI) with 4-nitrocatechol and tetrazolium blue chloride and its ap-

plication to extraction-spectrophotometric analysis of ferrous metallurgy products, J. Anal. Chem., 62, 2007, 122-125.

26. P. V. Racheva, K. B. Gavazov, V. D. Lekova, A. N. Dimitrov, Extraction equilibria and spectrophotometric determination of vanadium(V) with 4-nitrocatechol and the ion-pair reagent thiazolyl blue tetrazolium, J. Anal. Chem., 65, 2010, 21-25.

27. P. Racheva, K. Gavazov, V. Lekova, A. Dimitrov, Vanadium(IV,V) Speciation with a Catechol Derivative and a Tetrazolium Salt, J. Iranian Chem. Res., 1, 2008, 113-121.

28. K. Gavazov, V. Lekova, A. Dimitrov, Complex formation and extraction in the vanadium(IV) – 4-nitrocatechol – neotetrazolium chloride – water – organic solvent system, National Scientific Conference with International Participation under the heading "20 Years Union of Scientists in Bulgaria – Branch Smolyan", Scientific papers, Special Jubilee Edition, Smolyan, Bulgaria, 2006, 808-819.

29. P. Racheva, V. Lekova, T. Stefanova, A. Dimitrov, K. Gavazov, Complex formation and liquid-liquid extraction in the niobium(V) – 4-nitrocatechol – thiazolyl blue tetrazolium system, University of Plovdiv "Paissii Hilendarski"-Bulgaria, Scientific works. Chemistry, 37, 2010, 33-41.

30. V. Lekova, P. Racheva, K. Stojnova, A. Dimitrov, K. Gavazov, Ternary complexes of niobium(V) with nitroderivatives of catechol and tetrazolium salts. Extraction-spectrophotometric investigations, Chemija, 21, 2010, 106-111.

31. J.-P. Cornard, Rasmiwetti, J.-C. Merlin, Molecular structure and spectroscopic properties of 4-nitrocatechol at different pH: UV–visible, Raman, DFT and TD-DFT calculations, Chem. Phys., 309, 2005, 239-249.

32. A. Stefánsson, , Iron(III) Hydrolysis and Solubility at 25°C, Environmental Science & Technology, 41, 2007, 6117-6123.

33. Z. Zhiming, M. Dongsten, and Y. Cunxiao, Mobile equilibrium method for determining composition and stability constant of coordination compounds of the form M_mR_n, J. Rare Earths, 15, 1997, 216-219.

34. E. Asmus, Eine neue Methode zur Ermittlung der Zusammensetzung schwacher Komplexe, Fresenius' J. Anal. Chem., 178, 1960, 104-116.

35. A. Holme, F.J. Langmyhr, A modified and a new straight-line method for determining the composi-

tion of weak complexes of the form A_mB_n, Anal. Chim. Acta, 36, 1966, 383-391.

36. A.E. Harvey, D.L. Manning, Spectrophotometric Methods of establishing empirical formulas of colored complexes in solution, J. Am. Chem. Soc., 72, 1950, 4488-4493.

37. M.C. Mehra, M. Katyal, Use of tetrazolium salts as selective and sensitive reagents for iron in environmental samples, Bull. Environ. Contam. Toxicol., 30, 1983, 337-343.