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Ion-Association Between Some Ditetrazolium Cations and Anions Originated from 4-(2-Thiazolylazo)resorcinol or 4-(2-Pyridylazo)resorcinol[1]

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Abstract—Ion-associates between cations originating from ditetrazolium salts [DT: Neotetrazolium chloride (NT), Blue Tetrazolium chloride (BT), Nitro Blue Tetrazolium chloride (NBT), and Tetranitro Blue Tetrazolium chloride (TNBT)] and anions originating from azo compounds [AC: 4-(2-pyridylazo)resorcinol (H_2PAR) and 4-(2-thiazolylazo)resorcinol (H_2TAR)] were studied by water-chloroform extraction and spectrophotometry. Some key characteristics of the extracted compounds were determined. They can be expressed by different formulae depending on the ACs and DTs used: (1) $(DT^+)(HPAR^-)$ (for DT = BT, NBT or TNBT); (2) $(DT^{2+})(HTAR^-)_2$ (for DT = BT, NBT or TNBT); and (3) $(DT^+)_2(HAC^-)_2$ (for DT = NT).

Keywords: bis-tetrazolium salts, azo dyes, spectrophotometry, liquid–liquid extraction, ion-pair

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INTRODUCTION

2,3,5-Substituted mono- and ditetrazolium salts attract the researchers' attention not only due to their propensity to be converted to colored formazans under the action of various agents [1–5], but also due to their ability to generate bulky cations that are prone to associate with some anionic species [6–8]. The ion-associates thus formed are hydrophobic and can be utilized in various liquid-liquid extraction processes [7–14]. Their spectral properties are usually a combination of those of the constituent ions, especially when inorganic anions are involved [7]. However, different spectral characteristics (wavelength of maximum absorption, intensity and half width of the spectral line) have been observed for tetrazolium ion-associates with anionic chelate species [7, 15]. In order to explain the spectral differences and propose correct formulae of the obtained compounds there should be taken into account various processes: hydrolysis of the metal ion, oxidation/reduction, aggregation, formation of chelates with fully and/or partially deprotonated ligands, "intrusion" of anions, etc. [16-19]. The extraction-chromogenic systems containing bis-tetrazolium salts (ditetrazolium salts, DTs) are especially complicated, because it is not always clear whether the involved DTs act as monocations (DT^+) or dications (DT^{2+}) in the structures containing those [20].

In the publication [16] we have reported studies on water-chloroform systems containing monotetrazolium salts (MTs) and azocompounds (ACs), namely 4-(2-thiazolylazo)resorcinol (TAR, H_2TAR) and 4-(2-pyridylazo)resorcinol (PAR, H_2PAR). Elucidation of the processes taking place in the systems has been important because they govern the absorbance of the blank samples recorded in the course of extraction-spectrophotometric determination of metal ions [7, 9, 14, 21]. Interesting results initiated our decision to expand the scope of study of similar systems that involved DTs. Four widely used DTs were selected for the present study: (1) 3,3'-(4,4'-biphenylene)bis(2,5-diphenyl-2*H*-tetrazolium chloride) (Neotetrazolium chloride, NT); (2) 3,3'-(3,3'-dimetoxy-4,4'-biphenylene)bis(2,5-diphenyl-2H-tetrazolium chloride) (Blue Tetrazolium chloride, BT); (3) 3,3'-(3,3'-dimetoxy-4,4'-biphenylene)bis[2-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride] (Nitro Blue Tetrazolium chloride, NBT); and (4) 3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-bis[2,5-di(4-nitrophenyl)-2*H*-tetrazolium chloride] (Tetranitro Blue Tetrazolium chloride, TNBT).

[1] The text was submitted by the authors in English.

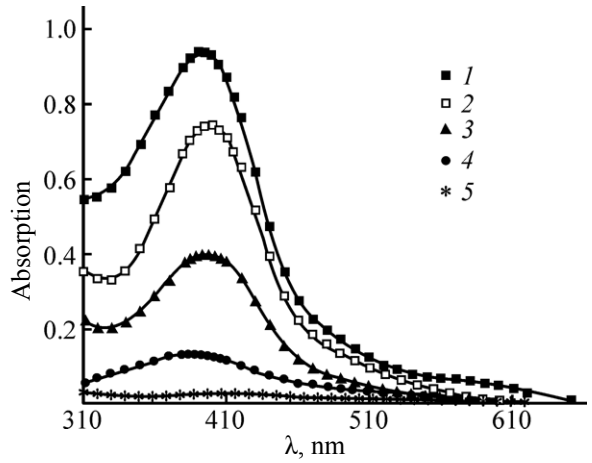

Fig. 1. Absorption spectra of chloroform extracts of DT-PAR ion-associates (curves *1–4*) against a blank (curve *5*; PAR). pH 9, $c_{PAR} = 5 \times 10^{-5}$ mol dm^{-3}, $c_{DT} = 2 \times 10^{-4}$ mol dm^{-3}. (*1*) NT-PAR, (*2*) BT-PAR, (*3*) NBT-PAR, (*4*) TNBT-PAR, and (*5*) PAR.

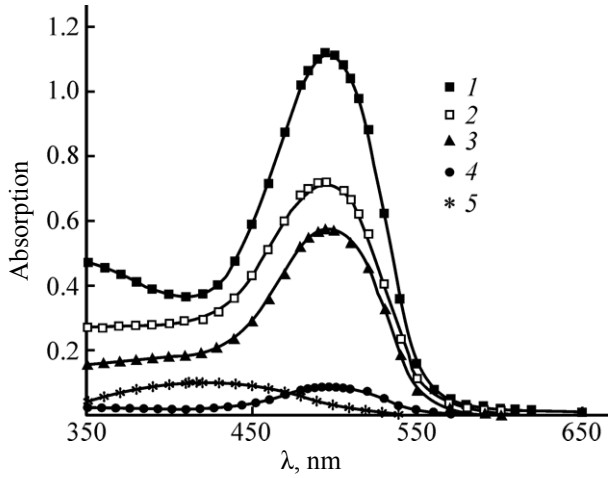

Fig. 2. Absorption spectra of chloroform extracts of DT-TAR ion-associates (curves *1–4*) against a blank (curve *5*; TAR). pH 9, $c_{TAR} = 5 \times 10^{-5}$ mol dm^{-3}, $c_{DT} = 2 \times 10^{-4}$ mol dm^{-3}. (*1*) NT-TAR, (*2*) BT-TAR, (*3*) NBT-TAR, (*4*) TNBT-TAR, and (*5*) TAR.

RESULTS AND DISCUSSION

Several optically different AC species (PAR and TAR): H_4PAR^{2+}, H_3AC^+, H_2AC, HAC^- and AC^{2-} are pH dependent in water solutions [22–24]. Upon chloroform extraction of these ACs [22] H_2PAR and H_2TAR pass into organic phase at pH 3.5-5 and 1.5-5, respectively. L. Marić, and M. Široki [22] pointed out that the concentrations of the neutral species were negligible at pH 9 [22], hence ACs could not be extracted in chloroform alone. However, ACs tended to form chloroform extractable ion-pairs in broad pH range when cationic ion-association reagents {e.g. tetraphenylphosphonium chloride (Ph_4PCl) [22], tetraphenylarsonium chloride (Ph_4AsCl) [22], and MTs [16]} were present in the solution.

Spectral characteristics at pH 9. The visible range spectra of the AC-DT compounds extracted under identical conditions (pH 9, $c_{AC} = 5.0 \times 10^{-5}$ mol dm^{-3}, and $c_{DT} = 2.0 \times 10^{-4}$ mol dm^{-3}) are presented in Fig. 1 (AC = PAR) and Fig. 2 (AC = TAR). Spectral

Table 1. Molar absorptivities (ε_{max}) and absorption maxima (λ_{max}) of extracted DT-AC and MT-AC compounds

	DT-AC compound			MT-AC compound [16]			
AC	DT	λ_{max}, nm	$\varepsilon_{max} \times 10^{-4}$, dm^3 mol^{-1} cm^{-1}	MT[a]	λ_{max}, nm	$\varepsilon_{max} \times 10^{-4}$, dm^3 mol^{-1} cm^{-1}	Formula
PAR	NT	390	3.79	TT	400	2.29	$(TT^+)(HPAR^-)$
	BT	394	2.82	MTT	385	3.50	$(MTT^+)(HPAR^-)$
	NBT	395	1.73	TV	399	3.56	$(TV^+)(HPAR^-)$
	TNBT	395	0.33	INT	388	3.93	$(INT^+)_2(HPAR^-)_2$
TAR	NT	496	2.86	TT	497	2.98	$(TT^+)(HTAR^-)$
	BT	495	2.08	MTT	489	2.96	$(MTT^+)(HTAR^-)$
	NBT	495	1.95	TV	496	3.21	$(TV^+)(HTAR^-)$
	TNBT	497	0.26	INT	482	1.96	$(INT^+)(HTAR^-)$

[a] Full names of the abbreviated monotetrazolium salts (MT): (TT) 2,3,5-triphenyl-2*H*-tetrazolium chloride; (MTT) 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2*H*-tetrazolium bromide; (TV) 3-(2-naphtyl)-2,5-diphenyl-2*H*-tetrazolium chloride; and (INT) 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride.

Table 2. Stoichiometric coefficients (*n* and *m*) determined by the mobile equilibrium method [25] and corresponding charges (z^+) of the ditetrazolium cations

HAC^- DT^{z+}	$HPAR^-$				$HTAR^-$			
	slopes for different *n* and *m*	stoichiometric coefficients found		z^+	slopes for different *n* and *m*	stoichiometric coefficients found		z^+
		n	*m*			*n*	*m*	
NT^{z+}	1.30 (*n* = 1, *m* = 1)	2	2	1	1.29 (*n* = 1, *m* = 1)	2	2	1
	2.04 (*n* = 1, *m* = 2)				2.21 (*n* = 1, *m* = 2)			
	2.00 (*n* = 2, *m* = 2)				2.07 (*n* = 2, *m* = 2)			
BT^{z+}	1.00 (*n* = 1, *m* = 1)	1	1	1	0.52 (*n* = 1, *m* = 1)	1	2	2
	1.47 (*n* = 1, *m* = 2)				1.04 (*n* = 1, *m* = 2)			
	1.42 (*n* = 2, *m* = 2)				0.71 (*n* = 2, *m* = 2)			
NBT^{z+}	1.00 (*n* = 1, *m* = 1)	1	1	1	0.67 (*n* = 1, *m* = 1)	1	2	2
	1.29 (*n* = 1, *m* = 2)				1.06 (*n* = 1, *m* = 2)			
	1.27 (*n* = 2, *m* = 2)				0.96 (*n* = 2, *m* = 2)			
$TNBT^{z+}$	1.04 (*n* = 1, *m* = 1)	1	1	1	0.60 (*n* = 1, *m* = 1)	1	2	2
	1.41 (*n* = 1, *m* = 2)				1.01 (*n* = 1, *m* = 2)			
	1.37 (*n* = 2, *m* = 2)				0.88 (*n* = 2, *m* = 2)			

characteristics (λ_{max} and ε_{max}) obtained at *i*-fold DT excesses (which are sufficient for maximum AC extraction; see below) are listed in Table 1. The following comments can be made regarding the figures, table, and published data [16, 22]:

(1) The recorded absorption maxima are quite close to those reported by us (for the ion-pairs of MTs^+ [16]) and Marić and Široki (for the ion-pairs of Ph_4P^+ and Ph_4As^+) [22]. The position of λ_{max} in all systems convincingly indicates that both ACs participate in the extracted species in the form of monoanions ($HPAR^-$ and $HTAR^-$). The dianionic AC species (PAR^{2-} and TAR^{2-}) have different spectral characteristics and they dominate at pH > 11.9 (for PAR) and 9.44 (for TAR) [22] in which DTs are unstable and tend to be reduced to formazans.

(2) In contrast to the systems with MTs [16], the molar absorptivities follow the same sequence for both ACs: $\varepsilon_{AC-NT} > \varepsilon_{AC-BT} > \varepsilon_{AC-NBT} > \varepsilon_{AC-TNBT}$.

Saturation curves, composition and stability of the extracted species. Relationship between the absorbance of extracted ion-associates and concentration of DT was studied at pH 9 and constant AC concentration ($c_{AC} = 5.0 \times 10^{-5}$ mol dm^{-3}). The following DT *i*-fold excesses (in parenthesis) appeared to be sufficient for maximum AC extraction: (1) AC=PAR: NT (30), BT (25), NBT (25), and TNBT (15); (2) AC = TAR: NT (25), BT (25), NBT (30), and

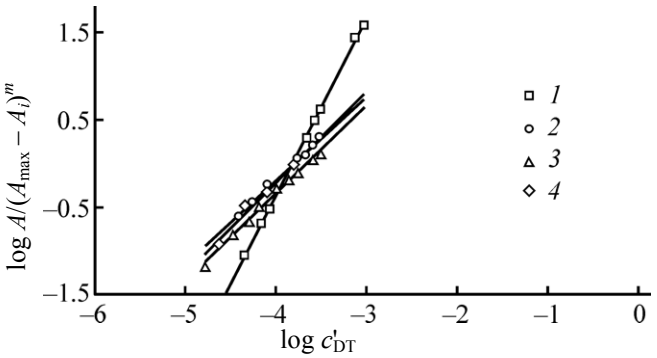

Fig. 3. Straight lines obtained by the mobile equilibrium method for $m = 1$ (lines *2–4*) and $m = 2$ (line *1*). $c_{PAR} = 5 \times 10^{-5}$ mol dm^{-3}, pH = 9. Straight line equations: (*1*) $y = 2.00x + 7.64$ ($R^2 = 0.998$); NT : PAR = 2 : 2, (*2*) $y = 1.00x + 3.80$ ($R^2 = 0.997$); BT : PAR = 1 : 1, (*3*) $y = 1.00x + 3.37$ ($R^2 = 0.986$); NBT : PAR = 1 : 1, and (*4*) $y = 1.04x + 3.94$ ($R^2 = 0.987$); TNBT : PAR = 1 : 1.

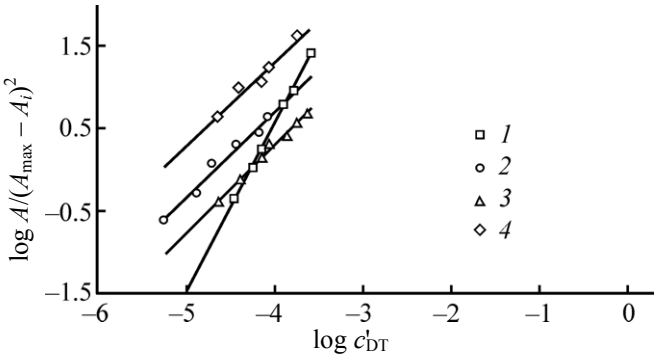

Fig. 4. Straight lines obtained by the mobile equilibrium method for $m = 2$. $c_{TAR} = 5 \times 10^{-5}$ mol dm^{-3}, pH = 9. Straight line equations: (*1*) $y = 2.07x + 8.86$ ($R^2 = 0.995$); NT : TAR = 2 : 2, (*2*) $y = 1.04x + 4.87$ ($R^2 = 0.980$); BT : TAR = 1 : 2, (*3*) $y = 1.06x + 4.52$ ($R^2 = 0.990$); NBT : TAR = 1 : 2, and (*4*) $y = 1.01x + 5.32$ ($R^2 = 0.974$); TNBT : TAR = 1 : 2.

TNBT (15). The saturation curves were used to determine the composition and stability of the DT-AC species by the mobile equilibrium method [25] recognized by giving reliable results for compounds of the type A_nB_m, where $n = m$ or $n \neq m$.

The following ion-association process was considered: $nDT^{z+} + mHAC^- \leftrightarrow (DT^{z+})_n(HAC^-)_m$.

Upon determining the stoichiometric coefficients n and m (Table 2, Figs. 3 and 4) the values of z^+ were figured out (Table 2). On the basis of the resulting data the following formulae of the individual DT^{z+}–HAC^- compounds: $(NT^+)_2(HPAR^-)_2$, $(NT^+)_2(HTAR^-)_2$, $(BT^+)(HPAR^-)$, $(BT^{2+})(HTAR^-)_2$, $(NBT^+)(HPAR^-)$, $(NBT^{2+})(HTAR^-)_2$, $(TNBT^+)(HPAR^-)$, and $(TNBT^{2+})\cdot(HTAR^-)_2$ were elucidated. The Holme–Langmyhr [26] and the Harvey–Manning [27] methods were used as alternative approaches to constants of association (β) of the monomers (i.e. compounds with the composition different from 2 : 2) (Table 3).

EXPERIMENTAL

All reagents were purchased from Sigma–Aldrich Chemie GmbH: PAR (96%), TAR (97%), NT and BT ("for microbiology" grade), NBT (98%), and TNBT (≥85%). The corresponding solutions ($c = 2 \times 10^{-3}$ mol dm^{-3} and/or 5×10^{-4} mol dm^{-3}) were prepared in distilled water (slightly alkalized with KOH when ACs solutions were prepared). Buffer solutions, pH 9, were prepared daily by mixing 2 mol dm^{-3} aqueous solutions of CH_3COOH and NH_4OH. The actual pH was verified by a Hanna HI-83140 pH meter. Redistilled chloroform was used. The absorbance data were measured with a Camspec M508 spectrophotometer (UK), equipped with 10 mm path-length cells.

Procedure. 1 mL of the NH_4OH–CH_3COOH solution and aliquots of AC solution (PAR or TAR) and DT solution (NT, BT, NBT or TNBT) were placed

Table 3. Constants of associations (β) calculated for a probability of 95%

Ion-associates of $HPAR^-$	log β	Ion-associates of $HTAR^-$	log β
$(NT^+)_2(HPAR^-)_2$	11.9±0.1[a]	$(NT^+)_2(HTAR^-)_2$	12.7±0.2[a]
$(BT^+)(HPAR^-)$	3.8±0.1[a]; 3.9±0.1[b]; 3.9±0.1[c]	$(BT^{2+})(HTAR^-)_2$	8.7±0.2[a]; 8.7±0.2[c]
$(NBT^+)(HPAR^-)$	3.7±0.2[a]; 3.7±0.1[b]; 3.6±0.1[c]	$(NBT^{2+})(HTAR^-)_2$	8.3±0.2[a]; 8.3±0.1[c]
$(TNBT^+)(HPAR^-)$	3.8±0.2[a]; 3.8±0.1[b]; 3.7±0.1[c]	$(TNBT^{2+})(HTAR^-)_2$	8.4±0.3[a]; 8.3±0.2[c]

[a] Calculated by the mobile equilibrium method. [b] Calculated by the Holme–Langmyhr method. [c] Calculated by the Harvey–Manning method.

into 100-cm^3 separatory funnels. The volumes were adjusted to 5 cm^3 with distilled water. 5 cm^3 of chloroform were added. The funnels were shaken for 2 min. Upon separation of the layers, the organic extracts were filtered into cells. The absorbance was measured against chloroform or blank containing AC.

CONCLUSIONS

$HACs^-$ form ion-associates with DTs^{z+} (z = 1 or 2) that can be expressed by different formulae depending on the individual ACs and DTs used. PAR and TAP, that are reagents with similar potential to form ion-associates, can behave differently depending on the nature of the substituents that they contain (pyridyl- or thiazolyl-). The present study demonstrates for the first time that DTs act as monocations (z = 1) in the processes of ion-association. This seems to be consistent with the Tôei's conception of low charge (z = 1) of the ion-associable ions [28]. However, $HTAR^-$ forms ion-associates with participation of DT^{2+}: $(BT^{2+})(HTAR^-)_2$, $(NBT^{2+})(HTAR^-)_2$, and $(TNBT^{2+})(HTAR^-)_2$. This, in turn, is in agreement with the reviews [6–8, 10] that denote the participation of DTs as DTs^{2+} in the ion-associates.

The accumulated results reveal that the ion-associates deriving from NT (the DT which does not contain polar substituents, such as $–NO_2$ and $–OCH_3$) are dimers of the types: $(NT^+)_2(HPAR^-)_2$ and $(NT^+)_2(HTAR^-)_2$. However, the absence of polar substituents in the NT is not the main reason for dimerization, since such a phenomenon has been observed in the system PAR–2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride [16].

The current study data offer an alternative explanation of some debatable experimental results concerning the composition of ternary complexes involving PAR and DTs [19, 29, 30]. These can lead to better understanding of processes taking place in many ion-association systems.

REFERENCES

1. Sabnis, R.W., *Handbook of Biological Dyes and Stains: Synthesis and Industrial Applications*, Hoboken: Wiley, 2010.
2. Shugalei, I.V., Ivanova, A.A., Ilyushin, M.A., Tselinskii, I.V., and Sokolova, V.V., *Russ. J. Gen. Chem*., 2010, vol. 80, no. 4, p. 829. DOI: 10.1134/s1070363210040250.
3. Şenöz, H., *Hacettepe J. Biol. Chem*., 2012, vol. 40, no. 3, p. 293.
4. Zhivich, A.B., Koldobskii, G.I., and Ostrovskii, V.A., *Chem. Heterocycl. Comp*., 1990. vol. 26, no. 12, p. 1319. DOI: 10.1007/bf00473958
5. Pikaev, A.K. and Kriminskaya Z.K., *Russ. Chem. Rev.*, 1998, vol. 67, no. 8, p. 671. DOI: 10.1070/RC1998v067n08ABEH000392.
6. Alexandrov, A., *Tertazolium Salts for Solvent Extraction of Elements as Ion Association Complexes. Review*, Saarbrücken: Universität des Saarlandes, 1984.
7. Gavazov, K.B., Dimitrov, A.N., and Lekova, V.D., *Russ. Chem. Rev.*, 2007, vol. 76, no. 2, p. 169. DOI: 10.1070/RC2007v076n02ABEH003655.
8. Kamburova, M., *Doctoral (Chem.) Dissertation*, Sofia, 1999.
9. Dimova, L.M. and Morgen, E.A., *Zavod. Lab*., 1984, vol. 50, no. 10, p. 7.
10. Alexandrov, A., *Doctoral (Chem.) Dissertation,* Sofia 1987.
11. Bashammakh, A.S., *E-Journal of Chemistry*, 2011, vol. 8, no. 3, p. 1462.
12. Kostova, D.V. and Kamburova M.A., *J. Int. Sci. Publ. Ecol. Saf.*, 2010, vol. 4, no. 1, p. 162.
13. Boggs, M., Gribat, L., Boele, C., and Wall, N., *J. Radioanal. Nucl. Chem*., 2012, vol. 293, no. 3, p. 843. DOI: 10.1007/s10967-012-1744-8.
14. Stefanova, T.S., Simitchiev, K.K., and Gavazov, K.B., *Chem. Pap.*, in press. DOI: 10.1515/chempap-2015-0048.
15. Gavazov, K.B., Racheva, P.V., Lekova, V.D., Dimitrov, A.N., Turkyilmaz, M., and Genc, F., *Croat. Chem. Acta*, 2012, vol. 85, no. 1, p. 53. DOI: 10.5562/cca1885.
16. Gavazov, K.B. and Toncheva, G.K., *J. Advances in Chemistry*, 2013, vol. 5, no. 2, p. 641.
17. Gavazov, K.B., *Chemistry*, 2013, vol. 22, no. 2, p. 222.
18. Fletcher, R.J., Gans, P., Gill, J.B., and Geyer, C., *J. Mol. Liq*., 1997, vols. 73–74, p. 99. DOI: 10.1016/S0167-7322(97)00060-3.
19. Divarova, V.V., Stojnova, K.T., Racheva, P.V., Lekova, V.D., and Dimitrov, A.N., *J. Serb. Chem. Soc.*, 2014, vol. 79 (in press); DOI: 0.2298/JSC140514102V *http://www.doiserbia.nb.rs/Article.aspx?id=0352513914-00102V#.VF90fpVZrIU*.
20. Genç, F., Gavazov, K.B., and Türkyilmaz, M., *Cent. Eur. J. Chem*., 2010, vol. 8, no. 2, p. 461. DOI: 10.2478/s11532-009-0119-7.
21. Divarova, V.V., Racheva, P.V., Lekova, V.D., Gavazov, K.B., and Dimitrov, A.N., *J. Chem. Technol. Metall*., 2013, vol. 48, no. 6, p. 623.
22. Marić, L. and Široki, M., *Anal. Chim. Acta*, 1996, vol. 318, no. 3, p. 345. DOI: 10.1016/0003-2670(95)00444-0.

23. Russeva, E., Kuban V., and Sommer, L., *Coll. Czech. Chem. Commun.*, 1979, vol. 44, no. 2, p. 374. DOI: 10.1135/cccc19790374.
24. Lobanov, F.I., Nurtaeva, G.K. and Ergozhin, E.E., *Ekstraktsiya kompleksov ionov metallov s piridinovymi oksiazosoedineniyami* (Extraction of Metal Ion Complexes with Pyridine Oxyazo Compounds), Alma-Ata: Nauka, 1983, p.8.
25. Zhiming, Z., Dongsten, M., and Cunxiao, Y., *J. Rare Earths*, 1997, vol. 15, no. 3, p. 216.
26. Holme, A., and Langmyhr, F.J., *Anal. Chim. Acta*, 1966, vol. 36, p. 383. DOI: 10.1016/0003-2670(66)80066-1.
27. Harvey, A. E., and Manning, D. L., *J. Am. Chem. Soc.*, 1950, vol. 72, no. 10, p. 4488. DOI: 10.1021/ja01166a044.
28. Tôei, K., *Anal. Sci*., 1987, vol. 3, no. 6, p. 479. DOI: http://dx.doi.org/10.2116/analsci.3.479.
29. Stojnova, K.T., Gavazov, K.B., and Lekova, V.D., *Acta Chim. Slov*., 2013, vol. 60, no. 2, p. 390.
30. Stefanova, T.S. and Gavazov, K.B., *Cent. Eur. J. Chem*., 2013, vol. 11, no. 2, p. 280. DOI: 10.2478/s11532-012-0149-4.