Extraction-Chromogenic Systems Containing Iron(III), 4-(2-Thiazolylazo) Resorcinol and Ditetrazolium Salts

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ABSTRACT

Liquid-liquid extraction systems containing Fe^{III}, 4-(2-thiazolylazo)resorcinol (TAR), and a ditetrazolium salt (DTS: Neotetrazolium chlortide, Blue Tetrazolium chloride, or Nitro Blue Tetrazolium chloride), water and chloroform were investigated. The optimum conditions for iron extraction, some equilibrium constants (association constants, distribution constants, and extraction constants), and characteristics (molar absorptivities, Sandell's sensitivities, limits of detection and quantification) were found. The extracted species are ternary complexes with a general formula of $(DT^{2+})[Fe^{II}(TAR)_2]$. In this formula the metal ion is in oxidation state II, the azo dye is in doubly deprotonated form (TAR^{2-}), and the ditetrazolium cation (DT) has a charge of 2+.

Key words: iron(III), azo dye, bis(tetrazolium) salt, liquid-liquid extraction, ternary complex.

INTRODUCTION

Tetrazolium salts are well-known compounds with multifunctional properties and various applications[1-7]. The most important 2,3,5-trisubstituted tetrazolium salts can be divided into two types: monotetrazolium(MTSs) and ditetrazolium (DTSs)[7]. Along with applications related to colour changes in biological or non-biological redox systems[6-10], corrosion inhibition[11], and dosimetry of ionization radiation[12], DTSs can be used as extraction reagents[4,13] by virtue of the ability of their cations (DT^{z+}; z=2 or 1) to form hydrophobic ion-association complexes. Ion-associates of DT^{z+} with many anions have been described in the literature, e.g. $[Cd(SCN)_4]^{2-}$ (14), $[Co(SCN)_4]^{2-}$(15), $[CrO_3Cl]^-$(16), $[Ga(OH)(PAR)_2]^{2-}$(17), $[MnO_4]^-$(18), $[MoO_2Cl_2(Cat)]^{2-}$ (Cat=catechol) (19), $[MoO_2(4NC)_2]^{2-}$ (4NC=4-nitrocatechol) (20), PAR^- (PAR=4-(2-pyridylazo) resorcinol) (21), $[ReCl_6]^{2-}$(22), $[ReO_4]^-$(23), TAR^- (TAR=4-(2-thiazolylazo)resorcinol) (21), $[VO(PAR)_2]^{2-}$ (24), $[VO_2(PAR)_2]^{3-}$(25),$[VO(OH)_2(TAR)]^{2-}$(25), $[VO_2(TAR)_2]^{3-}$(26), $[Zn(SCN)_4]^{2-}$(14), etc. However, to the best of our knowledge, ditetrazolium complexes with participation of iron have not been a subject of investigation until now.

In this work we describe studies on liquid-liquid extraction systems containing Fe^{III}, TAR, DTS, water, and chloroform. The most important commercially available DTSs were used, namely: (i) (3,3'-(4,4'-biphenylene)bis(2,5-diphenyl-2H-tetrazolium chloride;Neotetrazoliumchlortide, NTC); (ii) (3,3'-(3,3'-dimetoxy-4,4'-biphenylene)-bis(2,5-diphenyl-2H-tetrazolium) chloride; Blue Tetrazolium chloride, BTC); and (iii) (3,3'-(3,3'-dimetoxy-4,4'-biphenylene)-bis[2,5-di(4-nitrophenyl)-2H-tetrazolium] chloride; Nitro Blue Tetrazolium chloride, NBT).

Ion-association complexes of cations, deriving from MTSs and iron-containing chelate anions have been recently investigated[27-29]. The results for the extraction-chromogenic system Fe^{III}-TAR-MTS-water-chloroform[27] suggested that the initial oxidation state of Fe (i. e. III) lowers to II during the process of formation and extraction of the ternary complex. Some characteristics and applications of binary Fe-TAR complexes[30-35] and information concerning the Fe^{III} reduction under certain conditions[35-37] can be found in the literature.

MATERIALS AND METHODS

A stock iron(III) solution (1 mg mL^{-1}; 1 L) was prepared by dissolving 8.6350 g of FeNH$_4$(SO$_4$)$_2$·12H$_2$O (99.1%; Reanal, Hungry)in water containing 5 mL of conc. H$_2$SO$_4$. Working solutions (50 µg mL^{-1}) were prepared every day by suitable dilution of the stock solution with 0.01 mol L^{-1} H$_2$SO$_4$(27, 28). TAR (97%), NTC ("for microbiology" grade), BTC ("formicrobiology" grade), and NBT (98%) were purchased from Sigma–Aldrich Chemie GmbH. Aqueous solutions of the mentioned reagents were prepared: C$_{TAR}$=3×10^{-3}mol L^{-1}, and C$_{DTS}$=2×10^{-3}mol L^{-1}.The chloroform was additionally distilled before use. 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 pH was checked by a Hanna HI 83140 pH meter. A Camspec M507 spectrophotometer (United Kingdom), equipped with 0.5cm path-length cells, was used for reading the absorbance of the extracts.A Microwave Plasma – Atomic Emission Spectrometer Agilent 4200 MP-AES was employed for determining the iron content in residual aqueous phase obtained after extraction.

Procedure for establishing the optimum conditions

Aliquots of Fe(III) solution, TAR solution (up to 2.0 mL), DTS solution (up to 2.5 mL) and buffer solution (3 mL; pH ranging from 4.4 to 8.5) were introduced into 125-mL separatory funnels. The resulting mixtures were diluted with distilled water to a total volume of 10 mL. 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 by the UV-VIS spectrophotometer.

Procedure for determining the fractions extracted and constants of distribution

The fractions extracted (E%) and constants of distribution (K$_D$) were calculated by comparison of the iron content (determined by MP-AES at wavelengths 371.993, 373.486,and 302.064 nm) in the aqueous phase before and after the extraction. The initial concentration of Fe^{III} in the aqueous phase was 2.24×10^{-5}mol L^{-1} and the extraction was performed under the optimum conditions (Table 1; pH=6.3).

RESULTS AND DISCUSSION

Absorption spectra and effect of pH

Absorption spectra of the chloroform extracted ternary complexes are shown in Fig. 1. All three Fe-TAR-DTS complexes have maxima at *ca.* 618-620 nm (curves 1-3). Additional maxima can be observed in the spectra when the extraction is performed at relatively low pH: e.g. pH 4.1 for the systems with NTC and BTC (curves 1', 2'), or pH 5.7 for the system with NBT (curve 3').These maxima are the most pronounced for the NBT complex. This fact can be explained with the lowest stability and molar absorptivity of (NBT^{2+})(TAR$^-$)$_2$[21] - the salt-like compound which determines the absorption of the blank: Log β$_{NBT-TAR}$= 8.3, ε$_{max}$=1.95×10^4 L mol^{-1} cm^{-1}. The corresponding values for (NTC$^+$)$_2$(TAR$^-$)$_2$ and (BT^{2+})(TAR$^-$)$_2$are higher[21]. Hence, the resultant absorption A$_I$ = A$_{Fe-TAR-DTS}$– A$_{TAR-DTS}$ is strongly affected by the A$_{TAR-DTS}$, especially when TAR is predominantly in anionic form (pH>pK$_{TAR}$;pK$_{TAR}$=6.23 [38]) and λ< 550 nm. As a result, the peaks of the ternary Fe-TAR-DTS complexes in this spectral region disappear (curves 1-3).

At pH>pK$_{TAR}$, the maxima of the ternary Fe-TAR-DTS complexes appears at 618 or 620 nm (Table 1). The absorbance of the blank is negligible in this spectral region (Figure 2, curves 1'-3') and the obtained spectrophotometric results are stable in time and repeatable. Hence, these maxima were used for our further spectrophotometric measurements.

The effect of pH on the absorbance is shown in Fig. 2. It can be assumed that the shape of the pH-

Table 1: Optimum extraction-spectrophotometric conditions [a]

System	Organic solvent	λ, nm	pH	C_{TAR}, mol L^{-1}	C_{DTS}, mol L^{-1}	Extraction time, min
Fe^{III}-TAR-NTC	Chloroform	618	5.5-7.7	2.0×10^{-4}	4.0×10^{-4}	2
Fe^{III}-TAR-BTC	Chloroform	618	5.5-7.1	4.0×10^{-4}	2.0×10^{-4}	2
Fe^{III}-TAR-NBT	Chloroform	620	5.6-7.5	5.0×10^{-4}	2.0×10^{-4}	2

[a] - optimization experiments performed at room temperature (*ca.* 22°C) and C$_{Fe}$=2.24×10^{-5}mol L^{-1}

Table 2: Calculated values of the fractions extracted (E%), distribution constants (K$_D$), association constants (β), and extraction constants (K$_{ex}$)

Extraction system	E% [f]	Log K$_D$	Log β[f]	Log K$_{ex}$
Fe^{III}-TAR-NTC	89±1 (N=3)	0.9 [a]	4.7±0.2 (N=11) [b]; 4.6±0.2 (N=3)[c]; 4.7±0.2 (N=7) [d]	5.6 [e]
Fe^{III}-TAR-BTC	851 (N=3)	0.8 [a]	4.9±0.1 (N=9) [b], 4.9±0.2 (N=3) [c]; 4.9±0.2 (N=8) [d]	5.7 [e]
Fe^{III}-TAR-NBT	94±1 (N=3)	1.2[a]	4.9±0.1 (N=9) [b]; 5.0±0.2 (N=3) [c]; 4.9±0.2 (N=8) [d]	6.1 [e]

[a] - calculated by the equation K$_D$=E/(1-E); [b]- Holme-Langmyhr method; [c]- Harvey-Manning method; [d]- Mobile equilibrium method; [e] - by the equation Log K$_{ex}$ = Log K$_D$+ Log β; [f] - ±SD

Table 3: Slopes (a) [a], intercepts (b) [a], and R²-values of straight line equations obtained by the mobile equilibrium method

Extraction system	TAR:Fe=2:1	DTS:Fe=1:1
Fe^{III}-TAR-NTC	a=2.1±0.2; b=9.1±0.8; R^2=0.9710 (N=6)	a=1.05±0.05; b=5.0±0.2; R^2=0.9883 (N=7)
Fe^{III}-TAR-BTC	a=2.1±0.1; b=8.7±0.6; R^2=0.9647 (N=10)	a=1.00±0.05; b=4.9±0.2; R^2=0.9857 (N=8)
Fe^{III}-TAR-NBT	a=2.04±0.08; b=8.30.3; R^2=0.9855 (N=11)	a=0.97±0.03; b=4.8±0.1; R^2=0.9937 (N=8)

[a] - ±SD

Table 4: Characteristics concerning the application of the ion-association complexes for extractive-spectrophotometric determination of iron

Analytical characteristics	Fe^{III}-TAR-NTC	Fe^{III}-TAR-BTC	Fe^{III}-TAR-NBT
Linear range, µg mL^{-1}	up to 1.5	up to 3.2	up to 2.5
Molar absorptivity (ε), L mol^{-1} cm^{-1}	4.3×10^4	4.5×10^4	3.9×10^4
Sandell's sensitivity (SS), ng cm^{-2}	1.3	1.2	1.4
Limit of detection (LOD), µg mL^{-1}	0.07	0.07	0.09
Limit of quantification (LOQ), µg mL^{-1}	0.23	0.22	0.30

curves is governed by two factors: (i) doubly protonated TAR species predominate at low pH values; and (ii) Iron hydrolysis[39] exert noticeable effects on the complex formation at pH higher than pH_{opt}.

Effect of reagents concentration

The effect of TAR and DTS concentrations on the absorbance is shown in Fig. 3 and Fig. 4, respectively. The optimum reagents concentrations deduced from these figures are shown in Table 1.
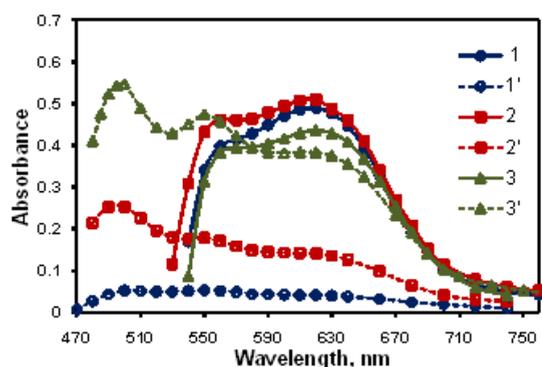
Effect of shaking time

The extraction equilibria in the Fe-TAR-DTS systems are reached for *ca.*90 seconds. We shook the funnels for 2 min in our further experiments.

Composition, molecular formulae and equilibrium constants

The molar TAR-to-Fe and DTS-to-Fe ratios were determined from the experimental results presented in Fig. 3 and Fig. 4, respectively. Two

Fig. 1: Absorption spectra of extracted ternary complexes. $C_{Fe(III)}=2.24\times10^{-5}$mol L^{-1}; $C_{TAR}=2.0\times10^{-4}$mol L^{-1}, $C_{NTC}=4.0\times10^{-4}$, (curves 1,1'); $C_{TAR}=4.0\times10^{-4}$mol L^{-1}, $C_{BTC}=2.0\times10^{-4}$(curves 2, 2'); $C_{TAR}=5.0\times10^{-4}$mol L^{-1}, $C_{NBT}=2.0\times10^{-4}$mol L^{-1} (curves 3,3'); pH=7 (curves 1,3), pH=6.3 (curve 2), pH=4.1 (curves 1',3'), pH=5.7 (curve 2') mol L^{-1}, λ=620 nm (curves 3,3')

Fig. 2: Effect of pH on the absorbance of extracted ternary complexes. $C_{Fe(III)}=2.24\times10^{-5}$mol L^{-1} (curves 1-3); $C_{TAR}=2.0\times10^{-4}$mol L^{-1}, $C_{NTC}=2.6\times10^{-4}$mol L^{-1}, λ=618 nm (curves 1,1'); $C_{TAR}=2.0\times10^{-4}$mol L^{-1}, $C_{BTC}=4.0\times10^{-4}$mol L^{-1}, λ=620 nm ; $C_{TAR}=2.0\times10^{-4}$mol L^{-1}, $C_{NBT}=4.0\times10^{-4}$
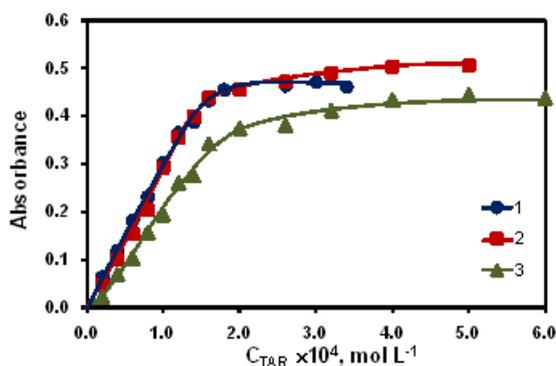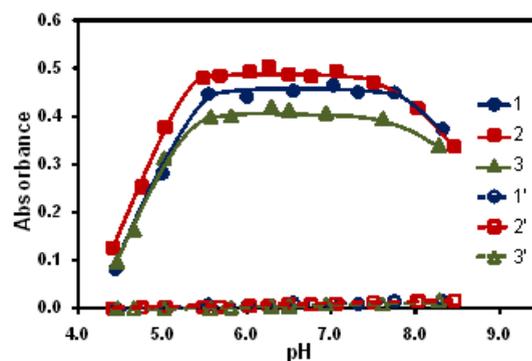

Fig. 3: Effect of the TAR concentration on the absorbance of extracted complexes. $C_{Fe(III)}=2.24\times10^{-5}$mol L^{-1} (curves 1-3); $C_{NTC}=2.6\times10^{-4}$mol L^{-1}, pH=7.0, λ=618 nm (curve 1); $C_{BTC}=2.0\times10^{-4}$mol L^{-1}, pH=6.3, λ=618 nm (curve 2); $C_{NBT}=2.0\times10^{-4}$mol L^{-1}, pH=5.7, λ=620 nm (curve 3)
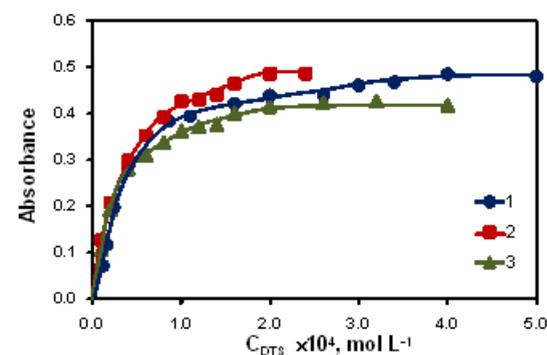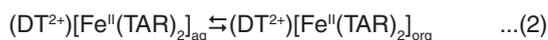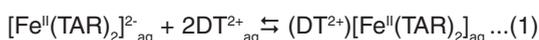

Fig. 4: Effect of the DTS concentration on the absorbance of extracted complexes. $C_{Fe(III)}=2.24\times10^{-5}$mol L^{-1} (curves 1-3); $C_{TAR}=2.0\times10^{-4}$mol L^{-1}, pH=7.0, λ=618 nm (curve 1); $C_{TAR}=4.0\times10^{-4}$mol L^{-1}, pH=6.3, λ=618 nm (curve 2); $C_{TAR}=2.0\times10^{-4}$mol L^{-1}, pH=5.7, λ=620 nm (curve 3)

different methods were used: the mobile equilibrium method[40] (Table 3) and the straight-line method of Asmus[41]. The results show that the molar ratio between the components of the ternary complex – Fe, TAR and DTS – is 1:2:1. This molar ratio, along with the spectral characteristics (which are similar to these for the Fe-TAR-MTS complexes[27]), give us grounds to assume that the iron ion has an oxidation state of II, TAR is in doubly deprotonated form (TAR^{2-}), and DT^{z+} has a charge of 2 (DT^{2+}).

If we assume that the Fe^{III} reduction takes place in the aqueous phase, we can write the following equations of ion-association (1) and distribution (2):

$$[Fe^{II}(TAR)_2]^{2-}_{aq} + 2DT^{2+}_{aq} \leftrightarrows (DT^{2+})[Fe^{II}(TAR)_2]_{aq} \dots(1)$$

$$(DT^{2+})[Fe^{II}(TAR)_2]_{aq} \leftrightarrows (DT^{2+})[Fe^{II}(TAR)_2]_{org} \quad \dots(2)$$

The constants of association (β) describing eq. 1 were determined by several methods: the Holme-Langmyhr method[42], the Harvey-Manning method[43], and the mobile equilibrium method[40]. The constants of distribution (K_D) describing eq. 2 were calculated by the formula $K_D=E/(1-E)$, where E is determined by MP-AES at the optimum extraction conditions (as described above). The attempts to determine these constants spectrophotometrically by comparison of the absorbance values obtained after single (A_1) and triple (A_3) extractions in equal final volumes[26-28] were unsuccessful, since A_1 was higher than A_3. This anomaly was greater than that observed in[27] for the Fe^{III}-TAR-TTC system (TTC=2,3,5-triphenyl-2H-tetrazolium chloride). It can be attributed to the formation of substances with different colour properties during the oxidation-reduction process involving iron or during the dilution of the organic extracts with chloroform.

The extraction constants (K_{ex}) were calculated by the formula $K_{ex}=K_D \times \beta$[44]. All experiments were performed at room temperature of ca. 22°C. The results are given in Table 2.

Beer's law and analytical characteristics

The adherence to Beer's law for each Fe^{III}–TAR–DTS–water–chloroform system was examined under the optimum extraction-spectrophotometric conditions. Then the molar absorptivities, Sandell's sensitivities, limits of detection (LOD) and limits of quantification (LOQ) were calculated. The results are given in Table 4.

CONCLUSION

Cations deriving from DTSs were studied for the first time as components of ion-association systems containing iron. These cations form chloroform-extractable ion-pairs with the anionic Fe-TAR chelate, which can be represented with the formula $[Fe^{II}(TAR^{2-})_2]^{2-}$, independently of the fact that the initial oxidation state of iron was III. The calculated equilibrium constants and characteristics suggest that the DTS-TAR couples can be applied for iron extraction. However, the greatest potential in this field has NBT which form the most stable and extractable complex.

ACKNOWLEDGMENTS

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