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Lecture

TERNARY ION-ASSOCIATION COMPLEXES BETWEEN THE IRON(III) WITH 4-(2-THIAZOLYLAZO)RESORCINOL AND SOME TETRAZOLIUM CATIONS

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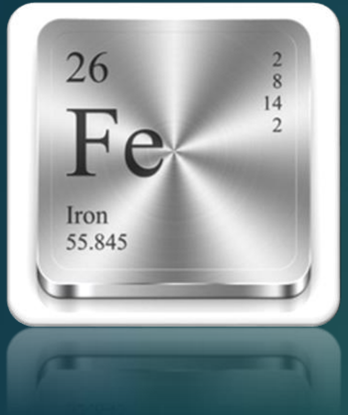
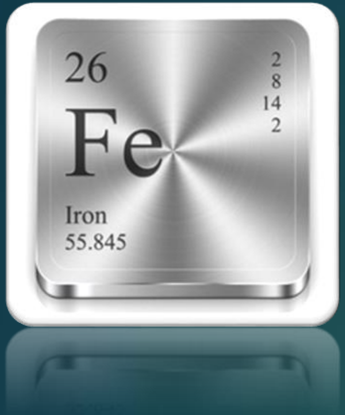


Iron characterization

- Iron (Fe) – silvery-gray, malleable metal with a shiny surface that oxidizes easily.
- The pure iron crystals are soft (softer than Al).
- Good conductor of electricity and heat, pronounced magnetic properties;
- High chemical reactivity;
- Forms compounds mostly in two oxidation states – Fe^{3+} and Fe^{2+} ;
- Specific properties and its alloys make iron "metal №1" in importance for humans;
- Often used iron-carbon alloys: steel (up to 2.14% C); cast iron (more than 2.14% C), as well as stainless (alloyed) steel with alloying metals (Cr, Mn, Ni and etc.).



Iron occurrence

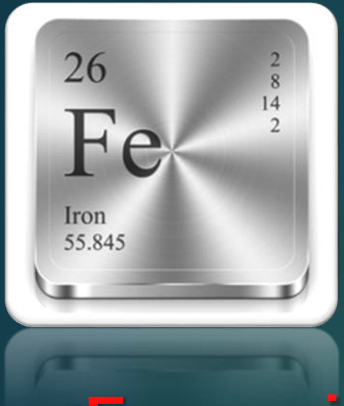
- Iron – one of the most common elements in the solar system, especially on the planets like the Earth.
- Much of the iron on Earth is found in the core of the planet.
- The Earth's core is believed to largely be composed of iron (80%), along with nickel (20%) and one or more light elements.

Terms concerning iron intake

The WHO and European Commission's Directorate-General for Health and Food Safety introduces:

- Recommended Dietary Allowances (RDAs)
- for adults – 20-50 year – 8 mg/day for males;
- for adults – 20-50 year – 18 mg/day for females.
- . Tolerable upper intake level (UL)
- for adults – 14-70 year – 45 mg/day.

The body of an adult contains about 3 - 4 g of iron (about 0.005%), of which only about 3.5 mg is in the blood plasma.



Iron toxicity

- **Excess iron** can enter the body along with rusty tap water (through cast iron pipes).
- The iron content in water more than 1-2 mg/L significantly impairs its organoleptic properties. Such water causes allergic reactions in humans.
- The maximum concentration limit for iron in water is 0.3 mg/L.
- **Iron overdose** stimulates the production of free radicals, inhibits the body's antioxidant system, and probably contributes to the development of atherosclerosis;

Excessive accumulation of iron in the body is toxic.

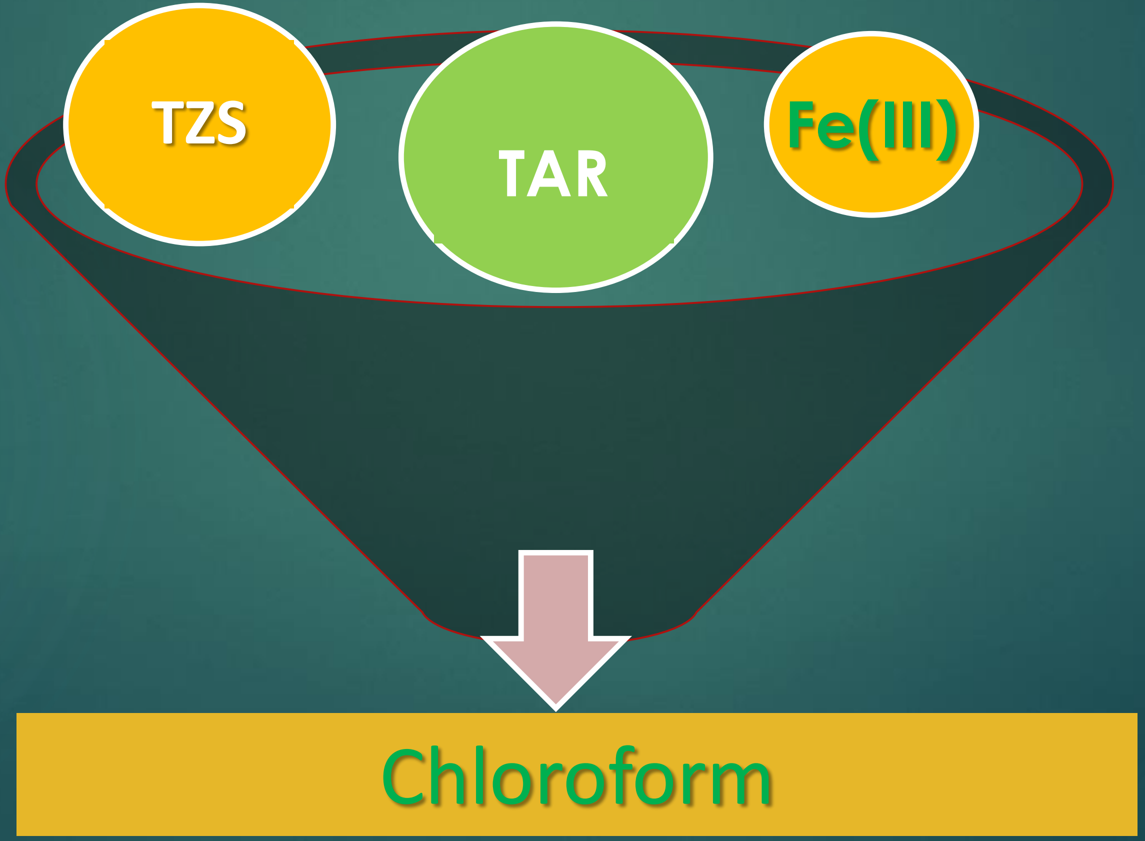
Iron in analytical techniques

Methods involving spectrophotometry

- simple and low-cost;
- can be easily combined with procedures for preliminary separation and concentration;
- can be easily combined with cloud point extraction;
- can be easily combined with liquid-liquid extraction.

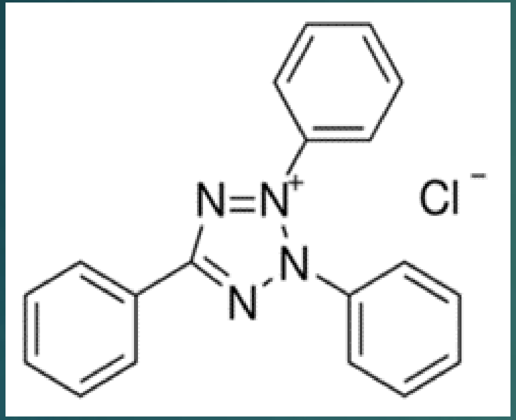
The present work aims at to study:

- water-chloroform extraction-chromogenic system for iron(III);
- Fe(III) – 4-(2-thiazolylazo)resorcinol (TAR) – tetrazolium salts(TZS)

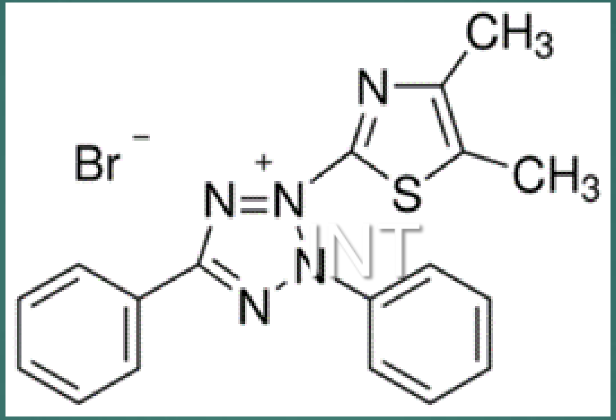


Reagents and apparatus

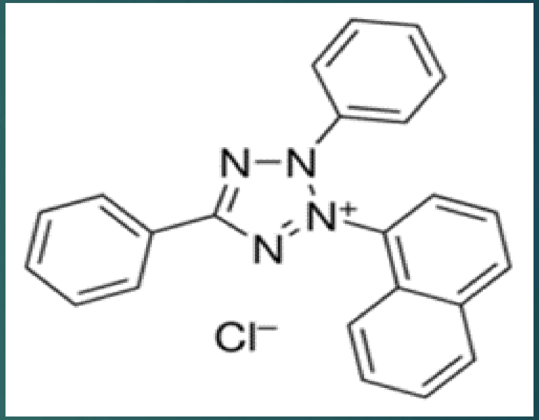
- . 4-(2-thiazolylazo)resorcinol (TAR) – a well-known analytical reagent;
- tetrazolium salts (TZS) – has been used in microbiology and analytical chemistry.



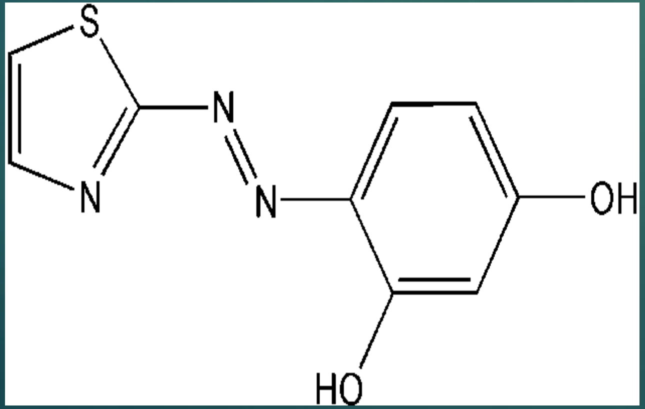
TTC



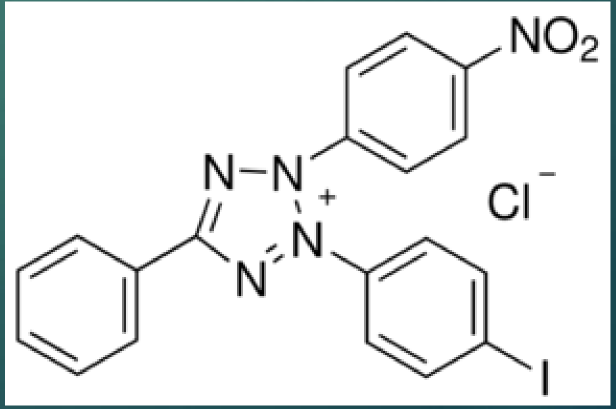
MTT



TV



TAR



INT

Reagents and apparatus

- Stock solution of Fe^{III} (1 mg dm^{-3}; pH ~2) prepared by dissolving 8.6350 g of $FeNH_4(SO_4)_2.12H_2O$ (99.1%; Reanal, Hungry) in water containing 5 cm^3 of conc. H_2SO_4. Working solutions (50 µg cm^{-3}) were prepared every day by suitable dilution of the stock solution with 0.01 mol dm^{-3} H_2SO_4.
- 4-(2-thiazolylazo)resorcinol (TAR) (Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)) – aqueous solutions with concentrations of 2×10^{-3} mol dm^{-3} were used.
- tetrazolium salts (TZS) – the concentrations were 2×10^{-3} mol dm^{-3} (TV and INT), 3×10^{-3} mol dm^{-3} (for MTT), and 4×10^{-3} mol dm^{-3} (for TTC).
- The acidity of the aqueous medium was set by the addition of buffer solution, prepared by mixing 2.0 mol dm^{-3} aqueous solutions of CH_3COOH and ammonia.
- Absorbance measurements were performed by using a Camspec M508 and a Ultrospec3300 pro UV-Vis spectrophotometers (UK).

Results and Discussion

1. Liquid–liquid extraction–spectrophotometric optimization

1.1. Absorption spectra of complexes in chloroform

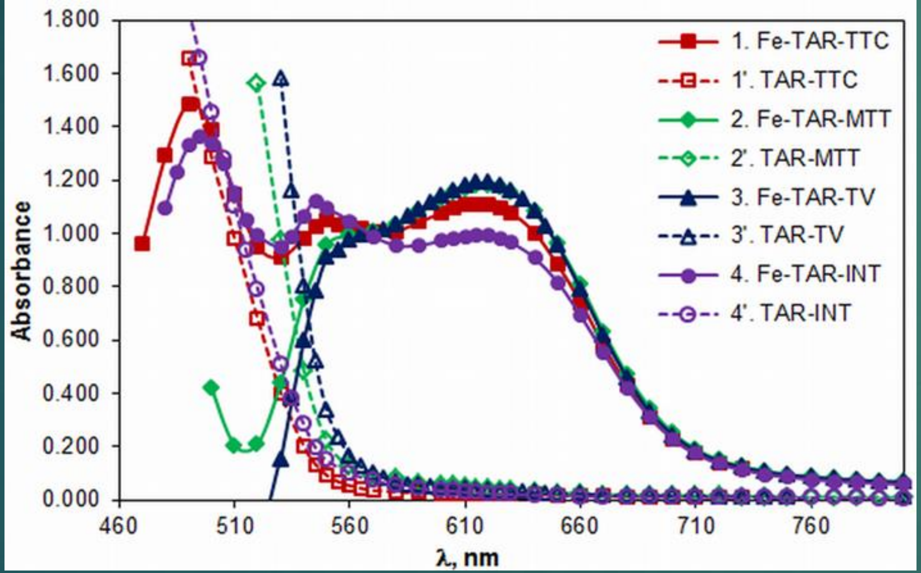


Figure 1. Absorption spectra of the ternary complexes (curves 1-4; $C_{Fe(III)}=2.24\times10^{-5}$ mol dm^{-3}) and blank samples (curves 1'-4') in chloroform at the optimum extraction conditions.
– TTC; – MTT; – TV; – INT.

The absorption spectra show that several complexes are formed in the system.

Iron(III) –TAR– TZS

1.2. Effect of pH on the extraction

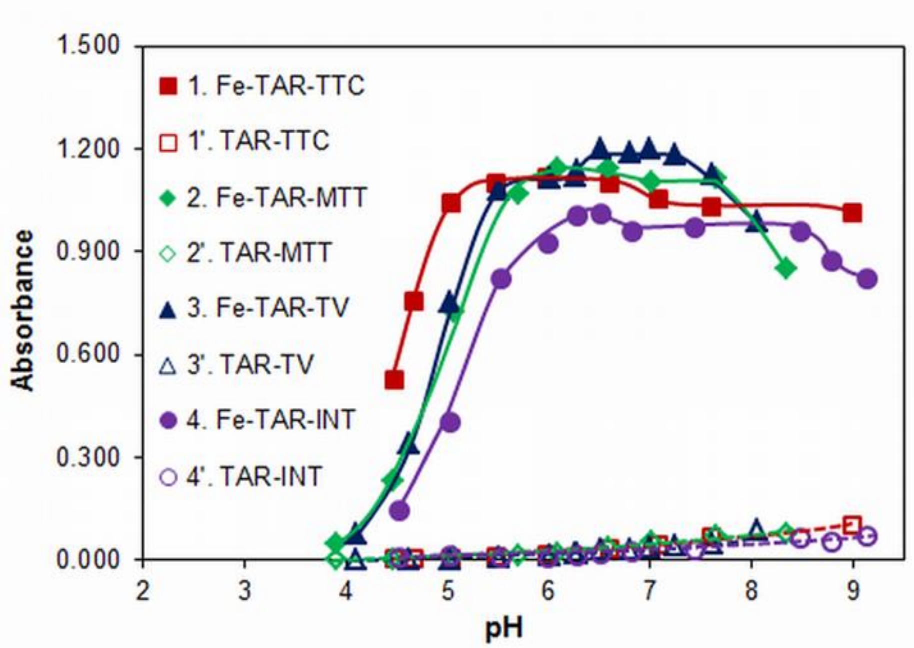
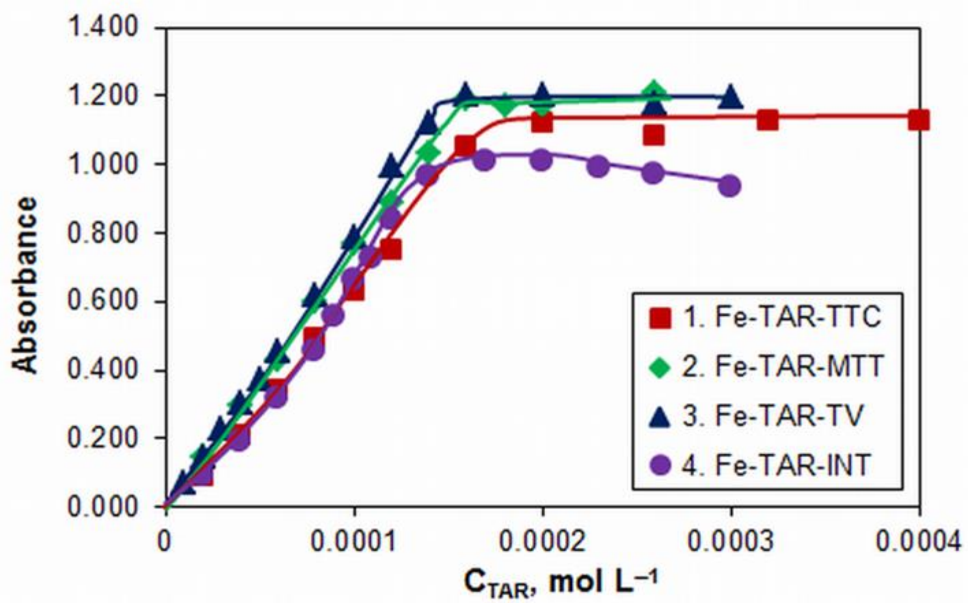


Figure 2. Absorbance of the ternary Fe-TAR-TZS complexes (curves 1-4; $C_{Fe(III)}=2.24\times10^{-5}$ mol dm^{-3}) and the blank samples (curves 1'-4') in chloroform vs. pH of the aqueous phase.
(1, 1') $C_{TAR}=3.0\times10^{-4}$ mol dm^{-3}, $C_{TTC}=8.0\times10^{-4}$ mol dm^{-3}, $\lambda=615$ nm;
(2, 2') $C_{TAR}=2.0\times10^{-4}$ mol dm^{-3}, $C_{MTT}=3.0\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm;
(3, 3') $C_{TAR}=2.0\times10^{-4}$ mol dm^{-3}, $C_{TV}=2.4\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm;
(4, 4') $C_{TAR}=2.0\times10^{-4}$ mol dm^{-3}, $C_{INT}=2.6\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm.

Absorbance is maximal in precisely defined ranges according to the complex.

Iron(III) –TAR– TZS

1.3. Effect of the reagents concentration



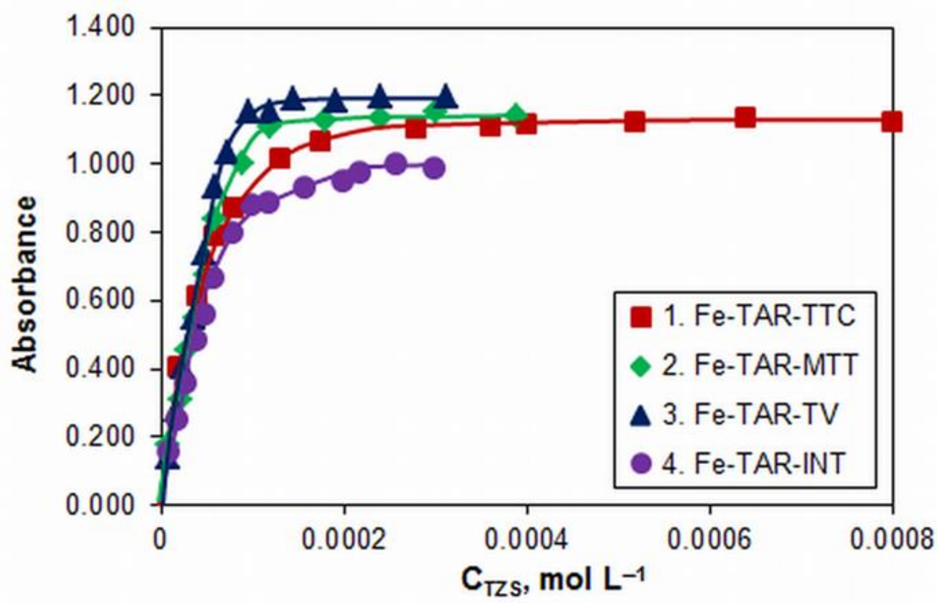


Figure 4. Absorbance of the extracted Fe-TAR-TZS complexes vs. concentration of the TAR plots; $C_{Fe(III)}=2.24\times10^{-6}$ mol dm^{-3}. (1) $C_{TTC}=6.0\times10^{-4}$ mol dm^{-3}, $\lambda=615$ nm; (2) $C_{MTT}=3.0\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm; (3) $C_{TV}=2.4\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm; (4) $C_{INT}=2.6\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm.

Figure 5. Absorbance of the extracted Fe-TAR-TZS complexes vs. concentration of the TZS plots; $C_{Fe(III)}=2.24\times10^{-6}$ mol dm^{-3}. (1) $C_{TAR}=3.0\times10^{-4}$ mol dm^{-3}, $\lambda=615$ nm; (2) $C_{TAR}=1.8\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm; (3) $C_{TAR}=2.0\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm; (4) $C_{TAR}=2.0\times10^{-4}$ mol dm^{-3}, $\lambda=620$ nm.

Iron(III) –TAR – TZS

Extraction system	λ/nm	pH	C_{TAR}/mol dm^{-3}	C_{TZS}/mol dm^{-3}	Extaction time/min
Fe(III)-TAR-TTC	615	5.5-6.6	$(2.0\text{-}3.0)\times10^{-4}$	$(4.0\text{-}8.0)\times10^{-4}$	1-2
Fe(III)-TAR-MTT	620	6.0-6.6	$(1.6\text{-}2.0)\times10^{-4}$	$(1.8\text{-}3.0)\times10^{-4}$	1-2
Fe(III)-TAR-TV	620	6.5-7.2	$(1.6\text{-}2.0)\times10^{-4}$	$(1.2\text{-}2.4)\times10^{-4}$	1-2
Fe(III)-TAR-INT	620	6.3-6.5	$(1.8\text{-}2.0)\times10^{-4}$	$(2.6\text{-}2.8)\times10^{-4}$	1-2

Table 1. LLE-spectrophotometric optimization of the **Fe^{III}–TAR–TZS**–water–chloroform system. The volume of the organic phase was 10 cm^3.

Iron(III) – TAR – TZS

2. Molar Ratios

Extraction system	**Squared correlation coefficient values (R^2) corresponding to molar ratios of 1, 2 and 3**	
	TAR:Fe	TZS:Fe
Fe(III)-TAR-TTC-water-chloroform	0.9879 (n=1)[a] 0.9939 (n=2)[a] 0.9594 (n=3)[a]	0.9676 (n=1)[e] 0.9994 (n=2)[e] 0.9817 (n=3)[e]
Fe(III)-TAR-MTT-water-chloroform	0.9888 (n=1)[b] 0.9914 (n=2)[b] 0.9793 (n=3)[b]	0.9351 (n=1)[f] 0.9862 (n=2)[f] 0.9643 (n=3)[f]
Fe(III)-TAR-TV-water-chloroform	0.9867 (n=1)[c] 0.9955 (n=2)[c] 0.9863 (n=3)[c]	0.9757 (n=1)[g] 0.9963 (n=2)[g] 0.9832 (n=3)[g]
Fe(III)-TAR-INT-water-chloroform	0.9670 (n=1)[d] 0.9865 (n=2)[d] 0.9657 (n=3)[d]	0.9653 (n=1)[h] 0.9929 (n=2)[h] 0.9419 (n=3)[h]

a – C_{TAR} varies from 6.0×10^{-5} mol dm^{-3} to 2.0×10^{-4} mol dm^{-3};
b – C_{TAR} varies from 8.0×10^{-5} mol dm^{-3} to 1.8×10^{-4} mol dm^{-3};
c – C_{TAR} varies from 8.0×10^{-5} mol dm^{-3} to 1.6×10^{-4} mol dm^{-3};
d – C_{TAR} varies from 6.0×10^{-5} mol dm^{-3} to 1.6×10^{-4} mol dm^{-3};
e – C_{TTC} varies from 8.0×10^{-5} mol dm^{-3} to 3.6×10^{-4} mol dm^{-3};
f – C_{MTT} varies from 3.9×10^{-5} mol dm^{-3} to 2.4×10^{-4} mol dm^{-3};
g – C_{TV} varies from 3.6×10^{-5} mol dm^{-3} to 9.6×10^{-5} mol dm^{-3};
h – C_{INT} varies from 3.0×10^{-5} mol dm^{-3} to 2.2×10^{-4} mol dm^{-3};
*– At reagent concentrations lower than the specified (references a, b, c, d, e, f, g, and h) the determined squared correlation coefficient values for n=1 were closest to 1.

Table 2. Determination of the TAR-to-Fe and TZS-to-Fe molar ratios by the stright-line method of Asmus

Iron(III) – TAR – TZS

3. Composition of the Complexes

The saturation curves presented in Figure 3 and Figure 4 allowed determination of the TAR-to-Fe and TZS-to-Fe molar ratios in the ternary complexes. The mobile equilibrium method [46], which is appropriate to distinguish mononuclear species from polynuclear species, and the straight-line method of Asmus [47] (Table 3) were used. The results led to the conclusion that mononuclear species are formed in all systems; the molar Fe:TAR:TZS ratio in these species is 1:2:2 if $C_{TAR}>(6.0\text{-}8.0)\times10^{-5}$ mol dm^{-3} and $CTZS>(3.0\text{-}8.0)\times10^{-5}$ mol dm^{-3} .

Extraction system	Log β	Log K_D	Log K_{ex}	R%
Fe(III)-TAR-TTC-H_2O-chloroform	9.08 ± 0.08^a, 9.0 ± 0.7^b	1.57 ± 0.01^c	10.65 ± 0.09^e	97.41 ± 0.05^f
Fe(III)-TAR-MTT-H_2O-chloroform	9.44 ± 0.08^a, 9.5 ± 0.6^b	1.45 ± 0.01^d	10.89 ± 0.09^e	96.58 ± 0.05^f
Fe(III)-TAR-TV-H_2O-chloroform	9.8 ± 0.2^a, 9.7 ± 1.3^b	1.53 ± 0.01^d	11.3 ± 0.2^e	97.14 ± 0.09^f
Fe(III)-TAR-INT-H_2O-chloroform	9.2 ± 0.1^a, 9.3 ± 0.2^b	1.011 ± 0.002^d	10.2 ± 0.1^e	91.12 ± 0.04^f

a – Calculated by the Holme-Langmihr method
b – Calculated by the Mobile equilibrium method
c – Calculated by the equation $K_D=\Delta A_1/(\Delta A_x-\Delta A_1)$; x=2
d – Calculated by the equation $K_D=\Delta A_1/(\Delta A_x-\Delta A_1)$; x=3
e – Calculated by the equation $K_{ex}=K_D\times\beta$, where β is the value obtained by the Holme-Langmihr method
f – Calculated by the equation $R\%=100\times K_D/(K_D+1)$

Table 3. Calculated values (P=95%) of the association constants (β), extraction constants (K_{ex}), distribution constants (K_D), and recovery factors (R%)

Iron(III) –TAR – TZS

4. Suggested Reaction Scheme and General Formula of the Ternary Complexes

The obtained Fe:TAR:TZS molar ratios (1:2:2 at the optimum extraction conditions) and the ability of TZS to form cations (TZ^+) in aqueous medium suppose a complex formation according to one of the following two schemes:

Scheme 1

$Fe^{III} + 2HTAR^- + e^- \leftrightarrow [Fe^{II}(TAR)_2]^{2-} + 2H^+$ (1.1)

$[Fe^{II}(TAR)_2]^{2-} + 2TZ^+ \leftrightarrow (TZ^+)_2[Fe^{II}(TAR)_2]$ (1.2)

Scheme 2

$Fe^{III} + 2HTAR^- + H_2O \leftrightarrow [Fe^{III}(OH)(TAR)_2]^{2-} + 3H^+$ (2.1)

$[Fe^{III}(OH)(TAR)_2]^{2-} + 2TZ^+ \leftrightarrow (TZ^+)_2[Fe^{III}(OH)(TAR)_2]$ (2.2)

Scheme 1 is better supported by the experimental facts (high molar absorptivity, stable results, lack of evidence for aggregation in the organic phase) and by the literature concerning iron-TAR and/or iron-PAR complexes.

Iron(III) –TAR – TSZ

5. Beer’s Law and Analytical Characteristics

Extraction system Analytical characteristics	Fe(III)-TAR-TTC-H_2O-$CHCl_3$	Fe(III)-TAR-MTT-H_2O-$CHCl_3$	Fe(III)-TAR-TV-H_2O-$CHCl_3$	Fe(III)-TAR-INT-H_2O-$CHCl_3$
Molar absorptivity (ε)/L mol^{-1} cm^{-1}	5.0×10^4	5.13×10^4	5.3×10^4	4.5×10^4
Sandell’s sensitivity (SS)/ ng cm^{-2}	1.12	1.09	1.06	1.25
Adherence to Beer’s law/ μg cm^{-3}	up to 1.3	up to 1.5	up to 1.5	up to 1.3
Squared correlation coefficient (R^2)	0.9986	0.9997	0.9979	0.9977
Limit of detection (LOD)/ μg cm^{-3}	0.049	0.031	0.073	0.070
Limit of quantification (LOQ)/ μg cm^{-3}	0.16	0.11	0.25	0.23
Absorbance of the blank at λ_{opt} (in parenthesis)	0.014±0.001 (615 nm)	0.029±0.001 (620 nm)	0.023±0.001 (620 nm)	0.020±0.001 (620 nm)

Table 4. Characteristics concerning the application of the ion-association complexes for extractive-spectrophotometric determination of iron(III)

Iron(III) –TAR – TZS

5. Beer’s Law and Analytical Characteristics

The adherence to Beer's law for each **Fe(III)–TAR–TZS system** was examined under the optimum extraction-spectrophotometric conditions (Table 2). Calculated molar absorptivities (ε) are listed in Table 4, along with some important characteristics concerning the application of the ternary complexes for extraction-spectrophotometric determination of iron.

The couples TZS–TAR ensure high sensitivity of determination. In this criterion, they are better than many reagents used in similar systems: TAR ($\varepsilon_{730}=2.9\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; aqueous medium) [21], TZS {$\varepsilon_{480}=2.2\times10^{3}$ dm^{3} mol^{-1} cm^{-1} (TZS=TTC), $\varepsilon_{555}=5.3\times10^{3}$ dm^{3} mol^{-1} cm^{-1} (TZS=MTT), $\varepsilon_{480}=1.1\times10^{4}$ dm^{3} mol^{-1} cm^{-1} (TZS=INT), $\varepsilon_{500}=2.2\times10^{3}$ dm^{3} mol^{-1} cm^{-1} (TZS=Neotetrazolium chloride); methyl isobutyl ketone extraction} [37], 4-nitrocatechol–TTC ($\varepsilon_{435}=2.7\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; chloroform extraction) [35], PAR–TTC ($\varepsilon_{540}=4.7\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; chloroform extraction) [20], PAR–zephiramine ($\varepsilon_{522}=(4.0\text{-}4.5)\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; chloroform extraction) [3-5], 4-(4-methyl-2-thiazolylazo)resorcinol ($\varepsilon_{735}=2.49\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; aqueous medium) [28], 2-(2-thiazolylazo)-5-dimethylaminophenol ($\varepsilon_{760}=2.70\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; chloroform extraction) [54], 2-(2-thiazolylazo)-4-methylphenol ($\varepsilon_{762}=1.37\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; chloroform extraction) [55], 2-(4,5-dimethyl-2-thiazolylazo)-4,6-dimethylphenol ($\varepsilon_{773}=1.38\times10^{4}$ dm^{3} mol^{-1} cm^{-1}; chloroform extraction) [56], and 1-(2-thiazolylazo)-2-naphthol {$\varepsilon_{787}=1.83\times10^{4}$ dm^{3} mol^{-1} cm^{-1} (aqueous medium) [57] or $\varepsilon_{786}=1.89\times10^{4}$ dm^{3} mol^{-1} cm^{-1} (chloroform extraction) [55]}.

Iron(III) –TAR – TZS

5. Conclusions

1. Fe(III) readily forms chloroform-extractable ternary ion-association complexes with TAR and TZSs. The anionic part of these complexes ensures intense brown coloration and the bulkiness of the cationic part (TZ^+) guarantees their good hydrophobic properties.
2. The complexes have a composition of 1:2:2 (Fe:TAR:TZS) under the optimum extraction conditions. The following general formula was suggested: $(TZ^+)_2[Fe^{II}(TAR)_2]$; in this formula TAR is doubly deprotonated (TAR^{2-}) and iron is in its 2^+ oxidation state.
3. The key equilibrium constants and analytical characteristics were determined. Linear relationships appear to exist between the logarithm of the molecular mass of the tetrazolium cation (Log $M_{TZ}+$) and some of the constants (Log β, Log K_{ex}, and $\varepsilon_{\lambda(opt)}$) for the complexes with TZ^+ which do not contain NO_2 groups (i.e. $TZ^+ = TT^+$, MTT^+ and TV^+).
4. The poorer extraction-spectrophotometric performance of $(INT^+)_2[Fe(TAR)_2]$ can be explained with a negative influence of the nitro group.

Thank you for your attention