Extraction-Spectrophotometric Studies on the Complex Formation of Iron(III) with 4-(2-Thiazolylazo)Resorcinol and Tetrazolium Salts

Kiril B. Gavazov* Teodora S. Stefanova & Galya K. Toncheva

Department of General and Inorganic Chemistry, University of Plovdiv “Paisii Hilendarskii”, 24 Tsar Assen Street, 4000 Plovdiv, Bulgaria

* Corresponding author: kgavazov@abv.bg

ABSTRACT

Four liquid-liquid extraction-chromogenic systems containing Fe(III), 4-(2-thiazolylazo)resorcinol (TAR), tetrazolium salt (TZS), water and chloroform were studied. 2,3,5-Triphenyl-2H-tetrazolium chloride (TTC), 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 3-(2-naphtyl)-2,5-diphenyl-2H-tetrazolium chloride (TV), and 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) were the examined TZSs. Optimization experiments for iron extraction were performed and the following parameters were found for each system: pH(opt), C_{TAR}(opt), C_{TZS}(opt), shaking time (opt), and ʎ(opt). Under the optimum conditions, the molar ratio of the reacting Fe(III), TAR and TZS is 1:2:2 and the general formula of the extracted species is (TZ^+)_2[Fe^{II}(TAR^–)_2]. Some equilibrium constants (constants of association, constants of distribution, and constants of extraction) and analytical characteristics (molar absorptivities, Sandell's sensitivities, Beer's law limits, etc.) were calculated. Linear relationships involving the molecular mass of TZ^+ were discussed.

Keywords: Iron-TAR chelate; Solvent extraction; Ion-association; Fe(III) reduction; Linear relationship.
INTRODUCTION

Iron(II) and iron(III) form colored complexes with heterocyclic azo derivatives of resorcinol, such as 4-(2-pyridylazo)resorcinol (PAR) [1-20], 4-(2-thiazolylazo)resorcinol (TAR) [8,14,16,21-26], 4-(5-nitro-2-pyridylazo)resorcinol [27], and 4-(4-methyl-2-thiazolylazo)resorcinol [28]. The complexes have high molar absorptivity and can be used for sensitive spectrophotometric determination of iron in various samples [2,7,11,14,21,28]. The anionic iron-PAR species, [Fe(III)(PAR)2]– and [Fe(II)(PAR)2]2–, which exist in aqueous solutions at defined pH and PAR concentration, are the most intensively colored. They can associate with bulky counterions Q+ such as tetradecyldimethylbenzylammonium [3-5,9,12] and 2,3,5-triphenyl-2H-tetrazolium [20], to form chloroform-extractable ternary complexes. The extraction equilibrium studies revealed some puzzling features of these complexes, including aggregation of Q[Fe(III)L2]– in the organic phase [9,20]. On the other hand, the investigations [5,10,14,18,29-31] showed the possibility of Fe(III)→Fe(II) reduction under the influence of PAR [5,10,14,18,29] or similar organic ligands [5,14,30,31]; they raise the question about the real oxidation state of iron in some analytical systems.

In this paper we describe results obtained during our liquid-liquid extraction-spectrophotometric investigations of systems containing Fe(III), TAR, tetrazolium salt (TZS), water, and chloroform. The preliminary investigations showed that the ternary complexes formed in such systems could compete successfully with similar Fe-R-Q complexes, where R is PAR, 1,10-phenanthroline [32], 2,2’-dipyridyl [32,33], 4,7-diphenyl-1,10-phenanthroline [32], thiocyanate [34], 4-nitrocatechol [35], 2,3-dihydroxynaphtalene [31], ferron [36], etc.

The following TZSs, which have been used as reagents in inorganic analysis [37,38], were selected for the present study: i) 2,3,5-triphenyl-2H-tetrazolium chloride (TTC); ii) 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT); iii) 3-(2-naphthyl)-2,5-diphenyl-2H-tetrazolium chloride (Tetrazolium violet, TV); and iv) 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT). Their structural formulae are shown in Table 1.

| Table 1. Tetrazolium salts used in the present study |
|-----------------------------------------------------|
| **Formula** | **Name of tetrazolium salt and abbreviation** | **Abbreviation and molecular mass of the tetrazolium cation** |
| ![Formula Image](image1.png) | 2,3,5-Triphenyl-2H-tetrazolium chloride (TTC) | TT+ MTT+=298.96 |
| ![Formula Image](image2.png) | 3-(4,5-Dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) | MTT+ MMT+=334.41 |
| ![Formula Image](image3.png) | 3-(2-Naphthyl)-2,5-diphenyl-2H-tetrazolium chloride (Tetrazolium violet, TV) | TV+ MTv+=349.41 |
| ![Formula Image](image4.png) | 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) | INT+ MINT+=470.25 |
EXPERIMENTAL

Reagents and apparatus

A stock iron(III) solution (1 mg cm⁻³; 1 dm³) was prepared by dissolving 8.6350 g of FeNH₄(SO₄)₂·12H₂O (99.1%; Reanal, Hungry) in water containing 5 cm³ of conc. H₂SO₄ [31,39]. Working solutions (50 µg cm⁻³) were prepared every day by suitable dilution of the stock solution with 0.01 mol dm⁻³ H₂SO₄. TAR and TV were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany); MTT, INT and TTC were purchased from Alfa Aesar (Karlsruhe, Germany), AppliChem GmbH (Darmstadt, Germany) and Loba Feinchemie GmbH (Fischamend, Austria), respectively. Aqueous solutions of these reagents were prepared. The concentrations were 2×10⁻³ mol dm⁻³ (for TAR, TV and INT), 3×10⁻³ mol dm⁻³ (for MTT), and 4×10⁻³ mol dm⁻³ (for TTC). Distilled chloroform was used. The acidity of the aqueous medium was set by the addition of buffer solution prepared by mixing 2 mol dm⁻³ aqueous solutions of CH₃COOH and NH₄OH. The resulting pH was checked by a HI-83140 pH meter. A Camspec M508 spectrophotometer (United Kingdom), equipped with 10 mm path-length cells, was employed for reading the absorbance.

Procedure for establishing the optimum conditions

Aliquots of Fe(III) solution, TAR solution (up to 2.0 cm³), TS solution (up to 2.0 cm³) and buffer solution (3 cm³; pH ranging from 3.9 to 9.2) were introduced into 100-cm³ separatory funnels. The resulting solutions were diluted with distilled water to a total volume of 10 cm³. Then 10 cm³ of chloroform were added and the funnels were shaken for a fixed time (up to 4.0 min). A portion of each organic extract was transferred through a paper filter into a cell and the absorbance was read against a blank.

Procedure for determining the constants of distribution

The distribution constants Kᵢ were found from the ratio Kᵢ = ΔAᵢ/ΔA₋₃₋₁ where ΔAᵢ is the absorbance (measured against a blank) after a single extraction under the optimum operating conditions (Table 2) and ΔA₋₃₋₁ is the absorbance obtained after a triple extraction (x=3; TZS=MTT, TV and INT) or double extraction (x=2; TZS=TTC) under the same conditions. The single extraction and the first stage of the double or triple extractions were performed with 10 cm³ of chloroform. The organic layers were transferred into 25 cm³ calibrated flasks and the flask for the single extraction was brought to volume with chloroform. The second stage was performed by adding 10 cm³ (x=2) or 7 mL (x=3) of chloroform to the aqueous phase that remained after the first stage. The shaking time was 2 min. The third stage (x=3) was performed in the same manner (with 7 cm³ of chloroform). The organic layers (x=2 or 3) were transferred to the flask containing the extract obtained during the first stage. The volume was brought to the mark with chloroform and shaken for homogenization.

Table 2. Optimum extraction-spectrophotometric conditions

| Extraction system | λ/nm  | pH      | C_TAR/mol dm⁻³ | C_TTZS/mol dm⁻³ | Extraction time/min |
|-------------------|-------|---------|----------------|-----------------|--------------------|
| Fe(III)-PAR-TTC   | 615   | 5.5-6.6 | (2.0-3.0)×10⁻³ | (4.0-8.0)×10⁻⁴ | 1-2                |
| Fe(III)-PAR-MTT   | 620   | 6.0-6.6 | (1.6-2.0)×10⁻³ | (1.8-3.0)×10⁻⁴ | 1-2                |
| Fe(III)-PAR-TV    | 620   | 6.5-7.2 | (1.6-2.0)×10⁻³ | (1.2-2.4)×10⁻⁴ | 1-2                |
| Fe(III)-PAR-INT   | 620   | 6.3-6.5 | (1.8-2.0)×10⁻³ | (2.6-2.8)×10⁻⁴ | 1-2                |

RESULTS AND DISCUSSION

The choice of the optimum wavelength

Spectra of the extracted ternary complexes are shown in Figure 1. Since the absorbance of the blank (curves 1', 2', 3' and 4') is high at λ<550 nm, the maxima of the Fe-TAR-TTC (curve 1) and Fe-TAR-INT (curve 4) complexes at about 495 nm were inappropriate for absorbance measurements. The maxima at about 545-550 nm of the same two complexes were also unsuitable; they are sharp, pH-dependent, and TAR-concentration dependent. The nature of the mentioned maxima can be understood if we take into account the competitive extraction of coloured ion-pairs (TZ')|TAR') [40] in the Fe-TAR-TTZS systems. As a result, minima appear in the recorded spectral curves of the complexes (ΔΔλᵢ = A_FE_TAR-TTS (λᵢ) – A_TAR-TZS (λᵢ)) when λᵢ is close to λ_max of (TZ')|TAR'). In the previous paper [40] we showed that the constants of association between TZ' and TAR follow the sequence β_{TT'}|INT(TAR) < β_{INT+INT(TAR)} < β_{MTT+INT(TAR)} < β_{TV+INT(TAR)}. The relatively unstable (TT')|TAR) and (INT)|TAR) cause small minima (λₘₑₙ=530 nm) in the resulting spectral curves 1 and 4. We consider these small minima responsible for the manifestation of the local maxima at λ 545-550 nm. The more stable (MTT')|TAR) causes deeper minimum (at about 515 nm) in the Fe-TAR-MTT spectral curve 2. As a result, there is no additional maximum in the mentioned spectral region. The same is valid for the system with TV (curve 3); TV produces the most stable ion-pair (TV')|TAR) and the resultant absorbance ΔA is even negative when λ<525 nm (curve 3).

Figure 1 shows that all four Fe-TAR-TTS complexes have maxima at about 615-620 nm. The absorbance of the blank is negligible in this spectral region and the obtained results are stable in time, reproducible and reliable. In addition, the recorded maxima of other investigated ternary M-TAR-TTZS complexes (M=V(V) [41,42], V(IV) [43], Ga(III) [44], and Co
These specifics gave us a reason to perform all further absorbance measurements at 615-620 nm ($\lambda_{\text{max}}=615$ nm when TZS=TTC; $\lambda_{\text{max}}=620$ nm when TZS=MTT, TV or INT).

Effect of pH

The effect of pH on the absorbance of the extracted ternary complexes is shown in Figure 2 (curves 1-4). The widest optimum pH-interval was recorded for the TTC-complex (pH 5.5-6.6; curve 1) and the narrowest optimum pH-interval was recorded for the INT-complex (pH 6.3-6.5; curve 4). Buffer solutions, prepared from acetic acid and ammonium hydroxide, were applied to control pH throughout the work.

Effect of Shaking Time

Extraction equilibrium is reached for about 35-40 seconds. However, in order to avoid accidental errors caused by a combination of short shaking times and different shaking rates, we extracted for 2 min in our experiments.
Effect of Reagents Concentration

The effect of TAR and TZS concentrations on the absorbance of the extracted species is illustrated in Figure 3 and Figure 4. The saturation is reached most easily in the extraction system containing TV (Figure 3, curve 3 and Figure 4, curve 3). The optimum reagents concentrations deduced from the Figures 3 and 4 are shown in Table 2.

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

**Fig 4**: Absorbance of the extracted Fe-TAR-TZS complexes vs. concentration of the TZS plots; $C_{\text{Fe(III)}}=2.24\times10^{-6}$ mol dm$^{-3}$. (1) $C_{\text{TAR}}=6.0\times10^{-4}$ mol dm$^{-3}$, $\lambda=615$ nm; (2) $C_{\text{TAR}}=1.8\times10^{-4}$ mol dm$^{-3}$, $\lambda=620$ nm; (3) $C_{\text{TAR}}=2.0\times10^{-4}$ mol dm$^{-3}$, $\lambda=620$ nm; (4) $C_{\text{TAR}}=2.0\times10^{-4}$ mol dm$^{-3}$, $\lambda=620$ nm.

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 $C_{\text{TAR}}>(6.0-8.0)\times10^{-5}$ mol dm$^{-3}$ and $C_{\text{TZS}}>(3.0-8.0)\times10^{-5}$ mol dm$^{-3}$ (see Table 3,*).
Table 3. Determination of the TAR-to-Fe and TZS-to-Fe molar ratios by the straight-line method of Asmus*

| Extraction system            | Squared correlation coefficient values (R²) corresponding to molar ratios of 1, 2 and 3 |
|-----------------------------|------------------------------------------------------------------------------------------|
| Fe(III)-TAR-TTC-water-chloroform |                                                                                         |
| Fe(III)-TAR-MTT-water-chloroform |                                                                                         |
| Fe(III)-TAR-TV-water-chloroform |                                                                                         |
| Fe(III)-TAR-INT-water-chloroform |                                                                                         |

a – $C_{\text{TAR}}$ varies from 6.0×10⁻⁵ mol dm⁻³ to 2.0×10⁻⁴ mol dm⁻³;

b – $C_{\text{TAR}}$ varies from 8.0×10⁻⁵ mol dm⁻³ to 1.8×10⁻⁴ mol dm⁻³;

c – $C_{\text{TAR}}$ varies from 8.0×10⁻⁵ mol dm⁻³ to 1.6×10⁻⁴ mol dm⁻³;

d – $C_{\text{TAR}}$ varies from 6.0×10⁻⁵ mol dm⁻³ to 1.6×10⁻⁴ mol dm⁻³;

e – $C_{\text{TAR}}$ varies from 8.0×10⁻⁵ mol dm⁻³ to 3.6×10⁻⁴ mol dm⁻³;

f – $C_{\text{MTT}}$ varies from 3.9×10⁻⁵ mol dm⁻³ to 2.4×10⁻⁴ mol dm⁻³;

g – $C_{\text{TV}}$ varies from 3.6×10⁻⁵ mol dm⁻³ to 9.6×10⁻⁵ mol dm⁻³;

h – $C_{\text{INT}}$ 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.

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**

$$\text{Fe}^{\text{III}} + 2\text{HTAR}^- + e^- \rightarrow [\text{Fe}^{\text{III}}(\text{TAR})_2]^{2-} + 2\text{H}^+$$  \hspace{1cm} (1.1)

$$[\text{Fe}^{\text{III}}(\text{TAR})_2]^{2-} + 2\text{TZ}^- \rightarrow (\text{TZ}')_2[\text{Fe}^{\text{III}}(\text{TAR})_2]$$  \hspace{1cm} (1.2)

**Scheme 2**

$$\text{Fe}^{\text{III}} + 2\text{HTAR}^- + \text{H}_2\text{O} \rightarrow [\text{Fe}^{\text{III}}(\text{OH})(\text{TAR})_2]^{2-} + 3\text{H}^+$$  \hspace{1cm} (2.1)

$$[\text{Fe}^{\text{III}}(\text{OH})(\text{TAR})_2]^{2-} + 2\text{TZ}^- \rightarrow (\text{TZ}')_2[\text{Fe}^{\text{III}}(\text{OH})(\text{TAR})_2]$$  \hspace{1cm} (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 [14] and/or iron-PAR [5,6,14,18] complexes. In this relation, the following statements seem particularly relevant to be highlighted:

i) “Once formed, the Fe²⁺L₂⁺ chelate reduces spontaneously in alkaline solution to Fe²⁺L₂⁻ [14];

ii) “No Fe³⁺ chelate was formed at low iron concentrations (up to ≈ 30 μmol) suggesting that Fe⁰ is reduced to Fe²⁺ by excess PAR.” [18] (p. 45);

iii) “The colour of PAR chelates of ... Fe(III) faded gradually. But, the color of PAR chelates of Cr(III), Fe(II), Co(III) ... did not change for two hours ... The inert nature of d⁸ (Cr(III)), diamagnetic d⁸ (Fe(II), Co(III)), and d⁶ (Ni(II)) is due to the large crystal field activation energies for the substitution reaction and the diamagnetic d⁸ system has the largest activation energy among these systems.” [5]
iv) "The IR data support that the ligand is coordinated to the metal ions [Fe\textsuperscript{II}, Cu\textsuperscript{II}, Zn\textsuperscript{II} and Cd\textsuperscript{II}] in a terdentate manner and complexation with 4-(2-thiazolylazo)resorcinol through the resorcinol OH, azo N and thiazole N.\textsuperscript{[25]}

v) "Co-ordination of two molecules of a terdentate ligand would lead to an octahedral complex, lacking a free position for attaching hydroxy groups."\textsuperscript{[6]}

It should be added, that our previous investigations on cobalt-containing extraction systems, namely Co(II)–TAR-TTC-water-chloroform \textsuperscript{[45]}, Co(II)-PAR-TTC-water-chloroform \textsuperscript{[48]}, Co(II)-PAR-INT-water-chloroform \textsuperscript{[48]}, and Co(II)-PAR-nitron-water-chloroform \textsuperscript{[49]} indicated fast and trouble-free oxidation of Co(II) (d\textsuperscript{2}) to Co(III) (d\textsuperscript{3}). Hence, the suggested Fe(III)→Fe(II) reduction, which ensures the same diamagnetic d\textsuperscript{6} electron configuration, can hardly even be regarded as a surprise.

**Equilibrium constants and recovery**

The constants of association (β), characterising the processes of association, were calculated by two different methods: the mobile equilibrium method \textsuperscript{[46]} and the Holme-Langmihr method \textsuperscript{[50]}. The constants of distribution (K\textsubscript{D}) were determined by the mobile equilibrium method. The recovery factors (R\%) and the constants of extraction (K\textsubscript{ex}) were determined by the formulae

\[ R\% = 100 \times (K_{ex}/K_{D} + 1) \]

and

\[ K_{ex} = \Delta A/\Delta A_{1} \]

The reason for this anomaly (ΔA\textsubscript{1}≤ΔA\textsubscript{1}) is probably a time-dependent transformation of the ternary complex during its dilution with chloroform \textsuperscript{[42,51]}. In fact, the complex deriving from TTC has the lowest association constant (Table 4); hence, it should be the most susceptible to alteration.

The recovery factors (R%) and the constants of extraction (K\textsubscript{ex}) were determined by the formula for the Holme-Langmihr method, and the constants of distribution (K\textsubscript{D}) were calculated by two different methods: the mobile equilibrium method and the Holme-Langmihr method. The recovery factors and constants of extraction (K\textsubscript{ex}) were determined by the formulae R\%=100×K\textsubscript{ex}/(K\textsubscript{D}+1) and K\textsubscript{ex}=K\textsubscript{D}/(K\textsubscript{D}+1), respectively. The results are presented in Table 4. All experiments were performed at room temperature of ~22°C and the calculations were carried out at a probability of 95%.

**Table 4. Calculated values (P=95%) of the association constants (β), extraction constants (K\textsubscript{ex}), distribution constants (K\textsubscript{D}), and recovery factors (R%)**

| Extraction system                  | Log β  | Log K\textsubscript{D} | Log K\textsubscript{ex} | R%    |
|-----------------------------------|-------|------------------------|-------------------------|-------|
| Fe(III)-TAR-TTC-H\textsubscript{2}O-chloroform | 9.08±0.08\textsuperscript{a}, 9.0±0.7\textsuperscript{b} | 1.57±0.01\textsuperscript{a} | 10.65±0.09\textsuperscript{a} | 97.41±0.05\textsuperscript{a} |
| Fe(III)-TAR-MTT-H\textsubscript{2}O-chloroform  | 9.44±0.08\textsuperscript{a}, 9.5±0.6\textsuperscript{d} | 1.45±0.01\textsuperscript{a} | 10.89±0.09\textsuperscript{a} | 96.58±0.05\textsuperscript{a} |
| Fe(III)-TAR-TV-H\textsubscript{2}O-chloroform  | 9.8±2.2\textsuperscript{a}, 9.7±1.3\textsuperscript{d} | 1.53±0.01\textsuperscript{a} | 11.3±0.2\textsuperscript{a} | 97.14±0.09\textsuperscript{a} |
| Fe(III)-TAR-INT-H\textsubscript{2}O-chloroform  | 9.2±0.1\textsuperscript{a}, 9.3±0.2\textsuperscript{d} | 1.01±0.002\textsuperscript{d} | 10.2±0.1\textsuperscript{c} | 91.12±0.04\textsuperscript{c} |

a – Calculated by the Holme-Langmihr method
b – Calculated by the Mobile equilibrium method
c – Calculated by the equation K\textsubscript{D}=ΔA/ΔA\textsubscript{1}; x=2
d – Calculated by the equation K\textsubscript{D}=ΔA\textsubscript{1}/ΔA\textsubscript{1}; x=3
e – Calculated by the equation K\textsubscript{ex}=K\textsubscript{D}/(K\textsubscript{D}+1), where β is the value obtained by the Holme-Langmihr method
f – Calculated by the equation R\%=100×K\textsubscript{ex}/(K\textsubscript{D}+1)

**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 absorbivities (ε) are listed in Table 5, along with some important characteristics concerning the application of the ternary complexes for extraction-spectrophotometric determination of iron. The couples FE–TAR ensure high sensitivity of determination. In this criterion, they are better than many reagents used in similar systems: TAR (ε\textsubscript{TAR}=2.9×10\textsuperscript{5} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; aqueous medium) \textsuperscript{[21]}, TTS (ε\textsubscript{TTS}=2.2×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; TTS=TTT, ε\textsubscript{TTS}=5.3×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; TTS=INT, ε\textsubscript{TTS}=1.1×10\textsuperscript{4} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; TTS=Neotetrazolium chloride; methyl isobutyl ketone extraction) \textsuperscript{[37]}, 4-nitrocatechol–TTC (ε\textsubscript{TCT}=2.7×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; chloroform extraction) \textsuperscript{[35]}, PAR–TTC (ε\textsubscript{TTC}=4.7×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; chloroform extraction) \textsuperscript{[20]}, PAR–zephiramine (ε\textsubscript{TCT}=4.0×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; chloroform extraction) \textsuperscript{[3–5]}, 4-(methyl-2-thiazolylazo)resorcin (ε\textsubscript{TAR}=2.49×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; aqueous medium) \textsuperscript{[28]}, 2-(2-thiazolylazo)-5-dimethylaminophenol (ε\textsubscript{TAR}=2.7×10\textsuperscript{3} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; chloroform extraction) \textsuperscript{[54]}, 2-(2-thiazolylazo)-4-methylphenol (ε\textsubscript{TAR}=1.37×10\textsuperscript{4} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; chloroform extraction) \textsuperscript{[55]}, 2-(4,5-dimethyl-2-thiazolylazo)-4,6-dimethylphenol (ε\textsubscript{TAR}=1.38×10\textsuperscript{4} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1}; chloroform extraction) \textsuperscript{[56]}, and 1-(2-thiazolylazo)-2-napthol (ε\textsubscript{TAR}=1.83×10\textsuperscript{4} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1} (aqueous medium) \textsuperscript{[57]}) or ε\textsubscript{TAR}=1.89×10\textsuperscript{4} dm\textsuperscript{3} mol\textsuperscript{-1} cm\textsuperscript{-1} (chloroform extraction) \textsuperscript{[55]}.
### Table 5. Characteristics concerning the application of the ion-association complexes for extractive-spectrophotometric determination of iron(III)

| Extraction system       | Fe(III)-TAR-TTC-H₂O-CHCl₃ | Fe(III)-TAR-MTT-H₂O-CHCl₃ | Fe(III)-TAR-TV-H₂O-CHCl₃ | Fe(III)-TAR-INT-H₂O-CHCl₃ |
|-------------------------|---------------------------|--------------------------|--------------------------|--------------------------|
| **Analytical characteristics** |                           |                          |                          |                          |
| Molar absorptivity (ε)/L mol⁻¹ cm⁻¹ | 5.0×10⁴                  | 5.13×10⁴                 | 5.3×10⁴                  | 4.5×10⁴                  |
| 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 (R²) | 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) |

### Relationships Involving the Molecular Mass of the Tetrazolium Cation

Continuous investigations on tetrazolium ion-association complexes have revealed that at least two factors noticeably influence the values of β: molecular mass (M) [58] (factor I) and the presence of nitro group(s) in the tetrazolium cation [59] (factor II). According to [58], a linear relationship exists between the M and β: the higher molecular mass, the higher the association constant. However, when TZ⁺ contains nitro group(s), the values of β are considerably lower [38,59-61] than those expected by the authors of Ref. [58].

The obtained in the present paper results confirm the significance of both factors (Fig. 5, points 1,1’ and 2,2’). The full markers (points 1 and points 2) represent the data for the ion-associates of TT⁺, MTT⁺ and TV⁺ (i.e., TZs⁺ which do not contain –NO₂ group) and the hollow markers (point 1’ and point 2’) represent the data for the ion-associate of INT⁺. Straight lines can be drawn through the points 1 or 2 with squared correlation coefficients of 1.000 (line 2) or close to 1 (line 1; R²=0.9401) (confirmation of factor I) and the points 1’ and 2’ are situated below these straight lines (confirmation of factor II).

The rest two experimental plots in Fig. 5 (3,3’ and 4,4’) show similar behaviour. The application of a linear model to the points 3 and 4 leads to high R² values (0.8504 and 0.9303, respectively) and the corresponding points for the NO₂-containing ion-associate (INT⁺)[Fe(TAR)₂] have lower ordinate values.

![Fig 5: Logarithm of K (left ordinate) and molar absorptivity ε (right ordinate; points 4,4’) vs. logarithm of the molecular mass of the tetrazolium cation (MTZ⁺) plots. K is the constants of extraction (points 3,3’) or the constant of association determined by the mobile equilibrium method (points 2,2’) or the Holme-Langmihr method (points 1,1’). Linear equations: (1) y=9.9948x-15.691 (R²=0.9401); (2) y=10.324x-16.559 (R²=1.000); (3) y=8.6791x-10.876 (R²=0.8504); and (4) y=4.0054x-4.938 (R²=0.9303).](image-url)

### 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:2ZS) under the optimum extraction conditions. The following general formula was suggested: \((TZ')_2[Fe(TAR)_2]\); in this formula TAR is doubly deprotonated \((TAR^{−})\) and iron is in its 2+ oxidation state.

3. The key equilibrium constants and analytical characteristics were determined: constants of association \(\beta\), constants of distribution \(K_0\), constants of extraction \(K_{ex}\), recovery factors \(R\%), molar absorptivities \(\varepsilon_{\lambda(\text{opt})}\), Sandell’s sensitivities, Beer’s law upper limits, limits of detection, and limits of quantification. Linear relationships appear to exist between the logarithm of the molecular mass of the tetrazolium cation \((\log M_{TZ^+})\) and some of the constants \((\log \beta, \log K_{ex},\) and \(\varepsilon_{\lambda(\text{opt})}\) for the complexes with \(TZ^+\) which do not contain NO₂ groups (i.e. \(TZ^+ = \text{T'T}, \text{MT'T}^+\) and \(\text{TV}^+\)); the higher \(M_{TZ^+}\), the higher the constant. The poorer extraction-spectrophotometric performance of \((\text{INT})_2[Fe(TAR)_2]\) can be explained with a negative influence of the nitro group.

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