



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

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### 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  $\lambda(opt)$ . 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  $(TZ^+)_2[Fe^{II}(TAR^{2-})_2]$ . 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.



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## 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,  $[\text{Fe}^{\text{III}}(\text{PAR})_2]^-$  and  $[\text{Fe}^{\text{II}}(\text{PAR})_2]^{2-}$ , which exist in aqueous solutions at defined pH and PAR concentration, are the most intensively colored. They can associate with bulky counterions  $\text{Q}^+$ , 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  $\text{Q}[\text{Fe}^{\text{III}}\text{L}_2]^-$  in the organic phase [9,20]. On the other hand, the investigations [5,10,14,18,29-31] showed the possibility of  $\text{Fe}(\text{III}) \rightarrow \text{Fe}(\text{II})$  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-dihydroxynaphthalene [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
	2,3,5-Triphenyl-2H-tetrazolium chloride (TTC)	$\text{TT}^+$ $M_{\text{TT}^+} = 298.96$
	3-(4,5-Dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT)	$\text{MTT}^+$ $M_{\text{MTT}^+} = 334.41$
	3-(2-Naphtyl)-2,5-diphenyl-2H-tetrazolium chloride (Tetrazolium violet, TV)	$\text{TV}^+$ $M_{\text{TV}^+} = 349.41$
	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT)	$\text{INT}^+$ $M_{\text{INT}^+} = 470.25$



## EXPERIMENTAL

### Reagents and apparatus

A stock iron(III) solution ( $1 \text{ mg cm}^{-3}$ ;  $1 \text{ dm}^3$ ) was prepared by dissolving 8.6350 g of  $\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  (99.1%; Reanal, Hungry) in water containing  $5 \text{ cm}^3$  of conc.  $\text{H}_2\text{SO}_4$  [31,39]. Working solutions ( $50 \text{ } \mu\text{g cm}^{-3}$ ) were prepared every day by suitable dilution of the stock solution with  $0.01 \text{ mol dm}^{-3}$   $\text{H}_2\text{SO}_4$ . TAR and TV were purchased from Sigma-Aldrich Chemie GmbH (Schnellendorf, 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  $2 \times 10^{-3} \text{ mol dm}^{-3}$  (for TAR, TV and INT),  $3 \times 10^{-3} \text{ mol dm}^{-3}$  (for MTT), and  $4 \times 10^{-3} \text{ mol dm}^{-3}$  (for TTC). Redistilled chloroform was used. The acidity of the aqueous medium was set by the addition of buffer solution prepared by mixing  $2 \text{ mol dm}^{-3}$  aqueous solutions of  $\text{CH}_3\text{COOH}$  and  $\text{NH}_4\text{OH}$ . The resulting pH was checked by a HI-83140 pH meter. A CamSpec M508 spectrophotometer (United Kingdom), equipped with  $10 \text{ mm}$  path-length cells, was employed for reading the absorbance.

### Procedure for establishing the optimum conditions

Aliquots of Fe(III) solution, TAR solution (up to  $2.0 \text{ cm}^3$ ), TS solution (up to  $2.0 \text{ cm}^3$ ) and buffer solution ( $3 \text{ cm}^3$ , pH ranging from 3.9 to 9.2) were introduced into  $100\text{-cm}^3$  separatory funnels. The resulting solutions were diluted with distilled water to a total volume of  $10 \text{ cm}^3$ . Then  $10 \text{ cm}^3$  of chloroform were added and the funnels were shaken for a fixed time (up to  $4.0 \text{ min}$ ). 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  $K_D$  were found from the ratio  $K_D = \Delta A_1 / (\Delta A_x - \Delta A_1)$  where  $\Delta A_1$  is the absorbance (measured against a blank) after a single extraction under the optimum operating conditions (Table 2) and  $\Delta A_x$  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 extraction were performed with  $10 \text{ cm}^3$  of chloroform. The organic layers were transferred into  $25 \text{ cm}^3$  calibrated flasks and the flask for the single extraction was brought to volume with chloroform. The second stage was performed by adding  $10 \text{ cm}^3$  ( $x=2$ ) or  $7 \text{ mL}$  ( $x=3$ ) of chloroform to the aqueous phase that remained after the first stage. The shaking time was  $2 \text{ min}$ . The third stage ( $x=3$ ) was performed in the same manner (with  $7 \text{ cm}^3$  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	$\lambda/\text{nm}$	pH	$C_{\text{TAR}}/\text{mol dm}^{-3}$	$C_{\text{TZs}}/\text{mol dm}^{-3}$	Extraction time/min
Fe(III)-PAR-TTC	615	5.5-6.6	$(2.0-3.0) \times 10^{-4}$	$(4.0-8.0) \times 10^{-4}$	1-2
Fe(III)-PAR-MTT	620	6.0-6.6	$(1.6-2.0) \times 10^{-4}$	$(1.8-3.0) \times 10^{-4}$	1-2
Fe(III)-PAR-TV	620	6.5-7.2	$(1.6-2.0) \times 10^{-4}$	$(1.2-2.4) \times 10^{-4}$	1-2
Fe(III)-PAR-INT	620	6.3-6.5	$(1.8-2.0) \times 10^{-4}$	$(2.6-2.8) \times 10^{-4}$	1-2

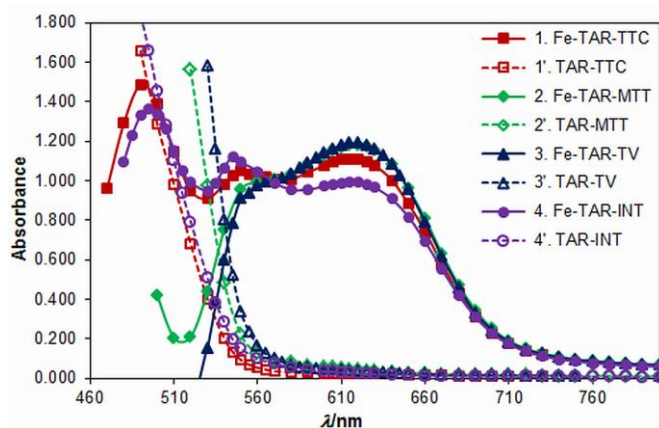
## RESULTS AND DISCUSSION

### 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  $\lambda < 550 \text{ nm}$ , the maxima of the Fe-TAR-TTC (curve 1) and Fe-TAR-INT (curve 4) complexes at about  $495 \text{ nm}$  were inappropriate for absorbance measurements. The maxima at about  $545\text{-}550 \text{ nm}$  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  $(\text{TZ}^+)(\text{TAR}^-)$  [40] in the Fe-TAR-TSZ systems. As a result, minima appear in the recorded spectral curves of the complexes ( $\Delta A_{\lambda_i} = A_{\text{Fe-TAR-TZS}}(\lambda_i) - A_{\text{TAR-TZS}}(\lambda_i)$ ) when  $\lambda_i$  is close to  $\lambda_{\text{max}}$  of  $(\text{TZ}^+)(\text{TAR}^-)$ . In the previous paper [40] we showed that the constants of association between  $\text{TZ}^+$  and  $\text{TAR}^-$  follow the sequence  $\beta_{(\text{TT}^+)(\text{TAR}^-)} < \beta_{(\text{INT}^+)(\text{TAR}^-)} < \beta_{(\text{MTT}^+)(\text{TAR}^-)} < \beta_{(\text{TV}^+)(\text{TAR}^-)}$ . The relatively unstable  $(\text{TT}^+)(\text{TAR}^-)$  and  $(\text{INT}^+)(\text{TAR}^-)$  cause small minima ( $\lambda_{\text{min}}=530 \text{ nm}$ ) in the resulting spectral curves 1 and 4. We consider these small minima responsible for the manifestation of the local maxima at  $\lambda$   $545\text{-}550 \text{ nm}$ . The more stable  $(\text{MTT}^+)(\text{TAR}^-)$  causes deeper minimum (at about  $515 \text{ nm}$ ) 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  $(\text{TV}^+)(\text{TAR}^-)$  and the resultant absorbance  $\Delta A$  is even negative when  $\lambda < 525 \text{ nm}$  (curve 3).

Figure 1 shows that all four Fe-TAR-TZS complexes have maxima at about  $615\text{-}620 \text{ nm}$ . 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-TZS complexes ( $\text{M}=\text{V(V)}$  [41,42],  $\text{V(IV)}$  [43],  $\text{Ga(III)}$  [44], and  $\text{Co}$

[45];  $\lambda_{V(IV,V)-TAR-TZS}=550$  nm [41-43],  $\lambda_{Ga-TAR-TTC}=530$  nm [44], and  $\lambda_{Co-TAR-TTC}=525$  nm [45]) are rather far from this region. These specifics gave us a reason to perform all further absorbance measurements at 615-620 nm ( $\lambda_{max}=615$  nm when TZS=TTC;  $\lambda_{max}=620$  nm when TZS=MTT, TV or INT).

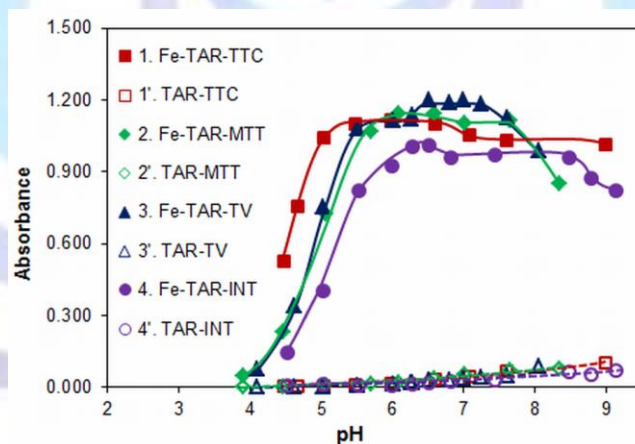


**Fig 1: Absorption spectra of the ternary complexes (curves 1-4;  $C_{Fe(III)}=2.24 \times 10^{-5}$  mol dm $^{-3}$ ) and blank samples (curves 1'-4') in chloroform at the optimum extraction conditions.**

- (1, 1')  $C_{TAR}=2.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{TTC}=4.7 \times 10^{-4}$  mol dm $^{-3}$ , pH=6.0;  
 (2, 2')  $C_{TAR}=1.8 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{MTT}=3.0 \times 10^{-4}$  mol dm $^{-3}$ , pH=6.3;  
 (3, 3')  $C_{TAR}=2.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{TV}=2.1 \times 10^{-4}$  mol dm $^{-3}$ , pH=6.7;  
 (4, 4')  $C_{TAR}=2.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{INT}=2.6 \times 10^{-4}$  mol dm $^{-3}$ , pH=6.4.

### 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.



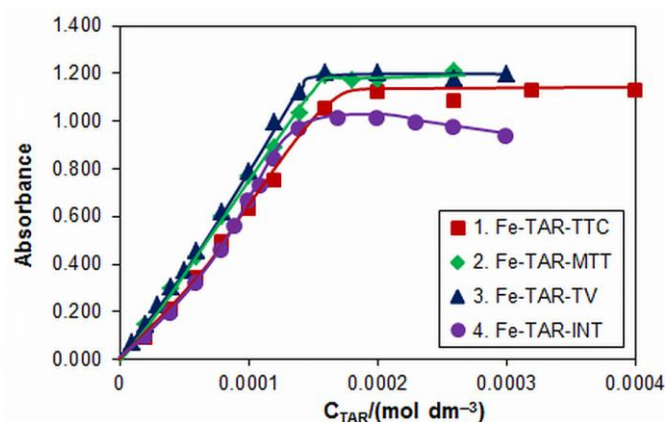
**Fig 2: Absorbance of the ternary Fe-TAR-TZS complexes (curves 1-4;  $C_{Fe(III)}=2.24 \times 10^{-5}$  mol dm $^{-3}$ ) and the blank samples (curves 1'-4') in chloroform vs. pH of the aqueous phase. (1, 1')  $C_{TAR}=3.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{TTC}=8.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $\lambda=615$  nm; (2, 2')  $C_{TAR}=2.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{MTT}=3.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $\lambda=620$  nm; (3, 3')  $C_{TAR}=2.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{TV}=2.4 \times 10^{-4}$  mol dm $^{-3}$ ,  $\lambda=620$  nm; (4, 4')  $C_{TAR}=2.0 \times 10^{-4}$  mol dm $^{-3}$ ,  $C_{INT}=2.6 \times 10^{-4}$  mol dm $^{-3}$ ,  $\lambda=620$  nm.**

### Effect of Shaking Time

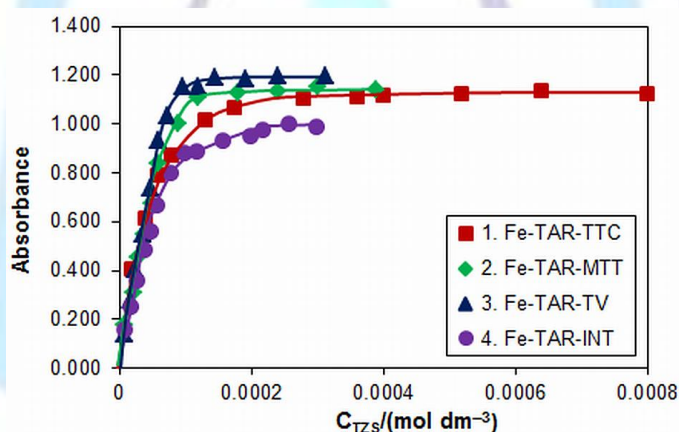
Extraction equilibrium is reached for about 35-40 seconds. However, in order to avoid accidental errors caused by a combination of short shaking times and different shaking rates, we extracted for 2 min in our experiments

## 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;  $C_{Fe(III)}=2.24 \times 10^{-6}$  mol dm<sup>-3</sup>. (1)  $C_{TTC}=6.0 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=615$  nm; (2)  $C_{MTT}=3.0 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=620$  nm; (3)  $C_{TV}=2.4 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=620$  nm; (4)  $C_{INT}=2.6 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=620$  nm.



**Fig 4:** Absorbance of the extracted Fe-TAR-TZS complexes vs. concentration of the TZS plots;  $C_{Fe(III)}=2.24 \times 10^{-6}$  mol dm<sup>-3</sup>. (1)  $C_{TAR}=3.0 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=615$  nm; (2)  $C_{TAR}=1.8 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=620$  nm; (3)  $C_{TAR}=2.0 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=620$  nm; (4)  $C_{TAR}=2.0 \times 10^{-4}$  mol dm<sup>-3</sup>,  $\lambda=620$  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  $C_{TAR} > (6.0-8.0) \times 10^{-5}$  mol dm<sup>-3</sup> and  $C_{TZS} > (3.0-8.0) \times 10^{-5}$  mol dm<sup>-3</sup> (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 ( $R^2$ ) corresponding to molar ratios of 1, 2 and 3	
	TAR:Fe	TZS:Fe
Fe(III)-TAR-TTC-water-chloroform	0.9879 (n=1) <sup>a</sup>	0.9676 (n=1) <sup>e</sup>
	0.9939 (n=2) <sup>a</sup>	0.9994 (n=2) <sup>e</sup>
	0.9594 (n=3) <sup>a</sup>	0.9817 (n=3) <sup>e</sup>
Fe(III)-TAR-MTT-water-chloroform	0.9888 (n=1) <sup>b</sup>	0.9351 (n=1) <sup>f</sup>
	0.9914 (n=2) <sup>b</sup>	0.9862 (n=2) <sup>f</sup>
	0.9793 (n=3) <sup>b</sup>	0.9643 (n=3) <sup>f</sup>
Fe(III)-TAR-TV-water-chloroform	0.9867 (n=1) <sup>c</sup>	0.9757 (n=1) <sup>g</sup>
	0.9955 (n=2) <sup>c</sup>	0.9963 (n=2) <sup>g</sup>
	0.9863 (n=3) <sup>c</sup>	0.9832 (n=3) <sup>g</sup>
Fe(III)-TAR-INT-water-chloroform	0.9670 (n=1) <sup>d</sup>	0.9653 (n=1) <sup>h</sup>
	0.9865 (n=2) <sup>d</sup>	0.9929 (n=2) <sup>h</sup>
	0.9657 (n=3) <sup>d</sup>	0.9419 (n=3) <sup>h</sup>

a –  $C_{TAR}$  varies from  $6.0 \times 10^{-5}$  mol dm<sup>-3</sup> to  $2.0 \times 10^{-4}$  mol dm<sup>-3</sup>;

b –  $C_{TAR}$  varies from  $8.0 \times 10^{-5}$  mol dm<sup>-3</sup> to  $1.8 \times 10^{-4}$  mol dm<sup>-3</sup>;

c –  $C_{TAR}$  varies from  $8.0 \times 10^{-5}$  mol dm<sup>-3</sup> to  $1.6 \times 10^{-4}$  mol dm<sup>-3</sup>;

d –  $C_{TAR}$  varies from  $6.0 \times 10^{-5}$  mol dm<sup>-3</sup> to  $1.6 \times 10^{-4}$  mol dm<sup>-3</sup>;

e –  $C_{TTC}$  varies from  $8.0 \times 10^{-5}$  mol dm<sup>-3</sup> to  $3.6 \times 10^{-4}$  mol dm<sup>-3</sup>;

f –  $C_{MTT}$  varies from  $3.9 \times 10^{-5}$  mol dm<sup>-3</sup> to  $2.4 \times 10^{-4}$  mol dm<sup>-3</sup>;

g –  $C_{TV}$  varies from  $3.6 \times 10^{-5}$  mol dm<sup>-3</sup> to  $9.6 \times 10^{-5}$  mol dm<sup>-3</sup>;

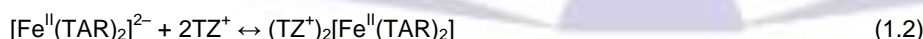
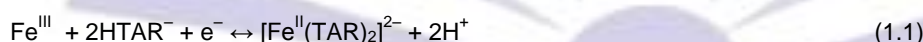
h –  $C_{INT}$  varies from  $3.0 \times 10^{-5}$  mol dm<sup>-3</sup> to  $2.2 \times 10^{-4}$  mol dm<sup>-3</sup>;

\* – 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.

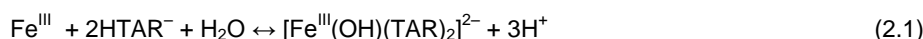
### Suggested Reaction Scheme and General Formula of the Ternary Complexes

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

#### Scheme 1



#### Scheme 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  $\text{Fe}^{\text{III}}\text{L}_2^{2-}$  chelate reduces spontaneously in alkaline solution to  $\text{Fe}^{\text{II}}\text{L}_2^{2-}$ " [14];
- ii) "No  $\text{Fe}^{\text{III}}$  complex was formed at low iron concentrations (up to  $\approx 30 \mu\text{mol}$ ) suggesting that  $\text{Fe}^{\text{III}}$  is reduced to  $\text{Fe}^{\text{II}}$  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  $d^3$  (Cr(III)), diamagnetic  $d^6$  (Fe(II), Co(III)), and  $d^8$  (Ni(II)) is due to the large crystal field activation energies for the substitution reaction and the diamagnetic  $d^6$  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<sup>II</sup>, Cu<sup>II</sup>, Zn<sup>II</sup> and Cd<sup>II</sup>] in a terdentate manner and complexation with 4-(2-thiazolylazo)resorcinol through the resorcinol OH, azo N and thiazole N." [25]

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

It should be added, that our previous investigations on cobalt-containing extraction systems, namely Co(II)-TAR-TTC-water-chloroform [45], Co(II)-PAR-TTC-water-chloroform [48], Co(II)-PAR-INT-water-chloroform [48], and Co(II)-PAR-nitron-water-chloroform [49] indicated fast and trouble-free oxidation of Co(II) (d<sup>7</sup>) to Co(III) (d<sup>6</sup>). Hence, the suggested Fe(III)→Fe(II) reduction, which ensures the same diamagnetic d<sup>6</sup> electron configuration, can hardly even be regarded as a surprise.

### Equilibrium constants and recovery

The constants of association ( $\beta$ ), characterising the processes of association, were calculated by two different methods: the mobile equilibrium method [46] and the Holme-Langmihr method [50]. The constants of distribution ( $K_D$ ) characterizing the processes of distribution of the ternary complexes between the aqueous and chloroform phases were determined as described above. It should be mentioned that the triple extraction cycle for the Fe-TAR-TTC system was found inappropriate for calculating  $K_D$  ( $K_D = \Delta A_1 / (\Delta A_3 - \Delta A_1)$ ;  $\Delta A_3 \leq \Delta A_1$ ). The reason for this anomaly ( $\Delta A_3 \leq \Delta A_1$ ) is probably a time-dependent transformation of the ternary complex during its dilution with chloroform [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 ( $K_{ex}$ ) were determined by the formulae  $R\% = 100 \times K_D / (K_D + 1)$  and  $K_{ex} = K_D \times \beta$  [52,53], 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 ( $\beta$ ), extraction constants ( $K_{ex}$ ), distribution constants ( $K_D$ ), and recovery factors (R%)**

Extraction system	Log $\beta$	Log $K_D$	Log $K_{ex}$	R%
Fe(III)-TAR-TTC-H <sub>2</sub> O-chloroform	9.08±0.08 <sup>a</sup> , 9.0±0.7 <sup>b</sup>	1.57±0.01 <sup>c</sup>	10.65±0.09 <sup>e</sup>	97.41±0.05 <sup>f</sup>
Fe(III)-TAR-MTT-H <sub>2</sub> O-chloroform	9.44±0.08 <sup>a</sup> , 9.5±0.6 <sup>b</sup>	1.45±0.01 <sup>d</sup>	10.89±0.09 <sup>e</sup>	96.58±0.05 <sup>f</sup>
Fe(III)-TAR-TV-H <sub>2</sub> O-chloroform	9.8±0.2 <sup>a</sup> , 9.7±1.3 <sup>b</sup>	1.53±0.01 <sup>d</sup>	11.3±0.2 <sup>e</sup>	97.14±0.09 <sup>f</sup>
Fe(III)-TAR-INT-H <sub>2</sub> O-chloroform	9.2±0.1 <sup>a</sup> , 9.3±0.2 <sup>b</sup>	1.011±0.002 <sup>d</sup>	10.2±0.1 <sup>e</sup>	91.12±0.04 <sup>f</sup>

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  $\beta$  is the value obtained by the Holme-Langmihr method

f – Calculated by the equation  $R\% = 100 \times K_D / (K_D + 1)$

### 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 absorptivities ( $\epsilon$ ) 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 TZS-TAR ensure high sensitivity of determination. In this criterion, they are better than many reagents used in similar systems: TAR ( $\epsilon_{730} = 2.9 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; aqueous medium) [21], TZS ( $\epsilon_{480} = 2.2 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  (TZS=TTC),  $\epsilon_{555} = 5.3 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  (TZS=MTT),  $\epsilon_{480} = 1.1 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  (TZS=INT),  $\epsilon_{500} = 2.2 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  (TZS=Neotetrazolium chloride); methyl isobutyl ketone extraction) [37], 4-nitrocatechol-TTC ( $\epsilon_{435} = 2.7 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; chloroform extraction) [35], PAR-TTC ( $\epsilon_{540} = 4.7 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; chloroform extraction) [20], PAR-zephiramine ( $\epsilon_{522} = (4.0-4.5) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; chloroform extraction) [3-5], 4-(4-methyl-2-thiazolylazo)resorcinol ( $\epsilon_{735} = 2.49 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; aqueous medium) [28], 2-(2-thiazolylazo)-5-dimethylaminophenol ( $\epsilon_{760} = 2.70 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; chloroform extraction) [54], 2-(2-thiazolylazo)-4-methylphenol ( $\epsilon_{762} = 1.37 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; chloroform extraction) [55], 2-(4,5-dimethyl-2-thiazolylazo)-4,6-dimethylphenol ( $\epsilon_{773} = 1.38 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ; chloroform extraction) [56], and 1-(2-thiazolylazo)-2-naphthol ( $\epsilon_{787} = 1.83 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  (aqueous medium) [57] or  $\epsilon_{786} = 1.89 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  (chloroform extraction) [55]).

**Table 5. Characteristics concerning the application of the ion-association complexes for extractive-spectrophotometric determination of iron(III)**

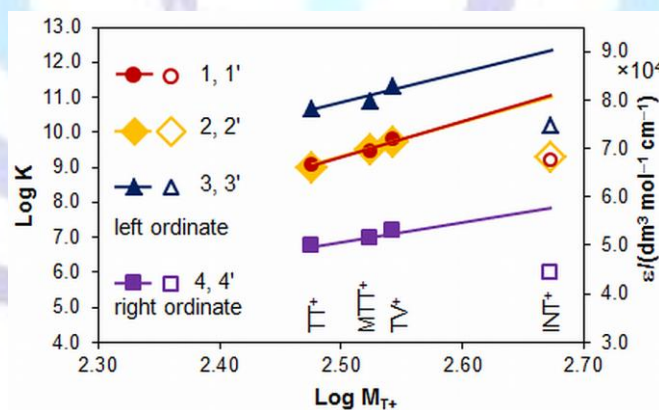
Extraction system	Fe(III)-TAR-TTC-H <sub>2</sub> O-CHCl <sub>3</sub>	Fe(III)-TAR-MTT-H <sub>2</sub> O-CHCl <sub>3</sub>	Fe(III)-TAR-TV-H <sub>2</sub> O-CHCl <sub>3</sub>	Fe(III)-TAR-INT-H <sub>2</sub> O-CHCl <sub>3</sub>
<b>Analytical characteristics</b>				
Molar absorptivity ( $\epsilon$ )/L mol <sup>-1</sup> cm <sup>-1</sup>	5.0×10 <sup>4</sup>	5.13×10 <sup>4</sup>	5.3×10 <sup>4</sup>	4.5×10 <sup>4</sup>
Sandell's sensitivity (SS)/ ng cm <sup>-2</sup>	1.12	1.09	1.06	1.25
Adherence to Beer's law/ $\mu$ g cm <sup>-3</sup>	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)/ $\mu$ g cm <sup>-3</sup>	0.049	0.031	0.073	0.070
Limit of quantification (LOQ)/ $\mu$ g cm <sup>-3</sup>	0.16	0.11	0.25	0.23
Absorbance of the blank at $\lambda_{opt}$ (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  $\beta$ : 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  $\beta$ : the higher molecular mass, the higher the association constant. However, when TZ<sup>+</sup> contains nitro group(s), the values of  $\beta$  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<sup>+</sup>, MTT<sup>+</sup> and TV<sup>+</sup> (i.e. TZs<sup>+</sup> which do not contain -NO<sub>2</sub> group) and the hollow markers (point 1' and point 2') represent the data for the ion-associate of INT<sup>+</sup>. 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;  $R^2=0.9401$ ) (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  $R^2$  values (0.8504 and 0.9303, respectively) and the corresponding points for the NO<sub>2</sub>-containing ion-associate (INT<sup>+</sup>)<sub>2</sub>[Fe(TAR)<sub>2</sub>] have lower ordinate values.



**Fig 5: Logarithm of K (left ordinate) and molar absorptivity  $\epsilon$  (right ordinate; points 4,4') vs. logarithm of the molecular mass of the tetrazolium cation ( $M_{TZ^+}$ ) 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)  $y=9.9948x-15.691$  ( $R^2=0.9401$ ); (2)  $y=10.324x-16.559$  ( $R^2=1.000$ ); (3)  $y=8.6791x-10.876$  ( $R^2=0.8504$ ); and (4)  $y=4.0054x-4.938$  ( $R^2=0.9303$ ).**

## 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<sup>+</sup>) 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: constants of association  $\beta$ , constants of distribution  $K_D$ , constants of extraction  $K_{ex}$ , recovery factors  $R\%$ , molar absorptivities  $\epsilon_{\lambda(opt)}$ , 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 ( $\text{Log } M_{TZ^+}$ ) and some of the constants ( $\text{Log } \beta$ ,  $\text{Log } K_{ex}$ , and  $\epsilon_{\lambda(opt)}$ ) for the complexes with  $TZ^+$  which do not contain  $NO_2$  groups (i.e.  $TZ^+ = TT^+$ ,  $MTT^+$  and  $TV^+$ ): the higher  $\text{Log } M_{TZ^+}$ , the higher the constant. The poorer extraction-spectrophotometric performance of  $(INT^+)_2[Fe(TAR)_2]$  can be explained with a negative influence of the nitro group.

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