Extraction-Spectrophotometric Studies on the Ion-Pairing Between Some 2,3,5-Substituted Monotetrazolium Cations and Anions Deriving from 4-(2-Thiazolylazo)resorcinol or 4-(2-Pyridylazo)resorcinol

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ABSTRACT

The ion-pairing between some 2,3,5-substituted monotetrazolium cations (T^+) and anions deriving from 4-(2-pyridylazo)resorcinol (PAR) or 4-(2-thiazolylazo)resorcinol (TAR) was studied by water-chloroform extraction and spectrophotometry. The following tetrazolium salts (TS) were used as a source of T^+: 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-naphtyl)-2,5-diphenyl-2H-tetrazolium chloride (Tetrazolium violet, TV); and iv) 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT). The spectral characteristics of the extracted species were established at different pH and TS concentration. The composition and stability of the ion-pairs were determined at pH 9, where the extraction of neutral PAR (H_2PAR) and TAR (H_2TAR) species was negligible. The results showed that the ion-pairs can be expressed by the following formulae (T^+)($HTAR^-$) (where T^+ = TT^+, MTT^+, TV^+ or INT^+), (T^+)($HPAR^-$) (where T+ = TT^+, MTT^+ or TV^+) and $[(INT^+)(HPAR^-)]_2$. Relationships involving the molecular masses of the ion-pairs (M_{IP}) or T^+ (M_{T+}) and the values of the constants of association (β) or conditional molar absorptivities (ε') were examined, namely $\text{Log } \beta = f(\text{Log } M_{IP})$ and $\varepsilon' = f(\text{Log } M_{T+})$. Some practical aspects concerning the investigation of metal complexes with TS-PAR/TS-TAR were discussed.

Indexing terms/Keywords

Tetrazolium salts; azo dyes; ion-association; liquid-liquid extraction; spectrophotometry.

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1. INTRODUCTION

2,3,5-Substituted tetrazolium salts (TS) are well-known analytical reagents [1-3]. They generate bulky cations in aqueous medium which are prone to associate with a number of simple and complex anionic species. The obtained ion-associates are poorly soluble in water; that is why TS are widely employed as components of liquid-liquid extraction systems [3, 4]. The chromophore properties of tetrazolium ion-associates with inorganic anions (e.g. BrO_3^-, ClO_3^-, ClO_4^-, CrO_3Cl^-, MnO_4^-, ReO_4^-, $TlCl_4^-$, HgI_3^-, $[Zn(SCN)_4]^{2-}$, $[Cd(SCN)_4]^{2-}$, and $[Co(SCN)_4]^{2-}$) usually represent a combination of chromophore properties of the constituent ions [1-3]; this suggests the predominantly ionic nature of the chemical bond. However, the spectral characteristics (wavelength of maximum absorption, intensity and half width of the spectral line) of tetrazolium ion-associates with a given anion can be different, especially when this anion is a complex chelate of the type MR_n^{m-}, where R derives from 4-(2-thiazolylazo)resorcinol (TAR) [5, 6], 4-(2-pyridylazo)resorcinol (PAR) [6-8], 4-nitrocatechol [3, 9, 10], 2,3-didydroxynaphthalene [3], pyrogallol [11], etc. The mentioned spectral differences, along with the observed under defined conditions "negative absorption values" ($\Delta A = A_{M+R+TS} - A_{R+TS}$; $A_{R+TS} > A_{M+R+TS}$) [12] and "saturation curves distortions" [13] can perplex the researcher. In order to find explanations, he should think over the possibilities of hydrolysis [5, 6], oxidation/reduction [13, 14], stepwise complex formation [13, 15], interaction of the reagents TS and R with formation of stable salt-like compounds, aggregation, etc. In this context, it will be useful for the future researchers to have in their disposal reliable information about the characteristics of the compounds between TS and R which govern the properties of the blank sample. Marić and Široki [16] studied the extraction of PAR and TAR from water to chloroform, as well as the chloroform extraction of these reagents in the presence of tetraphenylphosphonium chloride or tetraphenylarsonium chloride. In this paper, we share results obtained during our systematic investigations on eight water-chloroform extraction systems containing PAR or TAR and each of the following monotetrazolium salts: 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-naphtyl)-2,5-diphenyl-2H-tetrazolium chloride (Tetrazolium violet, TV); and iv) 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT). The mentioned R-TS systems have been repeatedly used in our laboratory in the course of extraction and spectrophotometric determination of vanadium(IV,V) [5, 17-24], indium(III) [25, 26], gallium(III) [26-28], cobalt(II,III) [29, 30], iron(III) [31], and zirconium [32]. The formulae of the used TS and molecular masses of their cations M_T^+ are given in Table 1.

2. EXPERIMENTAL

2.1. Reagents and apparatus

Azo derivatives of resorcinol (ADR) PAR (96%) and TAR (97%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 2×10^{-3} mol L^{-1} aqueous solutions of these ADRs were prepared by dissolving weighted amounts of PAR or TAR in water alkalised with KOH. The final pH of the obtained solutions was 7-8. More dilute ADR solutions (5×10^{-4} mol L^{-1}) were prepared by appropriate dilution with distilled water. MTT, TV, INT and TTC were purchased from Alfa Aesar (Karlsrue, Germany), Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany), AppliChem GmbH (Darmstad, Germany) and Loba Feinchemie GmbH (Fischamend, Austria), respectively. Aqueous solutions of these reagents were prepared. The concentrations were 2×10^{-3} mol L^{-1} (for TV and INT), 3×10^{-3} mol L^{-1} (for MTT), and 4×10^{-3} mol L^{-1} (for TTC). Redistilled chloroform was used. 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_3COOH and NH_4OH. The resulting pH was checked by HI-83140 pH meter. A Camspec M508 spectrophotometer (United Kingdom), equipped with 10 mm path-length cells, was employed for reading the absorbance.

2.2. Procedure

1 mL of buffer solution and aliquots of ADR solution (PAR or TAR) and TS solution (TTC, MTT, TV or INT) were pipetted into 100-mL separatory funnels. The volumes were made up to 5 mL with distilled water. Then 5 mL of chloroform were added. The funnels were closed with stoppers and shaken for 2 minutes. After separation of the layers, portions of the chloroform extracts were transferred through filter papers into cells. The absorbance was read against chloroform or an appropriate blank sample (containing buffer-ADR or buffer-MTT).

3. RESULTS AND DISCUSSION

3.1. Preliminary data and observations

Several optically different PAR and TAR species exist in aqueous solution in dependence on the acidity: H_4PAR^{2+}, H_3ADR^+, H_2ADR, $HADR^-$ and ADR^{2-} [16, 33-35] (Table 2). Studying the chloroform extraction of these reagents from buffered aqueous medium (pH 1-10) Marić and Široki [16] established maximum and practically constant extraction of PAR and TAR under the pH ranges of 3.5-5 and 1.5-5, respectively, where their neutral H_2ADR species predominate (Table 2). On the other hand, the concentration of H_2ADR is negligible at pH 9. As a result, PAR and TAR cannot be extracted with chloroform alone. However, they can be extracted as ion-pairs of the type $(Ph_4X^+)(HADR^-)$, where Ph_4X^+ is a cation deriving from tetraphenylphosphonium chloride and tetraphenylarsonium chloride [16]. Our preliminary investigations on the TS-ADR extraction systems at pH 9.0 suggested the formation of similar compounds. The recorded wavelengths of maximum absorption (λ_{max}) (Table 3) were quite close to these reported by Marić and Široki: $\lambda_{max}=400$ nm for $(Ph_4X^+)(HPAR^-)$ and $\lambda_{max}=498$ nm for $(Ph_4X^+)(HTAR^-)$ [16]. However, different conditional molar absorptivity (ε') rows were observed in both series (ADR=PAR and ADR=TAR) at equal conditions (Table 3): $\varepsilon'_{PAR-TTC} < \varepsilon'_{PAR-MTT} < \varepsilon'_{PAR-TV} < \varepsilon'_{PAR-INT}$ and $\varepsilon'_{TAR-INT} < \varepsilon'_{TAR-TTC} < \varepsilon'_{TAR-MTT} < \varepsilon'_{TAR-TV}$. The INT-PAR extracts exhibited the highest ε'-value, while the TAR

extracts with the same reagent (INT) exhibited the lowest ε'-value in its series (see Table 3 and Fig. 1). This anomaly showed that there must be some big difference between both extracted INT-containing compounds.

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)	TT^+ $M_{TT^+}=298.96$
	3-(4,5-Dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT)	MTT^+ $M_{MTT^+}=334.41$
	3-(2-Naphtyl)-2,5-diphenyl-2H-tetrazolium chloride (Tetrazolium violet,TV)	TV^+ $M_{TV^+}=349.41$
	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT)	INT^+ $M_{INT^+}=470.25$

Table 2. Optical and proton dissociation properties of PAR and TAR in aqueous solutions [16, 33-35]

Species / Reagent	H_3ADR^+	$\leftrightarrow$	H_2ADR	$\leftrightarrow$	$HADR^-$	$\leftrightarrow$	ADR^{2-}
ADR=PAR*	$\lambda_{max}=395$ nm	$pK_1=3.10$	$\lambda_{max}=385$ nm	$pK_2=5.60$	$\lambda_{max}=413$ nm	$pK_3=11.90$	$\lambda_{max}=490$ nm
ADR=TAR	$\lambda_{max}=488$ nm	$pK_1=0.96$	$\lambda_{max}=410$-440 nm	$pK_2=6.23$	$\lambda_{max}=470$-480 nm	$pK_3=9.44$	$\lambda_{max}=510$ nm

* In concentrated sulphuric acid PAR can form doubly protonated species H_4PAR^{2+}

Table 3. Optical properties of chloroform extracts of the ion-pairs of azoderivatives of resorcinol (ADR) with tetrazolium cations (T⁺).

ADR	T^+	$\lambda_{max}*$ [nm]	$\varepsilon'*$ [L mol^{-1} cm^{-1}]
PAR	TT^+	394	1840
	MTT^+	391	9280
	TV^+	399	12000
	INT^+	391	15740
TAR	TTC^+	497	7040
	MTT^+	491	12820
	TV^+	496	20040
	INT^+	480	6300

* Conditions: pH=9, $C_{ADR} = C_{TS} = 5.0 \times 10^{-5}$ mol L^{-1}, extraction time - 2 min.

Pay attention on the different sequences of the conditional molar absorptivities for the systems containing PAR ($\varepsilon_{PAR-TTC} < \varepsilon_{PAR-MTT} < \varepsilon_{PAR-TV} < \varepsilon_{PAR-INT}$) and TAR ($\varepsilon_{TAR-TTC} < \varepsilon_{TAR-MTT} < \varepsilon_{TAR-TV} > \varepsilon_{TAR-INT}$)
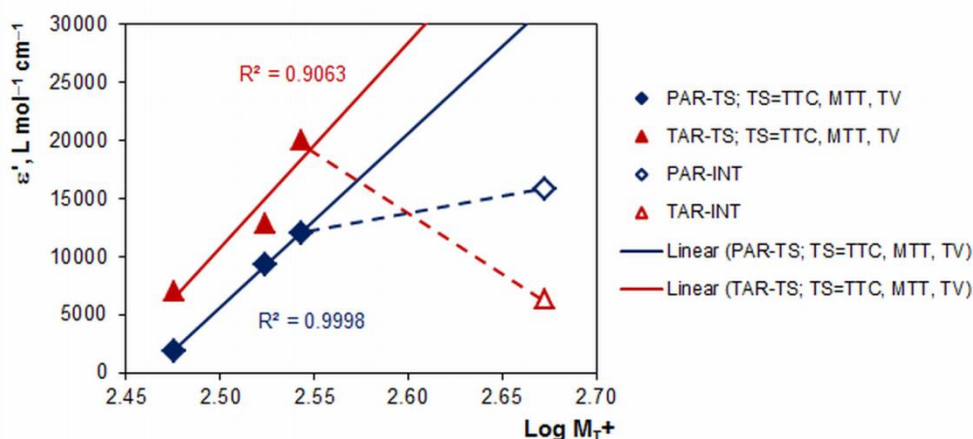
Figure 1. Dependence of the conditional molar absorptivity (ε') on the logarithm of the molecular mass of the studied tetrazolium cations (M_T+). Conditions: pH=9, $C_{ADR} = C_{TS} = 5.0 \times 10^{-5}$ mol L^{-1}, extraction time - 2 min. Linear relationships appear to exist in both series (ADR=PAR and ADR=TAR) for the tetrazolium cations T^+ which do not contain NO$_2$-groups ($T^+=TT^+$, MTT^+ and TV^+).

3.2. Saturation curves at pH 9

The dependences between the absorbance of extracted ion-pairs and concentration of TS were studied at pH 9 (Figure 2). The concentration of ADR (PAR or TAR) was kept constant in all series (C=5.0×10^{-5} mol L^{-1}) in order to ensure identical experimental conditions. The absorbances of the extracted species were recorded against blank samples containing ADR (curves 1, 3, 4, 1', 2', 3' and 4') or MTT (curve 2). The absorbance of the MTT-PAR species was found by a different manner due to the noticeable absorption of MTT at $\lambda_{max(MTT-PAR)}$. In contrast to the other studied TSs (which exhibit maxima in the UV region) MTT has an additional maximum at about 370-380 nm [1, 3, 36]. The results presented in Figure 2 show that the following TS n-fold excesses (in parenthesis) are necessary for maximum ADR extraction: i) ARD=PAR; TTC (48), MTT (18), TV (6.4), and INT (5); ii) ARD=TAR; TTC (48), MTT (30), TV (9.6), and INT (24). It should be mentioned that at TS excess conditions the recorded λ_{max} were slightly different from these reported in Table 3. The specific ε_{max} and λ_{max} values for each ADR-TS system are shown in the caption of Figure 2.
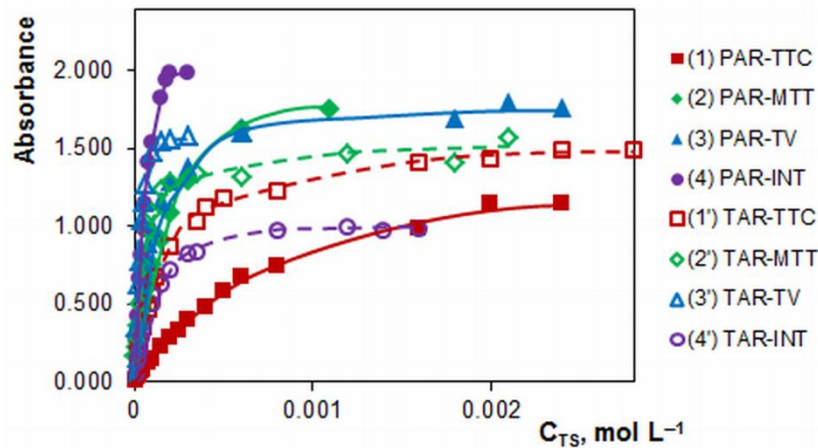
Figure 2. Absorbance of the extracted ion-pairs vs. concentration of the TS plots. pH = 9.0, C_{ADR} = 5.0 × 10⁻⁵ mol L⁻¹. The following molar absorptivity coefficients ε_{max} were calculated: (1)- 2.29×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=400 nm); (2) - 3.50×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=385 nm); (3) - 3.56×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=399 nm); (4) - 3.93×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=388 nm); (1') - 2.98×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=497 nm); (2') - 2.96×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=489 nm); (3') - 3.21×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=496 nm); (4') - 1.96×10⁴ L mol⁻¹ cm⁻¹ (λ_{max}=482 nm)

3.3. Composition of the ion-pairs

One can expect the following ion-pair reaction for the system of ADR and TS:

$$T^+ + ADR^- \Leftrightarrow (T^+)(ADR^-) \tag{1}$$

Job's isomolar curves [37] (Figure 3) and the saturation curves presented in Figure 2 were used to check the validity of Equation 1. The data shown in Figure 2 were processed by the straight-line method of Asmus [38], Bent-French method [39] and mobile equilibrium method [40]. The results confirmed the formation of $(T^+)(ADR^-)$ species in all systems except for the INT-PAR system. The mobile equilibrium method [40] (Figure 4 and Figure 5) and the dilution method [41] (Figure 6), which are appropriate to distinguish between 1:1 and 2:2 species, showed that the INT-PAR compound had a composition of 2:2. Hence, the ion-pair formation in this case can be expressed with Equation 2:

$$2INT^+ + 2PAR^- \Leftrightarrow [(INT^+)(PAR^-)]_2 \tag{2}$$
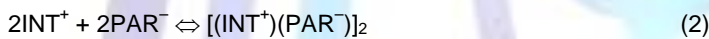
The established composition of 2:2 explains well the highest conditional molar absorptivity of PAR-INT in the PAR-TS series (Table 3): the dimerization leads to enhanced bulkiness and hydrophobicity of the compound and this improves its extraction behavior.
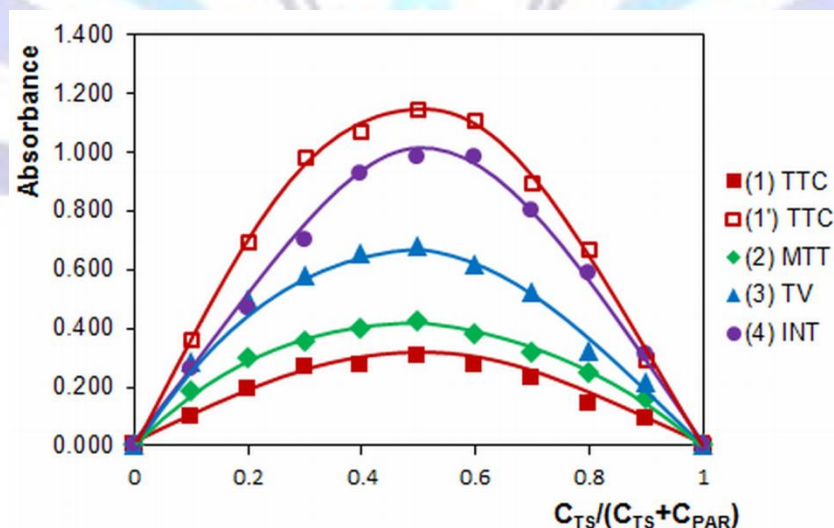

Figure 3. Continuous variation plots for the PAR-TS systems. $C_{TTC}+C_{PAR}$=2×10⁻⁴ mol L⁻¹ (curve 1), $C_{TTC}+C_{PAR}$=4×10⁻⁴ mol L⁻¹ (curve 1') and $C_{TS}+C_{PAR}$=1×10⁻⁴ mol L⁻¹ (curves 2, 3 and 4)
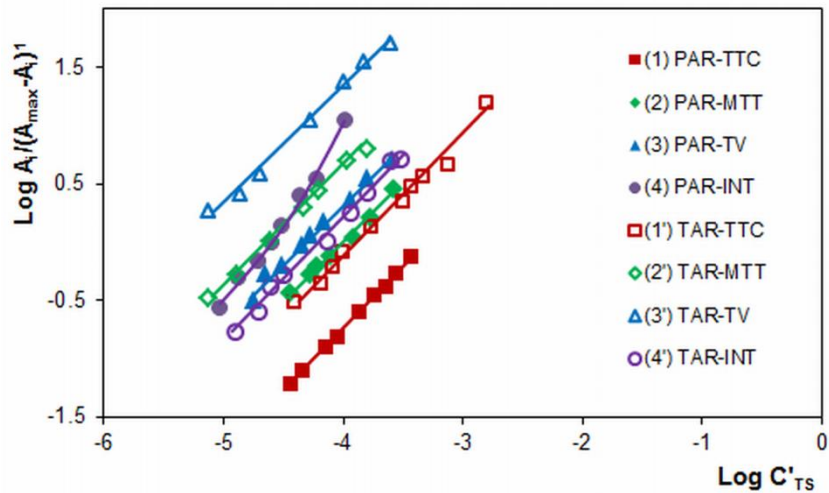
Figure 4. Straight lines (1, 2, 3, 1', 2', 3' and 4') and a curve (4) obtained by the mobile equilibrium method [40] for m=n=1. $C_{ADR} = 5.0 \times 10^{-5}$ mol L^{-1}, pH = 9.0. Straight line equations: (1) y=1.078x+3.535; (R^2=0.9985); (2) 1.016x+4.056; (R^2=0.9928); (3) 1.009x+4.356; (R^2=0.9920); (1') 1.022x+3.975; (R^2=0.9876); (2') 1.005x+4.643; (R^2=0.9978); (3') 1.009x+5.363; (R^2=0.9929); (4') 1.083x+4.516; (R^2=0.9950)
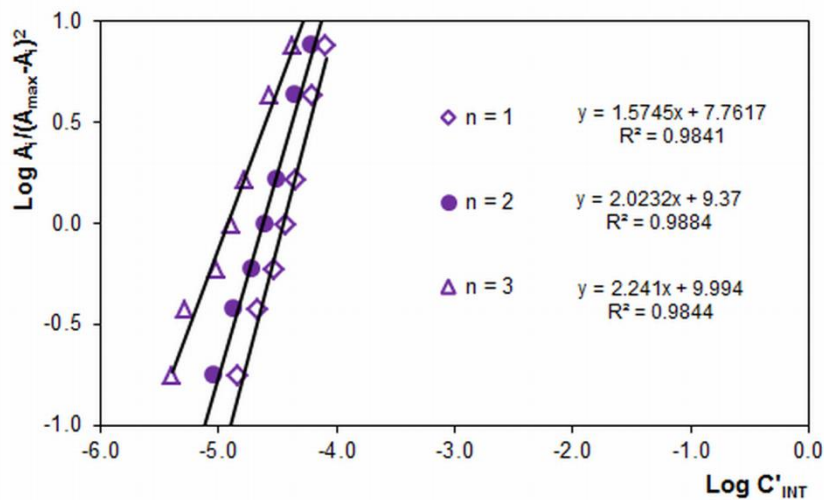

Figure 5. Straight lines for the INT-PAR system obtained by the mobile equilibrium method [40] for m=2. $C_{PAR} = 5.0 \times 10^{-5}$ mol L^{-1}, pH = 9.0

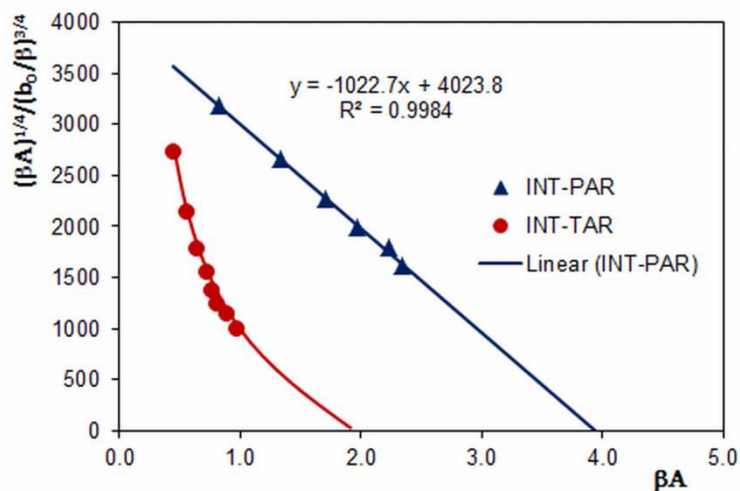**Figure 6. A straight line (INT-PAR system) and a curve (INT-TAR system) obtained for m=n=2 by the dilution method [41] at pH = 9.0**

3.4. Constants of association β

The equilibrium constants β characterizing the processes of ion-pairing described above (Equations 1 and 2) were calculated by several independent methods, namely the mobile equilibrium method (Figures 4 and 5) [40], the Harvey-Manning method [42], the Holme-Langmyhr method [43] and the dilution method [41] (Figure 6). The results are presented in Table 4.

Table 4. Calculated values (P = 95%) of the constants of association (β)

Ion-pair	Log β			
	Mobile equilibrium method [40]	Harvey-Manning method [42]	Holme-Langmyhr method [43]	Dilution method [41]
$(TT^+)(PAR^-)$	3.28±0.04	3.19±0.08	3.20±0.05	–
$(MTT^+)(PAR^-)$	4.0±0.1	4.00±0.05	4.02±0.04	–
$(TV^+)(PAR^-)$	4.3±0.1	4.26±0.09	4.23±0.04	–
$[(INT^+)(PAR^-)]_2$	13.6±0.3	–	–	13.52±0.04
$(TT^+)(TAR^-)$	3.89±0.09	3.91±0.05	3.92±0.04	–
$(MTT^+)(TAR^-)$	4.62±0.07	4.60±0.08	4.55±0.10	–
$(TV^+)(TAR^-)$	5.3±0.1	5.33±0.06	5.4±0.2	–
$(INT^+)(TAR^-)$	4.17±0.08	4.19±0.06	4.17±0.04	4.21±0.03

3.5. Relationships between Log β and Log M

At least two factors noticeably influence the stability of tetrazolium ion-associates. The first factor was formulated by Alexandrov et al [1, 44]: the higher the molecular mass (M), the higher the association constant (β). The second factor is bound up with the presence of nitrophenyl dubstituent(s) in the tetrazolium ring [3, 45]: The value of β is significantly lower than the expected one if the tetrazolium cation contains NO_2-group(s).

The results shown in Table 4 for Log $β_{(TT+)(ADR-)}$, Log $β_{(MTT+)(ADR-)}$ and Log $β_{(TV+)(ADR-)}$ and calculated logarithms of the molecular masses of the mentioned ion-pairs (Log M_{IP}) were used to check the validity of the first factor. The obtained squared correlation coefficients (R^2) were R^2=1.0000 (for ADR=PAR) and R^2=0.9524 for (ADR=TAR).

Figure 7 represents the dependences between Log M_{IP} and Log β for all studied ion-pairs, including $[(INT^+)(PAR^-)]_2$ and $(INT^+)(TAR^-)$. The points for the INT-containing ion-pairs have considerably different abscissa values, namely Log $M_{[(INT^+)(PAR-)]2}$=3.136 and Log $M_{(INT+)(TAR-)}$=2.839. The relative position of the point for $(INT^+)(TAR^-)$ was in accordance with our expectations based on the literature [3, 7, 23, 26, 45] concerning the second factor: Log $β_{(INT+)(TAR-)}$ < Log $β_{(TV+)(TAR-)}$. On the other hand, as a result of the dimerization in the PAR-INT system, a perfect straight-line (R^2=1.000) was obtained for the series PAR–T^+ (T^+=TT^+, MTT^+, TV^+ and INT^+; Figure 7).

y = 24.182x - 62.244
R^2 = 1

▲ PAR ion-pairs
● TAR ion-pairs
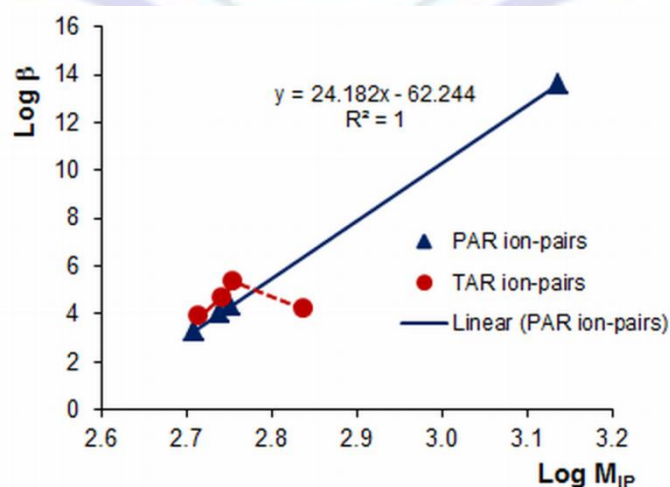— Linear (PAR ion-pairs)

Figure 7. Dependence of the logarithm of the association constant (β) on the logarithm of the molecular mass of the studied ion-pairs $[(T^+)(ADR^-)]_x$ (x=2 for the PAR-INT ion-pair; x=1 for all other ion-pairs)

3.6. Absorption spectra at pH 6.

At pH-values lower than 8 the spectral curves of the extracted ion-pairs were affected in some extent by the simultaneous extraction of neutral PAR and TAR species [16]. Having in mind the different stability of the extracted TS-ADR ion-pairs and the nature of the used blank samples (buffer-PAR or buffer-TAR) one can expect more remarkable spectral differences for the systems containing species with closer λ_{max} values. H_2PAR and H_2TAR have absorption maxima at 382 nm (Figure 8, curve 5) and 413 nm (Figure 9, curve 5), respectively. $HPAR^-$ and $HTAR^-$, in their turn, have absorption maxima at approximately 400 nm and 495-500 nm. Hence, the PAR-TS systems contain species with the closer spectral characteristics ($\Delta\lambda \approx 18$ nm); that is why the recorded λ_{max} values for PAR-TTC, PAR-MTT, PAR-TV and PAR-INT, appeared in relatively wide interval (see Figure 8). Contrariwise, the big difference between the maxima of H_2TAR and $HTAR^-$ ($\Delta\lambda \approx 84$ nm) resulted in a relative stability of the established λ_{max} values: λ_{max}=491 nm (TAR-MTT and TAR-INT systems), 492 nm (TAR-TV system) or 493 nm (TTC-TAR system). However, an additional effect can arise in these TAR-TS systems, namely negative absorbance values ($\Delta A = A_{TAR+TS} - A_{TAR}$; $A_{TAR} > A_{TAR+TS}$) recorded near the wavelength region of maximum H_2TAR absorption (Figure 9). This effect is expected to be stronger when C_{TAR} and β are higher. Figure 9 shows that the minimum in the spectral curve for the TAR-TV system is the deepest ($\lambda_{min} \approx 405$-410 nm, ΔA=−0.130); in fact, this system produces the most stable ion pair in the TAR-TS series. It should be mentioned that we observed similar minima in the spectra of some ternary complexes containing a metal ion (V, Co), TAR and TS [5, 30]. These minima caused noticeable distortion of the main spectral band of the complexes, especially when TV or TTC were used [5].

Independently of pH, MTT, which exhibits λ_{max}=370 nm in chloroform (Figure 8, curve 2'), contributes to the overall spectral pattern of the extracts. MTT causes hypsochromic shifts in both series ADR=TAR or TAR. However, in the first case (ADR=TAR) the shift is small (1-2 nm), while in the second case (ADR=TAR) it is bigger (12-15 nm): λ_{max}(PAR-MTT)=395 nm, λ_{max}(PAR-TTC)=410 nm, λ_{max}(PAR-TV)=407-408 nm.

One can notice from Figure 8 that in contrast to the spectral curves (1) and (3), the PAR-INT spectral curve (4) is not symmetrical. The shoulder observed on the left side of the peak can be regarded as a sign of dimerization [46]. As a result of the overlapping of two bands, the half-width of the recorded complex band is wider and λ_{max} appears at shorter wavelengths (405 nm). Such an explanation fits well with the observed facts.

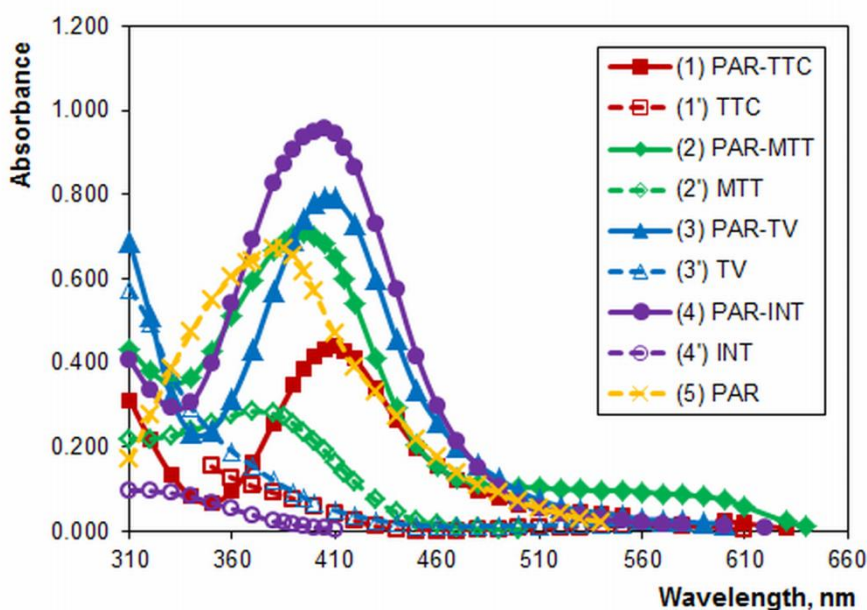**Figure 8. Absorption spectra at pH 6 against chloroform (curves 1', 2', 3', 4' and 5) or chloroform extract of PAR (curves 1, 2, 3 and 4). C_{PAR} = 5.0×10⁻⁵ mol L⁻¹ (curves 1, 2, 3, 4 and 5), C_{TTC}=2.4×10⁻³ mol L⁻¹ (curves 1 and 1'), C_{MTT}=3.2×10⁻⁴ mol L⁻¹ (curves 2 and 2'), C_{TV}=3.2×10⁻⁴ mol L⁻¹ (curves 3 and 3') and C_{INT}=2.5×10⁻⁴ mol L⁻¹ (curves 4 and 4'). The following λ_{max} were recorded: (1) - 410 nm; (2) - 395 nm; (3) - 407 nm; (4) - 405 nm; (5) - 382 nm; (2') – 370 nm**
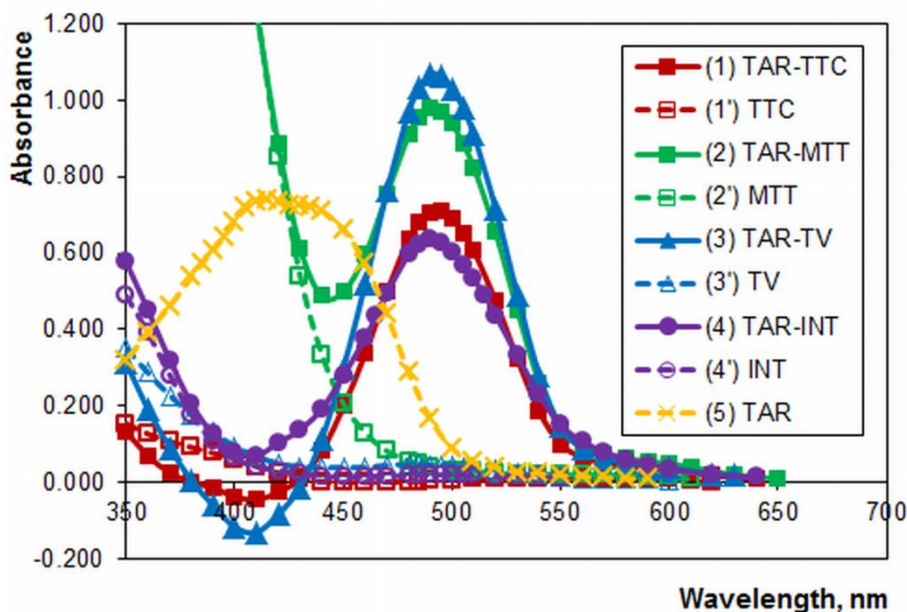
Figure 9. Absorption spectra at pH 6 against chloroform (curves 1', 2', 3', 4' and 5) or chloroform extract of TAR (curves 1, 2, 3 and 4). C_{TAR} = 5.0×10^{-5} mol L^{-1} (curves 1, 2, 3, 4 and 5), C_{TTC}=2.4×10^{-3} mol L^{-1} (curves 1 and 1'), C_{MTT}=1.5×10^{-3} mol L^{-1} (curves 2 and 2'), C_{TV}=4.8×10^{-4} mol L^{-1} (curves 3 and 3') and C_{INT}=1.2×10^{-3} mol L^{-1} (curves 4 and 4'). The following λ_{max} were recorded: (1) - 493 nm; (2) - 491 nm; (3) - 492 nm; (4) - 491 nm; (5) - 413 nm

4. CONCLUSIONS

The interaction between some widely used monotetrazolium salts (TS: TTC, MTT, TV and INT) and azoderivatives of resorcinol (ADR: PAR and TAR) was studied. The spectral characteristics of the extracted ion-pairs were established at conditions which are similar to these of formation of ion-association complexes of the type M-ADR-TS (where M is a metal ion): pH=6 or pH=9; extraction time – 2 min; concentration of ADR – 5×10^{-5} mol L^{-1}; concentration of TS (from 5×10^{-5} mol L^{-1} to 3.0×10^{-4} – 2.8×10^{-3} mol L^{-1}). The composition and stability of the ion-pairs were determined at pH 9, where the extraction of neutral ADR species (H$_2$ADR) was negligible. The following formulae of the extracted ion-pairs were established (T$^+$)(HTAR$^-$) (where T$^+$ = TT$^+$, MTT$^+$, TV$^+$ or INT$^+$), (T$^+$)(HPAR$^-$) (where T+ = TT$^+$, MTT$^+$ or TV$^+$) and [(INT$^+$)(HPAR$^-$)]$_2$. A well-defined linear relationship exists between Log β and Log $M_{IP(PAR)}$ (where β is the constant of ion-pairing and $M_{IP(PAR)}$ is the molecular mass of the ion-pair in the PAR-TS series). Partial linear relationships (violated for the INT-containing ion-pairs only) were established between Log β and Log $M_{IP(TAR)}$, and between the conditional molar absorptivity ε' (pH=9, $C_{ADR}=C_{TS}$) and Log M_{T+} (or Log M_{IP}). The relatively big differences between the studied ion-pairs can be attributed to the different nature of the substituents in tetrazolium ring. In contrast to the ion-pairs studied by Marić and M. Široki [16] (Ph$_4$X$^+$)(HADR$^-$), in which X is situated in the core of the cation (X=P or As), the different substituents in our case are located in the outside part of the tetrazolium cation T$^+$. Hence, they can contribute more dramatically to the overall performance of T$^+$ and the ion-pairs itself.

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