

Spectrophotometric Study of Equilibria Complexation of Copper(II), Zinc(II), and Iron(III) with 4-(1*H*-1,2,4-Triazolyl-3-azo)-2-methylresorcinol and Multicomponent Analysis Determination in Drugs

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Spectrophotometric study of Cu(II), Zn(II), and Fe(III) with 4-(1*H*-1,2,4-triazolyl-3-azo)-2-methylresorcinol (H₃L) and numerical analysis of data using a general least-squares program LETAGROP-SPEFO yields complexes ZnH₂L, ZnHL, Zn(HL)₂, CuH₂L, Cu(HL)₂, FeH₂L, FeH₂L(OH), and Fe(HL)₂ (charges omitted). The equilibria have been studied at $I(\text{NaNO}_3) = 0.25 \text{ mol dm}^{-3}$, 25 °C in 40 % methanol. Conditions for multicomponent spefo analysis were found, the determination is optimal at pH ≈ 9 , with a good sensitivity, i.e. numerical values of molar absorptivity values $\epsilon_i/(\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})$ 78400, 39500, and 47800 for Zn(II), Cu(II), and Fe(III), respectively. The reagent was used for the determination of these elements in drugs.

Pyridylazo- and thiazolylazophenols are frequently used in analytical chemistry [1–3] for the spectrophotometric and FIA determination [4] or HPLC separation and determination [5] of heavy metal ions.

The reagent, 4-(1*H*-1,2,4-triazolyl-3-azo)-2-methylresorcinol (TrAMeR), has been synthesized and first used for the determination of vanadium [6], cobalt [7], and vanadium and cobalt in steels [8]. It gives sensitive reactions with most of the heavy metal ions and with respect to this is competitive with PAR (4-(2-pyridylazo)resorcinol), for example.

The spectrophotometry in the visible spectra region using dye reagents is one of the basic methods for the determination of low concentration of heavy metal ions. Establishing of the optimum conditions for the determination is the main requisite for obtaining accurate and precise results. So in this work the complex reaction mechanisms between Cu(II), Zn(II), and Fe(III) and TrAMeR were studied by numerical analysis of spectrophotometric data with the aim to search for the best conditions for the determination and to work out the method of multicomponent analysis.

EXPERIMENTAL

Nitrates of copper, zinc, and iron (anal. grade, Merck) were prepared in water as 0.1 M solutions.

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In case of iron nitrate the solutions contained 0.1 M-HNO₃ to prevent hydrolysis. Solutions were standardized using standard EDTA titrations using PAR (for Cu and Zn) or sulfosalicylic acid as indicators [9]. The content of metal was checked by AAS using standard solutions for the calibration.

The ionic strength was adjusted by adding suitable amounts of 2.5 M sodium nitrate. A 0.025 M sodium tetraborate—sodium hydroxide buffer solution was used.

All reagents and solvent were of anal. grade and water was double-distilled from a quartz apparatus.

Reagent has been synthesized and purified as described earlier [6]. Elemental analysis is in a good agreement with theory. According to elemental analysis the purity is 100 % \pm 0.25 %.

Radiometer pHM84 (Copenhagen, Denmark) and combined glass-saturated calomel electrode (Metrohm 6.2104.070) were used while two standard buffers of pH 7.00 and 4.01 were used to calibrate the electrode. No correction for the presence of methanol and ionic strength was applied.

Absorbance was measured using a diode array spectrophotometer HP 8452A (Hewlett—Packard, USA) and 1 cm quartz cell.

The equilibrium data were evaluated using LETAGROP-SPEFO program [10], distributions diagrams were calculated using HALTAFALL [11] and either VAX/UMX 11/780(v.4) computer in "Centro de Cálculo", Universidad de La Laguna, or its PC version [12] for IBM PC XT/AT personal computer.

Multicomponent data were calculated using a PC version of partial least-squares program written according to the published algorithms [13, 14].

Conditions for Equilibrium Study

To a 25 cm³ calibrated flask were added in sequence adequate volume of the ions solution, 2.0 cm³ of methanolic 0.40 mM-TrAMeR, 8 cm³ of absolute methanol to obtain $\varphi_r = 40\%$ in the mixture and 2.5 cm³ of 2.5 M sodium nitrate. The absorbance—pH curves were measured after diluting the stock solutions with double-distilled water, the pH was adjusted with nitric acid (Merck) or diluted sodium hydroxide (Merck) stirring the solutions in order to avoid possible local hydrolysis. Only completely clear solutions were used for the absorbance measurements and immediately if any faint turbidity occurred, the experiment was stopped. In the determinations, 2.5 cm³ of buffer were added and volume fixed up to the mark with double-distilled water.

Procedure for the Simultaneous Spectrophotometric Determination of Cu(II), Zn(II), and Fe(III)

1—5 pills (Micebrina, Derly) were dissolved in the mixture nitric acid—hydrochloric acid ($\varphi_r = 1:1$), precipitation was filtered off and an aliquot part of the reagent (1 cm³ of 1.25×10^{-3} M-TrAMeR in pure methanol) was added to 2.5 cm³ of buffer and after filling up to the mark in 25 cm³ measuring flask the spectra were recorded in 1 cm cell in the range $\lambda = 350$ —800 nm.

RESULTS AND DISCUSSION

Absorption spectra show that TrAMeR (H₃L) has seven coloured forms H₆L³⁺, H₅L²⁺, H₄L⁺, H₃L, H₂L[−], HL^{2−}, and L^{3−} with pK_{a1} — pK_{a6} . In the pH range 0—10 in which we were working only pK_{a3} — pK_{a6} are important and these were determined spectrophotometrically. The determination of pK_a of TrAMeR under the working conditions was carried out at λ/nm : 370, 430, 450, and 476, and the absorbance was measured for 53 pH values. For data evaluation LETAGROP-SPEFO program [10, 11] has been used.

Optical properties of the reagent and the other results obtained are given in Table 1.

The complex formation equilibria can be formulated according to the general scheme

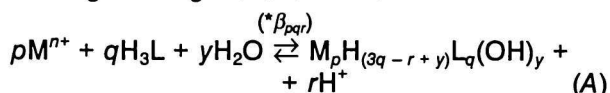


Table 1. Optical Characteristics and pK_a Values for 3-(1H-1,2,4-Triazolyl-3-azo)-2-methylresorcinol in Methanol—Water Medium ($\varphi_r = 40\%$) at $I(\text{NaNO}_3) = 0.25 \text{ mol dm}^{-3}$

Species	λ/nm	$\epsilon/(\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})$	$pK_a^* \pm s$ (pK_a)
H ₃ L	370	21100	0.70 ± 0.03
H ₂ L [−]	450	17000	6.52 ± 0.03
HL ^{2−}	430	28100	9.31 ± 0.02
L ^{3−}	476	30500	11.69 ± 0.01

s — standard deviation of pK_a (3σ value as obtained by LETAGROP-SPEFO is given).

*Evaluated from data for λ/nm 370, 430, 450, and 476.

where M^{n+} is the specific metal ion under study and H₃L is the neutral form of the reagent. Symbol $*\beta_{pqr}$ is the conditional equilibrium constant. The protons split off are either from the reagent or are due to the hydrolysis under the formation of mixed hydroxo species.

Equilibria were studied by a spectrophotometric method, measuring absorbance *A* as a function of pH, and total metal c_M or total ligand c_L concentrations. Data $A = f(c_M, c_L, \text{pH})$ were evaluated using a general least-squares procedure of LETAGROP-SPEFO, minimizing the sum of squares of residuals, *U*,

$$U = \sum_{j=1}^{N_\lambda} \sum_{i=1}^{N_p} (A_{\text{exp}} - A_{\text{calc}})^2 = \min \quad (1)$$

where A_{exp} are experimental and A_{calc} the calculated values of absorbances of solutions and summation is taken over all N_p experimental points and N_λ numbers of wavelengths. The program also calculates the standard deviation of absorbance defined as $\sigma(A) = [U/(N_p - n)]^{1/2}$, where *n* is the number of parameters estimated. The “best” model is that one for which the lowest values of *U* and $\sigma(A)$ are obtained.

Copper(II) forms with the reagent complexes with absorption maximum around $\lambda = 504 \text{ nm}$ and therefore this wavelength and $\lambda = 510 \text{ nm}$ were used for the equilibrium study.

Absorbance—pH curves have been measured for solutions in excess of the metal ion and solutions with an excess of the reagent. Figs. 1 and 2 show the curves obtained at $\lambda = 504 \text{ nm}$.

Computation of data for solutions with an excess of metal ion confirms the 1:1 complex, CuH_2L^+ , formed according to the general scheme (A), i.e. indices *p*, *q*, *r* are equal to 1 and *y* = 0. The formation of the same CuH_2L^+ complex was shifted to higher pH values in solutions with an excess of the ligand (Fig. 2, curves 1, 2) in the pH range 1—4, and further in the pH range 7.5—10 the complex formation of $\text{Cu}(\text{HL})_2^{2-}$ has been proved. The results of computations are given in Table 2.

In the solutions with excess of the ligand also complexes LHCu and $(\text{LH}_2)_2\text{Cu}$ have been accepted.

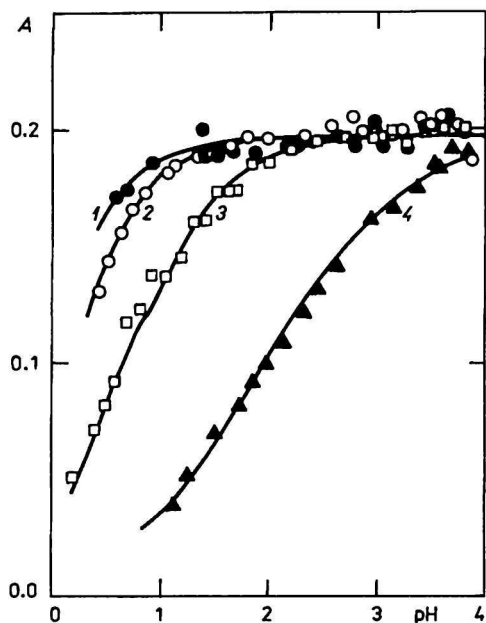


Fig. 1. Absorbance—pH curves for the system Cu(II)—TrAmE. $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$, $\phi_r(\text{methanol—water}) = 40 \%$, $I(\text{NaNO}_3) = 0.25 \text{ mol dm}^{-3}$, $\lambda = 504 \text{ nm}$. $c_M/(\text{mol dm}^{-3}) = 1. 2.63 \times 10^{-3}$; 2. 1.27×10^{-3} ; 3. 2.41×10^{-4} ; 4. 2.53×10^{-5} . Solid lines are theoretical curves calculated for the best model obtained from LETAGROP-SPEFO.

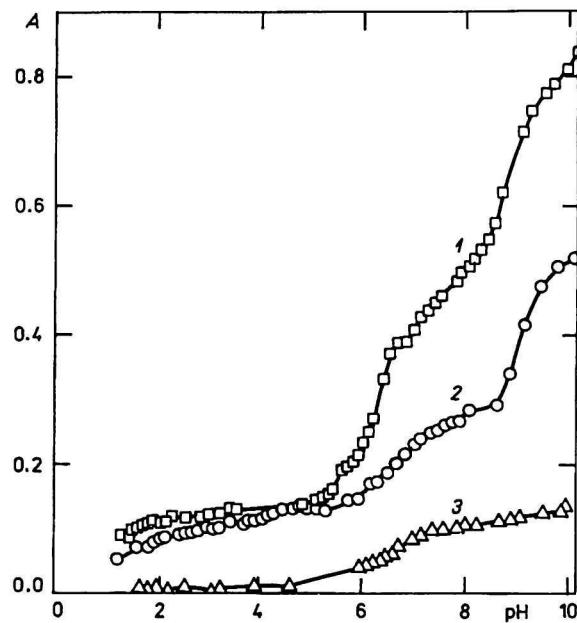


Fig. 2. Absorbance—pH curves for the system Cu(II)—TrAmE. $\phi_r(\text{methanol—water}) = 40 \%$, $I(\text{NaNO}_3) = 0.25 \text{ mol dm}^{-3}$, $\lambda = 504 \text{ nm}$. 1. $c_M = 8 \times 10^{-6} \text{ mol dm}^{-3}$, $c_L = 1.2 \times 10^{-4} \text{ mol dm}^{-3}$; 2. $c_M = 8 \times 10^{-6} \text{ mol dm}^{-3}$, $c_L = 6 \times 10^{-5} \text{ mol dm}^{-3}$; 3. $c_M = 0$, $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$. Solid lines are theoretical curves calculated for the best model obtained from LETAGROP-SPEFO.

Table 2. Equilibria Analysis in the Cu(II)—TrAmE System. Values of U , $\sigma(A)$, $\log^* \beta$, and $\varepsilon(504 \text{ nm})$ for the Different Models Tested by LETAGROP-SPEFO Method. λ/nm : 504 and 510

Model	Species	$\log^* \beta \pm 3\sigma(\log^* \beta)$	$U \cdot 10^3$	$\sigma(A) \cdot 10^3$	$\frac{\varepsilon \pm \sigma(\varepsilon)}{\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}}$
1	CuHL	2.23 ± 0.20	3.93	11.65	7660 ± 120
2	CuH ₂ L ⁺	$4.54 \text{ MAX} = 4.38$	0.25	3.04	3580 ± 600
	CuHL	$3.46 \text{ MAX} = 4.38$			8140 ± 250
3	CuH ₂ L ⁺	2.97 ± 0.09	0.74	5.06	8200 ± 170
Conditions: $N_p = 56$ $c_M > c_L$ $0.20 < \text{pH} < 4.05$					
4	CuHL	$1.32 \text{ MAX} = 1.58$	5.93	14.82	6800 ± 310
5	CuH ₂ L ⁺	$3.09 \text{ MAX} = 3.32$	0.31	3.60	6300 ± 230
	CuHL	$0.30 \text{ MAX} = 0.82$			7770 ± 125
6	CuH ₂ L ⁺	2.85 ± 0.06	0.74	4.60	8390 ± 300
Conditions: $N_p = 34$ $c_M = c_L$ $0.20 < \text{pH} < 4.05$					
7	CuH ₂ L ⁺	2.23 ± 0.14	8.90	9.79	10660 ± 410
8	CuH ₂ L ⁺	2.79 ± 0.20 (fixed)	1.25	5	6390 ± 375
	CuHL	$0.50 \text{ MAX} = -0.11$			11180 ± 290
9	CuH ₂ L ⁺	2.79 ± 0.20 (fixed)	12.4	5	6625 ± 1450
	Cu(LH ₂) ₂	$3.76 \text{ MAX} = 4.05$			11850 ± 270
Conditions: $N_p = 48$ $c_L > c_M$ $\text{pH} < 5.75$					
10	CuH ₂ L ⁺	$2.76 \text{ MAX} = 3.08$	12.56	25.71	7646 ± 210
	Cu(HL) ₂ ²⁻	$-17.83 \text{ MAX} = -17.47$			40100 ± 380
11	CuH ₂ L ⁺	$2.70 \text{ MAX} = 3.15$	6.32	18.40	8850 ± 175
	Cu(H ₂ L) ₂	4.94 ± 0.16			9215 ± 210
	Cu(HL) ₂ ²⁻	-13.12 ± 0.16			39500 ± 400
12	CuH ₂ L ⁺	2.79 ± 0.20 (fixed)	1.24	11.94	10610 ± 1450
	CuHL	$1.74 \text{ MAX} = 2.53$			9215 ± 210
	Cu(HL) ₂ ²⁻	$-10.09 \text{ MAX} = -9.24$			49270 ± 320
Conditions: $N_p = 98$ $c_L > c_M$ $1.24 < \text{pH} < 10.11$					

U is the sum of residuals as defined by eqn (1); $\sigma(A)$ is standard deviation of absorbance or of molar absorptivity; N_p is number of points (solutions); MAX is maximum value obtained which is given if $\sigma(\log^* \beta) > 0.25$ logarithmic unit.

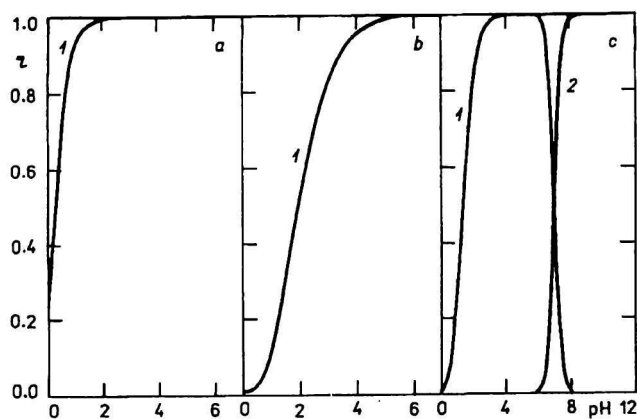


Fig. 3. Distribution diagrams ($\eta(\text{pH})$) of species. 1. CuH_2L^+ ; 2. $\text{Cu}(\text{HL})_2^{2-}$; a) $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$, $c_M = 2.41 \times 10^{-4} \text{ mol dm}^{-3}$; b) $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$, $c_M = 2.53 \times 10^{-5} \text{ mol dm}^{-3}$; c) $c_L = 6 \times 10^{-5} \text{ mol dm}^{-3}$, $c_M = 8 \times 10^{-6} \text{ mol dm}^{-3}$.

However, molar absorptivity values are not real, because these complexes are formed in a little amount. Thus finally these complexes have not been accepted.

Distribution diagrams calculated using values of $\text{p}K_{a3} - \text{p}K_{a5}$ of the reagent, $\log \beta_{111} = 2.97$, $\log \beta_{124} = -13.12$ for solutions with an excess of the metal ion, equimolar, and with the excess of the ligand are given in Fig. 3a–c.

It follows from the distribution diagram that in solutions with an excess of the metal ion only CuH_2L^+ complex predominates, while in equimolar solutions the complex CuH_2L^+ is formed in the pH range 3–8 and $\text{Cu}(\text{HL})_2^{2-}$ would be formed if pH were greater than 9. In excess of the reagent CuH_2L^+ complex is formed in a narrower pH range 1–3, and it predominates in the range 3–6.5 while at $\text{pH} > 8.5$ $\text{Cu}(\text{HL})_2^{2-}$ is formed.

Complexes of $\text{Zn}(\text{II})$ with TrAMeR show absorption maximum around $\lambda = 460 \text{ nm}$; however, in order to avoid the interference of the reagent which absorbs considerably at $\lambda = 460 \text{ nm}$, the measurement of $A = f(\text{pH})$ was performed at λ/nm : 470, 482, 490, and 510. The curves obtained at $\lambda = 482 \text{ nm}$ are presented in Fig. 4. In comparison to $\text{Cu}(\text{II})$ the complex formation is shifted to higher pH values.

Results of computations for four wavelengths are summarized in Table 3. In excess of the metal ion, ZnH_2L^+ complex is first formed, but at $\text{pH} > 5$ it is transformed to ZnHL one. In equimolar solutions where the complex formation starts at pH 6, $\log \beta$ for the first complex was necessary to fix at the value obtained from solutions with the excess of the metal ion, as the main species is ZnHL complex. The best model was obtained assuming ZnH_2L^+ , ZnHL , and also $\text{Zn}(\text{HL})_2^{2-}$ complexes. For solutions with an excess of the reagent the best model obtained was the same. Distribution diagrams calculated for the solu-

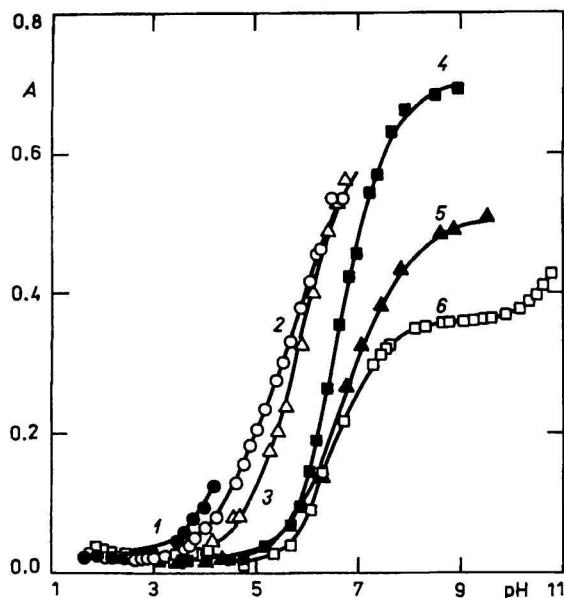


Fig. 4. Absorbance–pH curves for the system $\text{Zn}(\text{II})$ —TrAMeR. $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$, $\phi_1(\text{methanol—water}) = 40\%$, $I(\text{NaNO}_3) = 0.25 \text{ mol dm}^{-3}$, $\lambda = 480 \text{ nm}$. $c_M/(\text{mol dm}^{-3}) = 1. 2.46 \times 10^{-3}$; 2. 9.82×10^{-4} ; 3. 2.46×10^{-4} ; 4. 2.46×10^{-5} ; 5. 4.92×10^{-6} ; 6. 0. Solid lines are theoretical curves calculated for the best model obtained from LETAGROP-SPEFO.

tions with excess of the metal ion, equimolar, and solutions with excess of the ligand are given in Fig. 5.

Iron(III) and the reagent produce complexes with absorption maximum around $\lambda = 538 \text{ nm}$. Corresponding $A = f(\text{pH})$ curves were measured for λ/nm : 530, 538, and 546, while in Fig. 6 they are given only for the absorption maximum. While in solutions with an excess of the reagent the first complexation wave is observed in the pH range 0–4, in equimolar solutions the pH interval was 2–4, while at higher pH precipitation was observed. Only solutions without any precipitate and showing no Tyndall effect were used for the calculations.

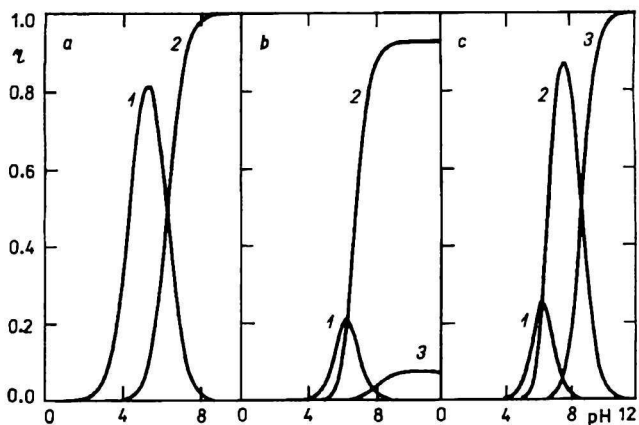


Fig. 5. Distribution diagrams ($\eta(\text{pH})$) of species. 1. ZnH_2L^+ ; 2. ZnHL ; 3. $\text{Zn}(\text{HL})_2^{2-}$; $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$. $c_M/(\text{mol dm}^{-3}) = \text{a) } 2.42 \times 10^{-4}$; b) 2.42×10^{-5} ; c) 6.06×10^{-6} .

Table 3. Equilibria Analysis in the Zn(II)—TrAMeR System. Values of U , $\sigma(A)$, $\log \beta$, and $\varepsilon(482 \text{ nm})$ for the Different Models Tested by LETAGROP-SPEFO Method. λ/nm : 470, 482, 490, and 510

Model	Species	$\log \beta \pm \sigma(\log \beta)$	$U \cdot 10^2$	$\sigma(A) \cdot 10^3$	$\frac{\varepsilon \pm \sigma(\varepsilon)}{\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}}$
1	ZnH_2L^+	-2.20 ± 0.09	27.4	34.16	23000 ± 620
2	ZnHL	-7.45 ± 0.18	4.25	42.55	19800 ± 490
3	ZnH_2L^+	-1.69 ± 0.06	1.65	8.48	10600 ± 160
	ZnHL	-7.78 ± 0.10			25790 ± 170
Conditions: $N_p = 240$ $c_M > c_L$ $1.66 < \text{pH} < 6.95$					
4	ZnHL	-8.40 ± 0.12	6.64	26.66	30700 ± 170
5	$\text{Zn}(\text{H}_2\text{L})_2$	-16.85 ± 0.25	14.2	38.97	55300 ± 1000
6	ZnHL	-8.32 ± 0.09	1.32	12.17	29400 ± 160
	$\text{Zn}(\text{HL})_2^{2-}$	18.46 ± 0.22			62200 ± 170
7	ZnH_2L^+	2.20 ± 0.09 (fixed)	1.22	11.93	10600 ± 710
	ZnHL	-7.95 ± 0.06			28600 ± 380
	$\text{Zn}(\text{HL})_2^{2-}$	-17.76 MAX = -17.48			65800 ± 5370
Conditions: $N_p = 99$ $c_M = c_L$ $2.60 < \text{pH} < 8.90$					
8	$\text{Zn}(\text{HL})_2^{2-}$	-13.30 MAX = -17.02	3.72	26.28	67000 ± 2150
9	ZnH_2L^+	2.20 (fixed)	0.34	8.36	10600 ± 160
	ZnHL	-8.14 ± 0.11 (fixed)			31300 ± 2010
	$\text{Zn}(\text{HL})_2^{2-}$	-18.38 MAX = -18.06			74700 ± 1490
Conditions: $N_p = 59$ $c_L > c_M$ $2.70 < \text{pH} < 9.50$					

The significance of symbols U , $\sigma(A)$, MAX, and N_p is the same as in Table 2.

The data for solutions with an excess of the metal ion and equimolar solutions could be well explained by the formation of the 1 : 1 complex $\text{FeH}_2\text{L}^{2+}$, which means the liberation of only one proton from the LH_3

form of the reagent. The results of the calculation are given in Table 4.

In solutions with an excess of the reagent two waves were observed. The first one was difficult to explain only by the formation of $\text{FeH}_2\text{L}^{2+}$, it was found that the number of protons split was equal to two, which means the formation of mixed $\text{FeH}_2\text{L}(\text{OH})^+$ complex, because the splitting of proton of p -hydroxy group is not possible at $\text{pH} < 5$.

Thus, the complete model of iron complexation consists of $\text{FeH}_2\text{L}^{2+}$, $\text{FeH}_2\text{L}(\text{OH})^+$, and $\text{Fe}(\text{HL})_2^-$ complexes. Distribution diagrams for this model are given in Fig. 7. The values of ε for $\text{FeH}_2\text{L}(\text{OH})^+$ seem to

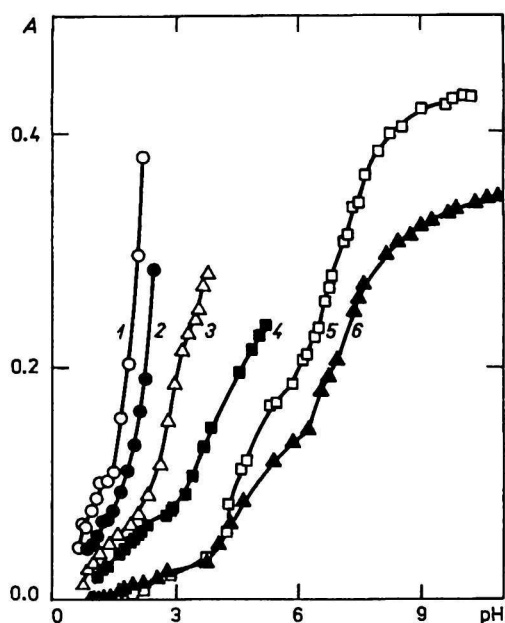


Fig. 6. Absorbance—pH curves for the system Fe(III)—TrAMeR. $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$, $\phi_r(\text{methanol—water}) = 40\%$, $I(\text{NaNO}_3) = 0.25 \text{ mol dm}^{-3}$, $\lambda = 538 \text{ nm}$. $c_M/(\text{mol dm}^{-3}) = 1, 1.21 \times 10^{-3}; 2, 6.06 \times 10^{-4}; 3, 2.42 \times 10^{-4}; 4, 2.42 \times 10^{-5}; 5, 6.06 \times 10^{-6}; 6, 0$. Solid lines are theoretical curves calculated for the best model obtained from LETAGROP-SPEFO.

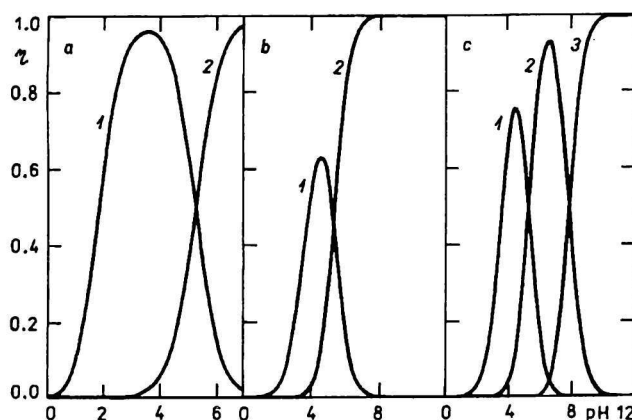


Fig. 7. Distribution diagrams ($\eta(\text{pH})$) of species. 1. $\text{FeH}_2\text{L}^{2+}$; 2. $\text{FeH}_2\text{L}(\text{OH})^+$; 3. $\text{Fe}(\text{HL})_2^-$; $c_L = 2.4 \times 10^{-5} \text{ mol dm}^{-3}$ $c_M/(\text{mol dm}^{-3}) = \text{a) } 2.42 \times 10^{-4}; \text{b) } 2.42 \times 10^{-5}; \text{c) } 6.06 \times 10^{-6}$.

Table 4. Equilibria Analysis in the Fe(III)—TrAMeR System. Values of U , $\sigma(A)$, $\log^* \beta$, and $\varepsilon(538 \text{ nm})$ for the Different Models Tested by LETAGROP-SPEFO Method. λ/nm : 530, 538, and 546

Model	Species	$\log^* \beta \pm \sigma(\log^* \beta)$	$U \cdot 10^2$	$\sigma(A) \cdot 10^3$	$\frac{\varepsilon \pm \sigma(\varepsilon)}{\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}}$
1	$\text{FeH}_3\text{L}^{3+}$	5.52 MAX = 5.94	19.0	60	4000 \pm 375
2	$\text{FeH}_2\text{L}(\text{OH})^+$	- 0.47 MAX = - 0.11	5.02	30.79	7190 \pm 315
3	$\text{FeH}_2\text{L}^{2+}$	1.10 \pm 0.15	0.99	13.68	10490 \pm 210
Conditions: $N_p = 55$ $c_M \geq c_L$ $0.20 < \text{pH} < 3.80$					
4	$\text{FeH}_2\text{L}(\text{OH})^+$	- 4.48 MAX = - 3.99	2.95	34.39	26000 \pm 1140
5	$\text{Fe}(\text{H}_2\text{L})_2(\text{OH})^+$	- 9.02 MAX = - 8.00	7.77	55.78	40400 \pm 2290
6	$\text{FeH}_2\text{L}^{2+}$	1.10 \pm 0.15 (fixed)	0.10	6.67	12000 (fixed)
	$\text{FeH}_2\text{L}(\text{OH})^+$	- 4.15 \pm 0.23 (fixed)			20700 \pm 290
	$\text{Fe}(\text{HL})_2^-$	- 13.76 MAX = - 13.50			47700 \pm 430
Conditions: $N_p = 27$ $c_L > c_M$ $\text{pH} < 4.24$					
7	$\text{FeH}_2\text{L}^{2+}$	1.10 \pm 0.15 (fixed)	0.04	4.52	12000 (fixed)
	$\text{FeH}_2\text{L}(\text{OH})^+$	- 4.15 \pm 0.23			19900 \pm 230
	$\text{Fe}(\text{HL})_2^-$	- 13.68 \pm 0.17			47600 \pm 290
Conditions: $N_p = 20$ $c_L > c_M$ $\text{pH} > 6.11$					

The significance of symbols U , $\sigma(A)$, MAX, and N_p is the same as in Table 2.

be reasonable. They are higher than those of $\text{FeH}_2\text{L}^{2+}$. It is well known that the replacement of coordinated water in Fe(III) coordination sphere leads to the high increase of the absorption of iron(III) in solution.

From the coordination chemistry viewpoint it can be concluded that TrAMeR reagent gives complexes with similar structure to those found with the other azo dyes. In solutions with an excess of the reagent 1 : 2 (metal : ligand) complexes are highly absorbing and can be used for the determination of those elements.

Analytical Applications of TrAMeR Reagent, Method of Simultaneous Spectrophotometric Determination of Cu(II), Zn(II), and Fe(III)

TrAMeR has been already used for the determination of Co, V and as it follows from the present study, the reaction with Cu, Zn, and Fe is also highly sensitive. Molar absorptivity numerical values ob-

tained $\varepsilon/(\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})$ 39500, 78400, and 47800 for Cu, Zn, and Fe 1 : 2 (metal : ligand) complexes enable to compare this reagent with PAR (4-(2-pyridylazo)resorcinol) or TAR (4-(2'-thiazolylazo)-resorcinol). Almost the same sensitivity has been reached. However, highly absorbing complexes are formed at pH 9 in solutions with an excess of the reagent where also the reagent itself absorbs due to the dissociation of H_2L^- to HL^{2-} form with λ_{max} at 430 nm, while λ_{max} of complexes are 460 nm, 504 nm, and 538 nm for Zn, Cu, and Fe, respectively.

The analytical method was established at pH \approx 9.2 according to the distribution diagrams, while tetraborate buffer ($0.025 \text{ mol dm}^{-3}$) was found not to interfere. High excess (10000) of chloride, perchlorate, nitrate, Ca(II), and Mg(II) does not interfere. Metal ions like Mn(II), Co(II), and Ni(II) interfere at the ratio 1 : 1 to any of Cu, Zn, and Fe metal ions. Phosphate interferes at the ratio $[\text{PO}_4^{3-}]/[\text{Fe}^{3+}]$ 0.5.

As metals under study are often present and used in pharmaceutical preparatives, like Micebrina, etc., the reagent was applied for the multicomponent analysis in synthetic mixtures of that kind of pills.

The data were evaluated using partial least-squares (PLS) calibration method. The calibration matrix has been designed according to two-level factorial design and consists of nine solutions with one central point in addition. We have used 24 wavelengths, i.e. from 470 nm to 480 nm with $\Delta\lambda = 2 \text{ nm}$ and from 480 nm to 552 nm with $\Delta\lambda = 4 \text{ nm}$.

The lowest and highest concentrations were: $c_{\text{Cu}} = 0.63 \mu\text{mol dm}^{-3}$ and $5.00 \mu\text{mol dm}^{-3}$, $c_{\text{Zn}} = 0.24 \mu\text{mol dm}^{-3}$ and $2.00 \mu\text{mol dm}^{-3}$, and $c_{\text{Fe}} = 0.91 \mu\text{mol dm}^{-3}$ and $10.98 \mu\text{mol dm}^{-3}$. Thus the dimension of the calibration matrix was 10×24 .

The results obtained are summarized in Tables 5

Table 5. Results of Simultaneous Determination of Cu(II), Zn(II), and Fe(III) in Synthetic Mixtures

Sample	$c(\text{added})/(\mu\text{mol dm}^{-3})$			$c(\text{found})/(\mu\text{mol dm}^{-3})$		
	Cu(II)	Zn(II)	Fe(III)	Cu(II)	Zn(II)	Fe(III)
1	1.05	0.73	5.45	0.97	0.74	5.48
2	1.05	0.73	5.45	1.07	0.63	5.43
3	1.05	0.73	5.45	1.06	0.66	5.56
4	0.42	0.61	2.73	0.39	0.59	2.71
5	0.52	0.92	1.82	0.47	0.90	1.84
6	0.84	0.31	7.27	0.81	0.31	7.27
7	1.58	0.49	9.09	1.50	0.52	8.95
8	0.63	0.37	3.63	0.61	0.39	3.63
Average relative error/%				± 4.88	± 5.20	± 0.79

Table 6. Results of Simultaneous Determination of Cu(II), Zn(II), and Fe(III) in Micebrina (Derly)

Sample	$c(\text{Cu(II)}, \text{found})/(\mu\text{mol dm}^{-3})$		$c(\text{Zn(II)}, \text{found})/(\mu\text{mol dm}^{-3})$		$c(\text{Fe(III)}, \text{found})/(\mu\text{mol dm}^{-3})$	
	Proposed method	AAS	Proposed method	AAS	Proposed method	AAS
1	0.58	0.61	0.76	0.89	10.84	10.76
2	0.60	0.63	0.65	0.87	10.92	10.75
3	0.54	0.61	0.81	0.90	11.03	10.76
Average	0.57 ± 0.03		0.74 ± 0.08		10.93 ± 0.09	

Concentrations in the solutions to analyze (5 pills dissolved in 1000 cm³ and 1 cm³ diluted to 25 cm³): $c(\text{Cu(II)}) = 0.63 \mu\text{mol dm}^{-3}$, $c(\text{Zn(II)}) = 0.91 \mu\text{mol dm}^{-3}$, $c(\text{Fe(III)}) = 10.74 \mu\text{mol dm}^{-3}$ calculated from the composition of pharmaceutical preparatives.

and 6. Even if all absorption bands of Zn, Cu, and Fe are highly interfering with each other, the results obtained are acceptable and a comparison with AAS gives sufficient agreement also with nominal values of the metal ions content in the medicament.

It can be concluded that TrAMeR reagent is comparable with "classical" azo dyes PAR, TAR and can be well used for the multicomponent analysis of heavy metal ions mixtures. The work for the determination of some other metal ions in waters takes its course in this laboratory.

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