

THE SIMULTANEOUS DETERMINATION OF Fe(III) AND Cu(II)  
IN THE PRESENCE OF Zn(II) USING  
2-(5-BROMO-2-PYRIDYLAZO)-5-(DIETHYLAMINO)PHENOL

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Spectrophotometric methods were employed to study the chelate formation between Fe(III) ions and 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (BrPADAP) in dimethylformamide ( $\varphi = 20\%$ ) and Triton X-100 ( $\varphi = 0.1\%$ ). The  $ML_2$  chelate with absorption maxima at 535 and 590 nm and molar absorption coefficients of 6.5 and 8.2  $m^2 \text{ mmol}^{-1}$  is formed quantitatively in the pH interval 3.5–4.0 for at least a seven-fold concentration excess of BrPADAP. Concentrations of  $c(\text{Fe}) = 1.6\text{--}8.1 \mu\text{mol l}^{-1}$  can be determined independently in 0.1M acetate buffer with pH 3.6. Iron and copper ions can be determined simultaneously in the presence of zinc ions in binary or ternary mixtures in the concentration ranges  $c(\text{Fe}) = 2\text{--}8 \mu\text{mol l}^{-1}$  and  $c(\text{Cu}) = c(\text{Zn}) = 3\text{--}16 \mu\text{mol l}^{-1}$ . The relative deviations in the calculated concentrations,  $\Delta c_r$ , are 1–4% for Fe and up to 5% for Cu in binary and ternary mixtures with equivalent concentrations up to a concentration excess of one component of five-fold in binary mixtures or of one or two components of up to three-fold in ternary mixtures. The simultaneous determination of Zn(II) is accompanied by a  $\Delta c_r$  value of up to 30–50% and cannot be recommended under the given experimental conditions.

Ferrous and ferric ions react with N-heterocyclic azodyes to form strongly coloured complexes<sup>1,2</sup>. The most sensitive reagents of this type include the halo-derivatives 2-(2-pyridylazo)-5-(dialkylamino)phenol (PADAP) with chelating groups of atoms identical with those of pyridylazoresorcinol (PAR). The reactions with a number of polyvalent metal ions are characterized by high sensitivity ( $\varepsilon = 8\text{--}15 \text{ m}^2 \text{ mmol}^{-1}$ ) and sharp colour contrast. The chelates with a number of ions are characterized by double absorption bands with maxima in the range 520–610 nm and marked differences in the absorption spectra. This fact makes these reagents especially suitable for the development of highly sensitive spectrophotometric methods for the determination of a number of elements, including iron ions and also in some cases for multicomponent analysis.

A number of highly sensitive methods have been described in the literature for the determination of Fe(II) and Fe(III) ions with BrPADAP or CIPADAP and their derivatives in weakly acidic to neutral media<sup>3–15</sup>, characterized by high precision and accuracy and high selectivity for Fe(II). However, these reactions are kinetically

slow and measurements can be carried out after attainment of constant absorption, after 5–30 minutes.

This work deals with the study of chelation equilibria of Fe(III) ions with BrPADAP in aqueous DMF solutions ( $\varphi = 20\%$ ) ( $\varphi$  is the volume fraction) and Triton X-100 ( $\varphi = 0.1\%$ ). Conditions for the spectrophotometric determination of Fe(III) were optimized, i.e. the presence of buffers, anionic and nonionic tensides and other factors affecting the formation of the ternary chelates. Optimization was aimed at the determination of Fe(III) in water and the possible simultaneous determination of Fe(III), Cu(II) and Zn(II) in blood serum.

## EXPERIMENTAL

### Chemicals and Apparatus

The stock solution of  $c(\text{BrPADAP}) = 0.1\text{--}1.0 \text{ mmol l}^{-1}$  was prepared by dissolving the chromatographically pure substance, 2-(5-bromo-2-pyridylazo)-5-(diethylamino)phenol (E. Merck, Darmstadt, F.R.G.) in dimethylformamide (DMF). Stock solutions of Fe(III), Cu(II) and Zn(II) ions with concentrations of  $c(\text{Fe}) = 201.7$ ,  $c(\text{Cu}) = 100.5$  and  $c(\text{Zn}) = 103.5 \text{ mmol l}^{-1}$  were prepared by dissolving the appropriate chlorides (Fe and Cu) or carbonate (Zn) of p.a. purity (Lachema, Brno, Czechoslovakia or Baker Analyzed Reagents, the Netherlands) in HCl to yield an approximate final concentrations of  $c(\text{HCl}) = 0.4$ ,  $0.1$  and  $0.25 \text{ mol l}^{-1}$ , respectively. The standard solution with  $c(\text{Fe(II)}) = 86.6 \text{ mmol l}^{-1}$  in  $0.2\text{M}$  hydrochloric acid was prepared by dissolving spectrally pure iron (Matthey and Johnson, London, Great Britain) in excess of concentrated HCl. The solutions were standardized by chelometric titration using murexide or xylenol orange indicator or gravimetrically. The stock solutions of trichloroacetic acid,  $c(\text{TCA}) = 3 \text{ mol l}^{-1}$  (Cambrian Chemicals, Croydon, Great Britain), sodium dodecylsulphate,  $c(\text{SDS}) = 50 \text{ mmol l}^{-1}$  (BDH Chemicals, Poole, Great Britain) and Triton X-100,  $\varphi(\text{TX100}) = 1\%$  (Koch Light, Colnbrook, Great Britain) were prepared by dissolving the p.a. commercial substances in water. The remaining chemicals were the p.a. commercial substances (Lachema, Brno, Czechoslovakia and Reanal, Budapest, Hungary) and Specpure NaOH (Reachim, Moscow, U.S.S.R.). Water was doubly distilled in a Bi-18 Destamat quartz apparatus (Heraeus Quarzschmelze, Hanau, F.R.G.).

The spectrophotometric measurements were carried out at laboratory temperature,  $23 \pm 1^\circ\text{C}$  using and SP 500 single-beam spectrophotometer (Pye Unicam, Cambridge, Great Britain) or a Specord M 40 computer-controlled double-beam recording spectrophotometer (C. Zeiss, Jena, G.D.R.) in quartz cuvettes with an optical pathway of 10 mm.

The solution acidity was measured using a digital pH meter, OP 208/1, with a combined glass and calomel electrode, OP 0808P (Radelkis, Budapest, Hungary). The instrument was regularly calibrated using standard S1306 and S1326 buffers with  $\text{pH } 1.68 \pm 0.01$  and  $7.00 \pm 0.01$  at  $25^\circ\text{C}$  (Radiometer, Copenhagen, Denmark). The conditional pH values in DMF medium,  $\varphi(\text{DMF}) = 20\%$  and  $\varphi(\text{TX100}) = 0.1\%$ , were not corrected.

The calibration curves were treated by the REG-1 program using the standard procedure for the linear least squares method<sup>16</sup>. Each point on the calibration curve ( $n = 10$ ), evenly distributed along the curve, was measured for two independent solutions and for 10 repeated absorbance measurements. The concentrations of the individual components,  $c_c$ , were calculated using the ORTHO program by numerical analysis of the absorption spectra of binary and ternary mixtures of Fe(III), Cu(II) and Zn(II) in the presence of BrPADAP.

The program is based on solution of a set of linear equations for the overall value of the absorbance of the reaction mixture,  $A_{ij} = \sum \varepsilon_{ij} c_i$ , for the individual wavelength values by orthogonalization of the columns in the absorbance matrix<sup>17</sup>. The number of linear equations employed could be varied during data treatment from a number identical with the number of components determined up to several times this value for overdetermined systems. The input data consisted of the values of the molar absorption coefficients,  $\varepsilon_{ij}$ , for all the absorbing components of the reaction mixture determined in an independent experiment and the values of the experimentally determined total absorbance for the individual wavelength values,  $A_{ij}$ . The precision and accuracy of the concentration values calculated,  $c_c$ , were controlled numerically or graphically<sup>18-20</sup>. All the calculations were carried out on a ZX Spectrum microcomputer (Sinclair, Great Britain).

Where not stated otherwise, measurements were carried out in DMF medium ( $\varphi = 20\%$ ) in the presence of TX-100 ( $\varphi = 0.1\%$ ), primarily at concentrations of  $c(\text{Fe}) = 6.5 \mu\text{mol l}^{-1}$  and  $c(\text{BrPADAP}) = 86 \mu\text{mol l}^{-1}$  at least 10 minutes after solution preparation. The solutions of the individual components of the reaction mixture were mixed in the order: acid Fe(III) solution plus an acid solution of interfering components where required, DMF, TX-100, water to 50% of the overall volume, reagent, followed by adjusting of the solution acidity with NaOH or a buffer to the required pH, and filling to the mark. This procedure yielded the highest absorbance values.

## RESULTS AND DISCUSSION

### *Chelating Equilibrium Between Fe(III) and BrPADAP*

In the presence of a concentration excess of BrPADAP ( $c_L \geq 10 \mu\text{mol l}^{-1}$ ) at  $\text{pH} > 1$ , the absorption spectra of solutions of ferric ions with concentration  $c(\text{Fe}) = 4.8 \mu\text{mol l}^{-1}$  exhibit a characteristic double absorption band with maxima at 535 and 590 nm ( $\varepsilon = 6.3$  and  $8.0 \text{ m}^2 \text{ mmol}^{-1}$ ). The position of the maximum is not affected by the magnitude of the concentration excess of the reagent and does not change in the presence of 0.1M acetate buffer with pH 3.6 or at  $c(\text{SDS}) = 5 \text{ mmol l}^{-1}$ . The chelate of Fe(III) with BrPADAP is formed slowly and the absorbance is constant in the range 10–30 min from preparation of the solution.

The absorbance–pH curves (Fig. 1),  $\Delta A = f(\text{pH})$  for a solution of Fe(III) ions with a concentration excess of BrPADAP ( $c_L = 86 \mu\text{mol l}^{-1}$ ) for a wavelength of 590 nm ( $\Delta A$  is the absorbance difference,  $A - A_{oL}$ ) confirm that the chelate begins to form at  $\text{pH} > 1$ . Quantitative formation is attained at pH 3.5–4.0. The absorbance decreases monotonously at higher pH values. Because of the higher selectivity of the reaction for polyvalent ions, the lower limit of the given pH range, pH 3.6, was chosen as optimal.

It follows for the concentration dependence  $\Delta A = f(c_L)$  that at least a seven-fold concentration excess of BrPADAP is essential for quantitative chelate formation ( $\varepsilon = 8.2 \text{ m}^2 \text{ mmol}^{-1}$ ) at  $\text{pH} \geq 3.6$ . At least a ten-fold concentration excess was employed in all practical applications, i.e.  $c_L \geq 86 \mu\text{mol l}^{-1}$ .

Formate buffer with pH 3.6 decreases the absorbance of a solution containing Fe(III) and BrPADAP by  $\Delta A_r \geq 2\%$  at buffer concentrations from  $0.05 \text{ mol l}^{-1}$ ,

where  $\Delta A_r$  is the relative change in absorbance in per cent ( $\Delta A_r = 100(A - A')/A'$ ). Acetate buffer increases the absorbance over the whole range 500–630 nm as a result of formation of the ternary chelate  $\text{FeL}_2\text{Ac}$ , demonstrated indirectly by measurements at various acetate concentrations. All subsequent measurements were carried out in the presence of 0.1M acetate buffer at pH 3.6, even though its buffering capacity is slightly lower. The ionic strength has no great effect on the absorbance of the solution ( $\Delta A_r \leq 2\%$ ) up to a value of  $I = 0.1 \text{ mol l}^{-1}$  (HCl + NaCl).

Anionic and nonionic tensides have negligible effect on the position of the absorption maxima of the Fe(III) chelate with BrPADAP. At TX100 concentrations of less than  $\varphi = 0.1 \pm 0.05\%$ , the solution foams, and at higher concentrations it becomes opalescent. In the presence of an anionic tenside the value of the molar absorption coefficient is lower and at the optimal concentration  $c(\text{SDS}) = 4 \text{ mmol l}^{-1}$  it is about 10% lower ( $\varepsilon = 7.2 \text{ m}^2 \text{ mmol}^{-1}$ ) than for  $\varphi(\text{TX100}) = 0.1\%$ .

The curves of continuous variation measured at 534, 590 and 610 nm for values of  $c_0 = c_M + c_L = 56.5 \text{ } \mu\text{mol l}^{-1}$  in a medium of 0.1M acetate buffer and in mixtures of HCl and NaCl ( $c = 0.1 \text{ mol l}^{-1}$ ) confirm formation of a chelate with stoichiometric ratio Fe(III) : BrPADAP = 1 : 2 at pH 3.6.

Under the above optimal conditions at pH 3.6 and a wavelength of 590 nm, the calibration plots are linear in the range  $c(\text{Fe}) 1.6$  to  $8.1 \text{ } \mu\text{mol l}^{-1}$  in 0.1M acetate buffer medium and in 0.1M HCl–NaCl mixture, as well as in this mixture in the presence of  $4 \text{ mmol l}^{-1}$  SDS. The highest molar absorption coefficient value ( $\varepsilon = 8.1 \text{ m}^2 \text{ mmol}^{-1}$ ) was attained in 0.1M acetate buffer medium, compared to values of 7.71 and 7.24 in the other media, respectively (Table I).

The threshold interference ( $\Delta A_r = \pm 2\%$ ) for a number of metal ions and selected reagents was determined graphically by interpolation of the absorbance dependence on the concentration of interferent at  $c(\text{Fe}) = 4.8 \text{ } \mu\text{mol l}^{-1}$ ,  $c_L = 86 \text{ } \mu\text{mol l}^{-1}$  in

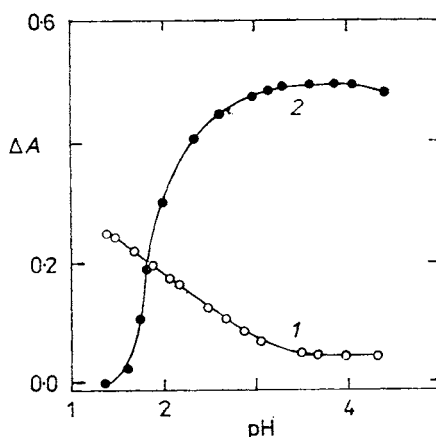


FIG. 1

The absorbance–pH curves  $\Delta A = f(\text{pH})$  for BrPADAP solutions (1) and the chelate with Fe(III) (2) in medium with  $\varphi(\text{DMF}) = 20\%$  and  $\varphi(\text{TX 100}) = 0.1\%$ ,  $c_L = 85.9 \text{ } \mu\text{mol l}^{-1}$ ,  $c(\text{Fe}) = 6.45 \text{ } \mu\text{mol l}^{-1}$ ,  $I = 0.1 \text{ mol l}^{-1}$  (HCl + NaCl), 590 nm

a 0.1M acetate buffer medium with pH 3.6. The threshold interfering concentrations are listed in Table II. To test the applicability of this method for determining Fe(III)

TABLE I

Statistical parameters of the calibration curves for the determination of Fe(III) using BrPADAP.  $c_M$  1.61 to 8.07  $\mu\text{mol l}^{-1}$  Fe(III),  $c_L$  = 85.9  $\mu\text{mol l}^{-1}$ , 0.1M acetate buffer, pH 3.6,  $\varphi(\text{DMF})$  = 20%,  $\varphi(\text{TX100})$  = 0.1%,  $n$  = 10, even distribution over the whole concentration interval, Specord M 40,  $l$  = 10 mm

Parameter	$\lambda$ , nm		
	545	575	590
$\varepsilon \pm d(\varepsilon)^a$	6.6948 $\pm$ 0.0243	6.9930 $\pm$ 0.0317	8.0734 $\pm$ 0.0280
$(A_0 \pm d(A_0)) \cdot 10^{3b}$	286.6 $\pm$ 1.3	98.4 $\pm$ 1.7	67.4 $\pm$ 1.5
$s_{xy} \cdot 10^{3c}$	1.2	1.6	1.4
$s_0 \cdot 10^{3d}$	3.2	4.3	1.5
DL	2.7	3.5	1.0

<sup>a</sup> Molar absorption coefficient,  $\text{m}^2 \text{mmol}^{-1}$ ; <sup>b</sup> absorbance of the blank; <sup>c</sup>  $s_{xy} = [(\sum(A_{\text{exp}} - A_{\text{calc}})^2 / (n - 2))]^{1/2}$ ; <sup>d</sup>  $s_0$  standard deviation of the blank, <sup>e</sup> DL = 10  $s_0$ , detection limit in  $\text{ng ml}^{-1}$ .

TABLE II

Interference threshold  $\Delta A_r = \pm 2\%$  for the determination of Fe(III) with BrPADAP.  $c_L$  = 85.9  $\mu\text{mol l}^{-1}$ ,  $c(\text{Fe})$  = 4.84  $\mu\text{mol l}^{-1}$ ,  $\varphi(\text{DMF})$  = 20%,  $\varphi(\text{TX100})$  = 0.1%, 0.1M acetate buffer, pH 3.6,  $\lambda$  590 nm

Ion X	Salt	$n_X/n_{\text{Fe}}^a$	$m_X/m_{\text{Fe}}$
$\text{IO}_4^-$	$\text{NaIO}_4$	83	316
$\text{ClO}_4^-$	$\text{NaClO}_4$	85	210
$\text{F}^-$	$\text{NaF}$	100	35
$\text{H}_2\text{PO}_4^-$	$\text{KH}_2\text{PO}_4$	165	290
$\text{SO}_4^{2-}$	$\text{K}_2\text{SO}_4$	5 200	8 880
$\text{NO}_3^-$	$\text{NaNO}_3$	12 400	13 800
$\text{Cl}^-$	$\text{NaCl}$	82 600	52 500
TCA	TCA	82 600	242 000
$\text{Zn}^{2+}$	$\text{ZnCl}_2$	33	39
$\text{Ca}^{2+}$	$\text{CaCl}_2$	620	445
$\text{Mg}^{2+}$	$\text{MgCl}_2$	725	315

<sup>a</sup> Cu(II), Ni(II), Co(II) and Mn(II) interfere at  $n_X/n_{\text{Fe}} < 1$ ;  $n_X/n_{\text{Fe}}$  and  $m_X/m_{\text{Fe}}$  are the material or mass ratios of the X and Fe ions.

ions with BrPADAP in blood serum, special attention was paid to the effect of trichloroacetic acid (TCA), which is the most commonly employed deproteination agent. A study was also carried out of the effect of hydrogen peroxide on the rate and quantitiveness of the oxidation of Fe(II) to Fe(III) ions and possible destruction of BrPADAP. The molar absorption coefficient value was identical with that given above up to  $\varphi(\text{H}_2\text{O}_2) = 1\%$  for an initial solution of Fe(III), a mixture of Fe(III) and Fe(II) and Fe(II) after oxidation by approx. 3–6%  $\text{H}_2\text{O}_2$  at about  $50^\circ\text{C}$  for 10 min. TCA has no marked effect up to a concentration of  $0.4 \text{ mol l}^{-1}$ .

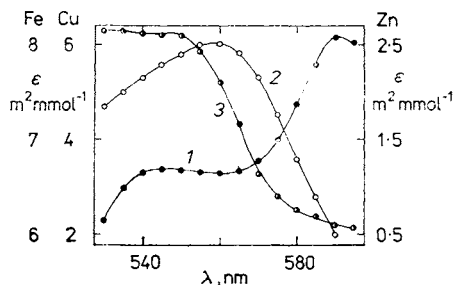
#### *Simultaneous Determination of Fe(III) and Cu(II) in the Presence of Zn(II) using BrPADAP*

The statistical parameters of the calibration curves for the determination of all three metal ions with BrPADAP were determined in the range 530–590 nm at constant steps of 5 nm on the basis of previous detailed spectrophotometric study of the reaction of BrPADAP with Cu(II), Zn(II) and other ions<sup>21,22</sup>, considering the requirement for a fast method for the simultaneous determination of Fe(III) and Cu(II) in the presence of roughly equimolar concentrations of Zn(II) in blood serum. The molar absorption coefficients (Fig. 2) were found for the regions of the absorption maxima of all three chelates.

The overall absorbance values  $A_{ij}$  for binary and ternary mixtures of Fe(III), Cu(II) and Zn(II) were measured under identical experimental conditions for concentration ratios of the individual components in the range from 1 : 1 up to a three- or five-fold concentration excess of Fe(III) or Cu(II) and Zn(II) in binary mixtures and in the range from 1 : 1 : 1 to a three-fold concentration excess of one or two components in ternary mixtures. The total concentration of metal ions was always less than 10% of the overall BrPADAP concentration to ensure a sufficient concentration excess of BrPADAP for quantitative formation of the  $\text{ML}_2$  or  $\text{ML}$  chelates.

The theoretical absorbance values  $A_t$  for the individual solutions were calculated from the molar absorption coefficient values (see Fig. 2) for the individual wave-

FIG. 2  
The dependence of the molar absorption coefficient values of the Fe(III), Cu(II) and Zn(II) chelates on the wavelength.  $\varphi(\text{DMF}) = 20\%$ ,  $\varphi(\text{TX100}) = 0.1\%$ ,  $0.1 \text{ M}$  acetate buffer, pH 3.6,  $c_M$  2 to  $9 \mu\text{mol l}^{-1}$  for Fe(III),  $c_M$  3 to  $16 \mu\text{mol l}^{-1}$  for Cu(II) and Zn(II),  $c_L = 86 \mu\text{mol l}^{-1}$ ; Specord M 40. Curve: 1 Fe(III), 2 Cu(II), 3 Zn(II)



lengths and corresponding metal ion concentrations. These values were compared with the experimentally determined values  $A_{ij}$  and the relative deviations  $\Delta A_r = 100(A_{ij} - A_i)/A_{ij}$  did not exceed 2%.

TABLE III

Comparison of the declared  $c_t$  and calculated  $c_c$  concentrations of Fe(III), Cu(II) and Zn(II) ions in binary and ternary mixtures with BrPADAP for various concentration ratios.  $c_L = 86 \mu\text{mol l}^{-1}$ ,  $c(\text{Fe}) = 3.22 \mu\text{mol l}^{-1}$ ,  $c(\text{Cu}) = 3.21 \mu\text{mol l}^{-1}$ ,  $c(\text{Zn})^a = 3.30 \mu\text{mol l}^{-1}$ , 0.1M acetate buffer,  $\varphi(\text{DMF}) = 20\%$ ,  $\varphi(\text{TX100}) = 0.1\%$ , pH 3.6

$c(\text{Fe}) : c(\text{Cu}) : c(\text{Zn})^b$	$c_c(\text{Fe})^c$ $\mu\text{mol l}^{-1}$	$\Delta c_r(\text{Fe})^d$ %	$c_c(\text{Cu})^c$ $\mu\text{mol l}^{-1}$	$\Delta c_r(\text{Cu})^d$ %	$c_t(\text{Zn})^c$ $\mu\text{mol l}^{-1}$
1 : 1 : 1	3.28	1.67	3.25	0.99	3.30
1 : 1 : 2	3.31	2.73	3.23	0.98	6.61
1 : 1 : 3	3.32	2.87	3.31	2.94	—
1 : 2 : 2	3.35	4.06	6.46	0.48	—
1 : 2 : 3	3.39	4.98	6.48	0.79	—
1 : 3 : 1	3.21	-0.23	9.75	1.11	—
1 : 3 : 2	3.09	-4.08	9.64	-0.01	—
1 : 3 : 3	3.22	-0.08	9.95	3.21	—
2 : 1 : 1	6.44	-0.26	3.37	4.90	—
2 : 1 : 2	6.25	-3.23	3.24	0.80	—
2 : 1 : 3	6.17	-4.34	3.21	3.88	—
2 : 2 : 1	6.21	-3.91	6.55	2.01	—
2 : 3 : 1	6.30	-2.41	9.44	-2.16	—
2 : 3 : 2	6.23	-3.45	9.45	-1.95	—
2 : 3 : 3	6.21	-3.77	9.72	0.85	—
3 : 1 : 2	9.65	-0.31	3.11	-3.09	—
3 : 2 : 1	9.47	-2.17	6.38	-0.66	—
1 : 1 : 0	3.28	1.82	3.05	-5.03	—
1 : 3 : 0	3.31	2.69	9.82	0.49	—
1 : 5 : 0	3.09	-4.20	16.07	0.90	—
2 : 1 : 0	6.44	-0.16	3.44	2.21	—
3 : 1 : 0	10.05	3.87	3.06	-4.68	—
1 : 0 : 1	3.25	0.75	—	—	3.30
1 : 0 : 3	3.32	2.99	—	—	9.91
1 : 0 : 5	3.31	2.66	—	—	16.52
2 : 0 : 1	6.45	0.10	—	—	3.30
3 : 0 : 1	9.68	-0.71	—	—	3.30

<sup>a</sup>  $c(\text{Zn})$  calculated for the other mixtures is not correlated; <sup>b</sup> ratio of the material amounts  
<sup>c</sup>  $c_c$  calculated material concentration; <sup>d</sup>  $\Delta c_r = 100(c_c - c_t)/c_t$ ,  $c_t$  theoretical material concentrations of Fe(III), Cu(III) and Zn(II).

The concentrations of the individual components in the various binary and ternary mixtures were calculated using the ORTHO program and the above values of the molar absorption coefficients and total absorbances  $A_{ij}$ . The Youden graphical method<sup>16</sup> confirms that a change in the wavelength combination in the range 530–590 nm has no effect on the accuracy of the results, provided that the number of wavelengths employed is at least twice the number of determined components. An increase in the number of wavelengths employed has no great effect on the accuracy of the results under the above assumptions for at least twice as many wavelengths compared to the number of components, when wavelengths are employed that correspond to the absorbance maxima of the Fe(III), Cu(II) and Zn(II). When the number of wavelengths employed is less than twice the number of determined components or when wavelengths are used outside the maxima for chelate absorbance, the accuracy of the calculated metal ion concentrations is decreased.

The maximal relative deviation,  $\Delta c_r = 100(c_c - c_t)/c_t$ , in the calculated concentrations of Fe(III) and Cu(II) in the range  $c(\text{Fe})$  2 to 9 and  $c(\text{Cu})$  3 to 16  $\mu\text{mol l}^{-1}$  from the declared concentration values for all the studied binary and ternary mixtures was not greater than 1–4% for Fe(III) and 5% for Cu(II) (see Table III). However, the accuracy of the calculated Zn(II) concentrations is always at least an order of

TABLE IV

Reproducibility of the determination Fe(III) and Cu(II) using BrPADAP by multicomponent analysis and the determination of Fe(III) with BrPADAP.  $c(\text{Cu}) = 3.22 \mu\text{mol l}^{-1}$ ,  $c(\text{Fe}) = 3.23 \mu\text{mol l}^{-1}$ ,  $c(\text{Zn}) = 3.30 \mu\text{mol l}^{-1}$ ,  $c_L = 86 \mu\text{mol l}^{-1}$ ,  $\varphi(\text{DMF}) = 20\%$ ,  $\varphi(\text{TX100}) = 0.1\%$ , 0.1M acetate buffer, pH 3.6,  $n = 10$ ,  $t_{0.95}(9) = 2.262$ ,  $\nu = 9$

Parameter	Fe(III)	Cu(II)	Fe(III) <sup>a</sup>	Fe(III) <sup>b</sup>
$\langle c_c \rangle$ , $\mu\text{mol l}^{-1}$ <sup>c</sup>	3.275	3.266	—	—
$s_c$ , $\text{nmol l}^{-1}$ <sup>d</sup>	33.1	32.1	11 <sup>j</sup>	5 <sup>j</sup>
$s_c$ , $\text{ng .l}^{-1}$ <sup>d</sup>	1.8	2.0	—	—
$s_r$ , % <sup>e</sup>	1.0	0.98	0.4	0.2
$s_c^*$ , $\text{nmol l}^{-1}$ <sup>f</sup>	74.8	72.7	2.5 <sup>k</sup>	1.1 <sup>k</sup>
$s_r^*$ , % <sup>g</sup>	2.3	2.2	0.9	0.4
$A \cdot 10^3$ <sup>h</sup>	—	—	324.1	391.0
$s_A \cdot 10^3$ <sup>i</sup>	—	—	1.3	0.7

<sup>a</sup> Determination of Fe(III), 545 nm,  $c(\text{Fe}) = 4.84 \mu\text{mol l}^{-1}$ ; <sup>b</sup> determination of Fe(III), 590 nm,  $c(\text{Fe}) = 4.84 \mu\text{mol l}^{-1}$ ; <sup>c</sup> average concentration value calculated by the ORTHO program; <sup>d</sup> standard deviation of the average values  $s_c = ((\sum c^2 - n \langle c \rangle^2)/n(n-1))^{1/2}$ ; <sup>e</sup> relative standard deviation of the average value,  $s_r = 100 s_c / \langle c_t \rangle$  %; <sup>f</sup> interval of reliability  $s_r^* = s_c t_\alpha(\nu)$ ; <sup>g</sup>  $s_r^* = 100 s_c^* / \langle c_c \rangle$  %; <sup>h</sup> mean absorbance value for  $c(\text{Fe}) = 4.84 \mu\text{mol l}^{-1}$ ; <sup>i</sup>  $s_A = ((\sum A^2 - n \langle A \rangle^2)/n(n-1))^{1/2}$ ; <sup>j</sup>  $s_c = s_A / \epsilon_\lambda$ ; <sup>k</sup>  $s_c^* = s_A t_\alpha(\nu) / \epsilon_\lambda$ .



magnitude worse, as the values of the molar absorption coefficients of the Zn(II) chelate with BrPADAP at pH 4.6 is at least 1 to 2 orders of magnitude smaller than the values for the Fe(III) and Cu(II) chelates. Nonetheless, neglecting of the presence of the Zn(II) chelate as an additional component in the calculations greatly decreases the accuracy of the calculated concentrations of the two remaining metal ions (often by as much as 5%).

The simultaneous determination of Fe(III) and Cu(II) is fast, precise and accurate for both ions even in relatively high concentration excesses of one component (Table IV). The method can be used in the presence of up to 5-fold concentration excess of Zn(II) and large concentration excesses of other ions. As the solution components interfere negligibly (see Table II), including TCA and H<sub>2</sub>O<sub>2</sub>, the method can be recommended for the simultaneous determination of both ions in multicomponent analysis in biological samples. The sensitivity of the method is higher than that of commonly used methods for the simultaneous determination of Fe(II) and Cu(I) ions using the mixed reagent disodium bathocuproin disulfonate with disodium bathophenanthroline disulfonate or ferrozine<sup>23</sup>.

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