SIMULTANEOUS DETERMINATION OF IRON AND COPPER IN WATERS BY MULTILIGAND FLOW INJECTION ANALYSIS

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Optical characteristics were measured and optimum conditions established for the simultaneous spectrophotometric determination of iron and copper with reagent mixtures of disodium bathocuproinedisulfonate (I) with disodium bathofenanthrolinedisulfonate (II) or ferrospectral (III) in static or dynamic (FIA) conditions. In the FIA method suggested, 30 μl water samples containing Fe and Cu in concentration ratios 1 : 10 to 10 : 1 are injected into a stream of I–II mixed reagent at $c(I) = 0.6$ mmol l^{-1} and $c(II) = 0.2$ mmol l^{-1} in a medium of 0.1M formate buffer pH 3·5 and 0·01M ascorbic acid; for the two metals, present in concentrations of 4 to 40 μmol l^{-1}, the relative standard deviations of determination do not exceed 2 and 5%, respectively. Alternatively, in the reverse FIA method using a multichannel diode array detector, 25 μl of the mixed reagent under the above conditions is injected into the acid sample stream; for samples containing Fe and Cu in concentrations of 1—43 μmol l^{-1} in ratios of 10 : 1 to 1 : 15, the relative standard deviation of determination is about 2%. The use of mixed reagents makes for a broader variability of the optical characteristics and conditional equilibrium constants of the reaction products as compared to methods employing a single nonselective reagent; owing to this, improved selectivity, accuracy and precision of determination are achieved with multi-component flow injection analysis (MC — FIA).

Flow injection analysis (FIA) with photometric detection is being used with success for the simultaneous determination of several components in a single injected volume of several microlitres[1–7].

Direct multicomponent FIA methods based on the measurement of absorption of UV or visible radiation by all the components pursued, without addition of any reagent, give good results both for majority and minority components (relative standard deviation $s_r < 1$ and 10%, respectively) provided that their absorption bands are sufficiently well separated[6]. The accuracy and precision of results of indirect multicomponent FIA methods using a single, nonselective organic analytical reagent which reacts with all of the components to be determined are frequently somewhat poorer due to the too small differences between the spectral parameters of the reaction products, mutually as well as with respect to the reagent[7]. It is rea-

sonable to expect the accuracy and precision to improve on the use of two or several highly selective organic analytical reagents, each reacting with a single component of the solution with a comparatively high value of the selective coefficient.

Among such reagents are disodium bathocuproinedisulfonate (*I*), disodium bathophenanthrolinedisulfonate (*II*) and ferrospectral (*III*), all of which contain the 1,10-phenanthroline functional analytical group forming stable coordination compounds with ions of *d*-elements in their lower oxidation states[3,8−10]. Particularly 2,9-dimethyl-1,10-phenanthroline and its derivatives (including *I*) do not form stable coordination compounds with Fe(II) ions whereas in the presence of reductants they easily form stable coloured complexes with Cu(I) ions. This difference in the selectivity of reactions with Fe and Cu ions can be used with advantage for the simultaneous determination of the two ions with a so-called mixed multiligand reagent.

In this work, *I–II* and *I–III* mixtures are used for the simultaneous determination of Fe and Cu in model mixtures containing the metals in various concentration ratios both in static and dynamic (FIA) conditions. The method is applied to the simultaneous determination of copper and iron in drinking and surface waters by multicomponent flow injection analysis.

EXPERIMENTAL

Chemicals and Apparatus

Standard iron and copper solutions with $c(\text{Fe(III)}) = 100\cdot8$ mmol l^{-1} and $c(\text{Cu(II)}) = 100\cdot5$ mmol l^{-1} were prepared by dissolving their chlorides of reagent grade purity (Lachema, Brno, Czechoslovakia) in $0\cdot2$M and $0\cdot1$M-HCl, respectively. Standard iron solution with $c(\text{Fe(II)}) = 86\cdot6$ mmol l^{-1} was prepared by dissolving iron metal of spectral purity (Specpure, Johnson Matthey, London, U.K.) in calculated volume of concentrated HCl and diluting with redistilled water so that the final concentration of HCl was $0\cdot2$ mol l^{-1}.

Stock solutions of *I* (disodium 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonate), *II* (disodium 4,7-diphenyl-1,10-phenanthrolinedisulfonate) and *III* (disodium 3-[2-pyridyl]--5,6-bis(4-sulfophenyl)-1,2,4-triazine) in concentrations of $1-5$, $1-2$ and $1-2$ mmol l^{-1}, respectively, were prepared by dissolving the chemicals of reagent grade purity (E. Merck, Darmstadt, F.R.G., and Serva, Heidelberg, F.R.G.) in water. Fresh stock solutions of $0\cdot1$M ascorbic acid in water (always before measurement) were obtained from the commercial preparation supplied by Farmakon, Olomouc, Czechoslovakia, whose purity conformed to Czechoslovak Pharmacopoeia 3. For the study of interactions of copper and iron ions with the reagents and their mixtures, the ionic strength of the solutions was adjusted to $I = 0\cdot1$mol l^{-1} with mixtures of HNO_3 and NH_3. All measurements were carried out in the presence of ascorbic acid in a concentration of 10 mmol l^{-1} unless stated otherwise. All chemicals used were of reagent grade or better purity (Lachema, Brno, Czechoslovakia), bidistilled water was prepared on a Bi-18 Destamat quartz still (Heraeus Quarzschmelze, Hanau, F.R.G.).

Acidity of solutions was measured with an OP 208/1 pH-meter fitted with an OP 0718P glass electrode and an OP 0830P calomel electrode and calibrated periodically with glycinate (K21) and phosphate (K71) buffers, pH $2\cdot18$ and $7\cdot04$, respectively, at 25°C (all Radelkis, Budapest, Hungary). Spectrophotometric measurements in the static mode were performed with a Specord

M40 spectrophotometer with a built-in printer (Carl Zeiss, Jena, G.D.R.) using quartz cells 10 mm optical pathlength at $24 \pm 1°C$.

Measurements in the dynamic FIA mode were partly conducted on a device consisting of a chromatographic six-way valve with a sample loop $V_1 = 30 \mu l$ volume and a Spekol 21 spectrophotometric detector with a flow cell 18 μl volume interfaced to a K 201 recorder (both Carl Zeiss, Jena, G.D.R.). The absorbance values were obtained from several repetitive measurements ($n \gtrsim 5$) at all individual wavelengths. Acid solution of sample containing Fe and Cu ions was injected into a continuous stream of the reagent mixture containing II or III in a concentration of $0.2 \text{ mmol } l^{-1}$ and I in a concentration of $0.6 \text{ mmol } l^{-1}$ or $2.0 \text{ mmol } l^{-1}$, respectively, in $0.1M$ acetate buffer at pH 3.5; flow rate in capillary system (i.d. $= 0.6$ mm, PTFE) was held constant at $F_m = 1.0 \text{ ml min}^{-1}$ by the hydrostatic pressure of reagent in the reservoir.

Reverse FIA measurements were performed with an apparatus[7] comprising a HPP 4 001 high pressure pump (Laboratorní přístroje, Prague, Czechoslovakia), a loop injector with the loop volume variable within the limits of $10-100 \mu l$, a PU 4 021 multichannel UV-VIS spectrophotometric diode array detector (Pye Unicam, U.K.), a TZ 4 200 two-line recorder (Laboratorní přístroje, Prague, Czechoslovakia) and a SAPI 1 process control computer (Tesla Eltos, Prague, Czechoslovakia).

To a pulseless continuous stream of acid solutions of both metals in water at a flow rate of $F_m = 1.0 \text{ ml min}^{-1}$ were added 25 μl of $I-II$ or $I-III$ reagent mixture. The absorbance values were recorded automatically in the peak maximum (autostore mode) within the $390-590$ nm range. Parameters of the two FIA devices (length of reaction coil L_r, flow rate F_m, sample volume V_i and inner diameter of PTFE capillary) were optimized following the methodology[7].

Parameters of the calibration plots and data sets were subjected to statistical processing by the LLSQM and STAT programs using standard procedures[11]. Concentrations of the components during the simultaneous determination of Fe and Cu ions with the multiligand mixed reagent were calculated by the ORTHO program[12] for overdetermined systems (with the number of linear equations exceeding the number of components to be determined). The program procedure is based on the use of the molar absorptivities of all components of the reaction mixture at the wavelengths chosen which are known from independent measurements.

RESULTS AND DISCUSSION

Interaction of Copper and Iron Ions with I

The reagent $\left(c = 200 \, \mu\text{mol } l^{-1}\right)$ reacts with Cu(II) ions $\left(c = 50 \, \mu\text{mol } l^{-1}\right)$ in the presence of ascorbic acid over a wide region of pH $2-10$ giving rise to an intensely yellow coloured chelate $\left(\varepsilon = 1.3 \text{ m}^2 \text{ mmol}^{-1}\right)$ with an absorption maximum at 482 nm (Fig. 1); the reagent itself does not absorb in the visible region. The reaction proceeds rapidly, absorbance is constant 1 min after the addition of I and ascorbic acid to the solution of Cu(I).

Absorbance–pH curves $\Delta A = f(\text{pH})$ for $c(\text{Cu}) = 50 \, \mu\text{mol } l^{-1}$ and $c(I) = 50$ to $400 \, \mu\text{mol } l^{-1}$ exhibt a broad plateau over the pH $2.5-8.0$ range, and the formation parts of the curve shift to the acidic region with increasing concentration of I. At lower concentrations of I $\left(50-120 \, \mu\text{mol } l^{-1}\right)$ the chelate does not form quantitatively, so that the absorbance in the region of the plateau lowers (Fig. 2). The con-

centration dependence $\Delta A = f(c(I))$ at $c(Cu) = 50\ \mu mol\ l^{-1}$ and pH 3·5 attains a constant absorbance at a 3·5-fold excess of I.

Iron ions in conditions of reaction of Cu(II) do not form coloured chelates with I and at pH < 4 do not interfere with the determination of copper $(50\ \mu mol\ l^{-1})$ even in a 50-fold excess. Ni(II) and Co(II) ions in a 5-fold and 20-fold excess, respectively, interfere. Calibration curves $\Delta A = f(c(Cu))$ at $c(I) = 200\ \mu mol\ l^{-1}$ in 0·1M formate buffer at pH 3·5 are linear over the region of $c(Cu) = 4-50\ \mu mol\ l^{-1}$. The reproducibility of measurement at $c(Cu) = 7·8\ \mu mol\ l^{-1}$ is very good, $s_r = 0·74\%$ for $n = 10$.

Interaction of Copper and Iron Ions with II *and* III

The two reagents react with Fe(III) or Fe(II) ions $(c(Fe) = 25\ \mu mol\ l^{-1}, c(II\ or\ III) = 200\ \mu mol\ l^{-1})$ in the presence of ascorbic acid over the wide region of pH 1·5 — 10 giving rise to red-violet chelates with absorption maxima at 535 nm $(\varepsilon = 2·2\ m^2 \cdot mmol^{-1})$ or 562 nm $(\varepsilon = 2·6\ m^2\ mmol^{-1})$, respectively, while the reagents themselves do not absorb in the visible region (Fig. 1).

FIG. 1

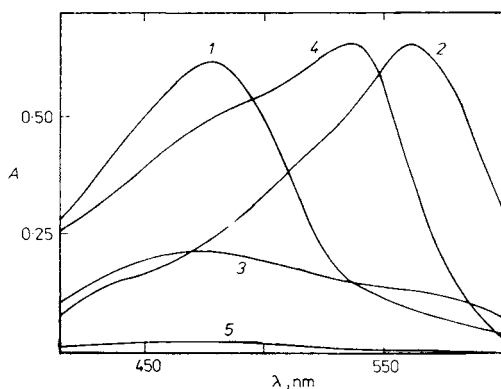Absorption spectra of *1* Cu–*I*, *2* Fe–*III*, *3* Cu–*III*, *4* Fe–*II*, and *5* Cu–*II* chelates; c(metal) $(\mu mol\ l^{-1})$: *1* 48, *2* 25, *3* 48, *4* 30, *5* 50; $c(I, II, III) = 200\ \mu mol\ l^{-1}$; c(ascorbic acid) $= 10\ mmol\ l^{-1}$; 0·1M formate buffer, pH 3·5
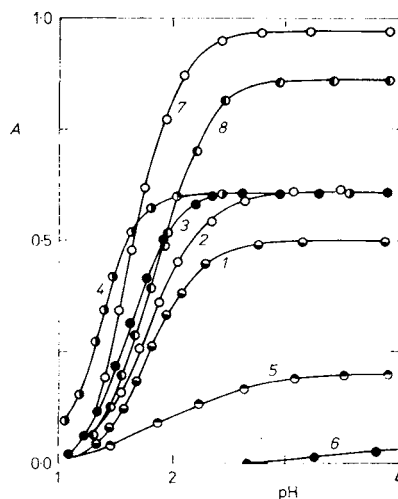
FIG. 2

Absorbance–pH curves of Cu–*I* (*1*—*4*), Cu–*III* (*5*), Cu–*II* (*6*), Fe–*III* (*7*), and Fe–*II* (*8*) systems; $c(Cu) = 50\ \mu mol\ l^{-1}$, $c(Fe) = 40\ \mu mol\ l^{-1}$, $I = 0·10$ (HNO₃ + NH₃), c(ascorbic acid) $= 10\ mmol\ l^{-1}$. $c_L\ (\mu mol\cdot l^{-1})$, λ(nm): *1* 120, 480; *2* 150, 480; *3* 200, 480; *4* 400, 480; *5* 200, 470; *6* 200, 430; *7* 200, 560; *8* 200, 535

The reaction rate for *III* depends on acidity the; chelation is complete in $3-4$ h at pH 2, in 45 min at pH 2·5, in $5-7$ min at pH 3 and in less than a minute at pH $\geq$ 3·5. Reagent *II* reacts virtually instantly on the mixing of the reaction components. The colour of the two chelates is steady for a minimum of 24 h.

The absorbance–pH curves for $c(Fe) = 40 \, \mu mol \, l^{-1}$ measured 12 h after the solution preparation exhibit a broad plateau at pH 2·5−7·0 for $c(III) = 150$ to $320 \, \mu mol \, l^{-1}$ and pH 2·0−8·0 for $c(II) = 150-500 \, \mu mol \, l^{-1}$. The chelation at pH < 3 is quantitative with a 3·5 − 4-fold concentration excess of reagent, as the pH curves (Fig. 2) and the concentration dependences $\Delta A = f(c(II \text{ or } III))$ at pH 3·5 demonstrate.

Calibration curves for $c(II \text{ or } III) = 200 \, \mu mol \, l^{-1}$ in 0·1M formate buffer at pH 3·5 are linear over the region of $c(Fe) = 4-40 \, \mu mol \, l^{-1}$. The reproducibility of measurement at $c(Fe) = 32 \, \mu mol \, l^{-1}$ for *III* or $c(Fe) = 40 \, \mu mol \, l^{-1}$ for *II* is characterized by $s_r = 1·02$ and 1·55%, respectively, at $n = 10$.

Copper ions $(c(Cu) = 48 \, \mu mol \, l^{-1})$ react with $c(III) = 200 \, \mu mol \, l^{-1}$ in the presence of ascorbic acid at pH 2−8 giving rise to a not very intensely absorbing chelate $(\varepsilon = 0·02 \, m^2 \, mmol^{-1})$ with an absorption maximum at 470 nm (Fig. 1, Table I). Interaction of copper ions $(c(Cu) = 50 \, \mu mol \, l^{-1})$ with *II* under these experimental conditions at pH < 3 leads to the formation of a coloured chelate $(\lambda_{max} \, 427 \, nm, \, \varepsilon = 0·4 \, m^2 \, mmol^{-1}$ at pH 7, $c(II) = 400 \, \mu mol \, l^{-1})$. At pH 3·5, which is the optimum for the simultaneous determination of Cu and Fe, the molar absorptivity of the copper chelate in the absorption maximum range does not exceed $0·1 \, m^2 \, mmol^{-1}$.

Thus, the reaction of the two reagents with iron ions is considerably more sensitive than the reaction with Cu(I), which makes their use in combination with *I* convenient for the simultaneous multiligand determination of the two ions.

Simultaneous Determination of Copper and Iron Ions
with I–II *or* I–III *Mixed Reagents*

Absorption spectra of products of interaction of Cu(II) ions $(c(Cu) = 40 \, \mu mol \, l^{-1})$ with the *I–II* or *I–III* mixed reagents at various concentration ratios of the two components $(c(II \text{ or } III) = 0-20 \, \mu mol \, l^{-1}, \, c(I) = 0-200$ and $0-60 \, \mu mol \, l^{-1}$, respectively) demonstrate (Figs 3 and 4) that in the absence of *I*, copper ions form weakly coloured chelates with *II* or *III* (curves 6 and 5 in Figs 3 and 4, respectively); at nonzero concentrations of *I*, Cu(II) ions react with *I* and *II* or *III* in dependence on the values of the conditional equilibrium constants, resulting in an absorbance increase in the range of maximum absorption of Cu(I) chelates with *I*; at a 3−4-fold excess of *I* with respect to *II* or a 10-fold excess with respect to *III*, the shape of the absorption spectrum approaches that for Cu(II) with *I* only (curves 1).

The simultaneous use of *I* with *II* or *III* in the presence of comparable concentra-

tions of Fe and Cu ions under otherwise identical experimental conditions leads to a lowering in the yield of the reaction of Fe(II) ions with *II* or *III*, whereupon the absorbance in the region of maximum absorption of the chelates of Fe(II) with *II* or *III* decreases slightly while that in the region of maximum absorption of Cu(I) chelates with the reagents increases slightly; at a 3–4-fold excess of *I* with respect to *II*
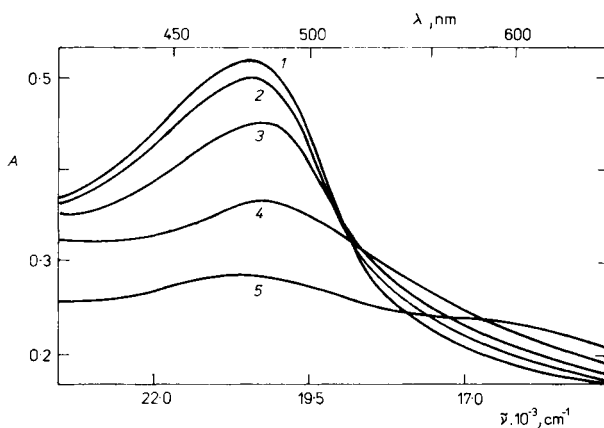
FIG. 3

Absorption spectra of Cu–*I*–*III* mixtures. $c(Cu) = 40 \,\mu mol\, l^{-1}$; c(ascorbic acid) $= 10$ mmol . $. l^{-3}$; 0·1M formate buffer, pH 3·5. $c(I)$, $c(III)$ ($\mu mol\, l^{-1}$): *1* 20, 0; *2* 200, 20; *3* 80, 20; *4* 20, 20; *5* 0, 20
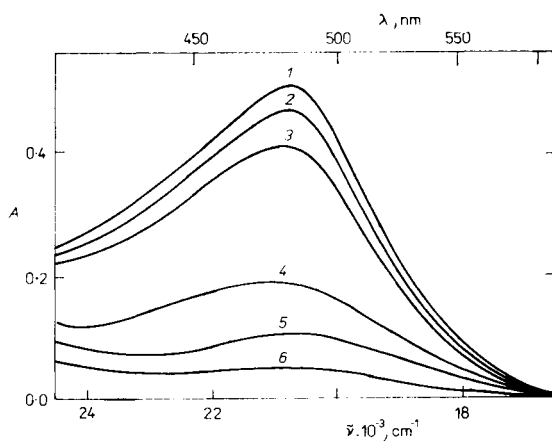
FIG. 4

Absorption spectra of Cu–*I*–*II* mixtures. $c(Cu) = 40 \,\mu mol\, l^{-1}$; c(ascorbic acid) $= 10$ mmol l^{-1}; 0·1M formate buffer pH, 3·5. $c(I)$, $c(II)$ ($\mu mol\, l^{-1}$): *1* 20, 0; *2* 60, 20; *3* 40, 20; *4* 16, 20; *5* 8, 20; *6* 0, 20

or a 10-fold excess of I with respect to III, however, this effect is negligible (<3 rel. %). Thus, a concentration excess of I is required for the simultaneous determination of Fe and Cu with the I–II or I–III mixed reagents. Concentrations $c(I) = 600$ μmol . . l^{-1} in the I–II system and 2 000 μmol l^{-1} in the I–III system can be regarded as optimum at $c(II$ or $III) = 200$ μmol l^{-1}.

Statistical parameters of the $\Delta A = f(c_M)$ dependences for Fe(II) and Cu(I) and the I–II or I–III mixed reagents are given in Table I. The molar absorptivities differ from those found for the metal–ligand binary mixtures about 8% for Cu with I, about 5% for Fe with II and $1-2\%$ for Fe with III.

The resulting concentrations of Cu and Fe calculated by the ORTHO program[12] for model two-component mixtures with concentration ratios of the two ions ranging from 1 : 10 to 10 : 1 and the two mixed reagents exhibit errors up to 7% (Table II). When using the molar absorptivities for Cu and Fe mixtures using the I–III reagent, the least accurate results for Cu (error of $1-5\%$) were obtained at Fe: Cu ratios greater than 2, whereas at a concentration excess of Cu the error did not exceed $1\cdot5\%$. Simultaneous determination of Fe is associated with an error which is largely lower than 3%. The number of wavelengths used plays no major role, the accuracy of

TABLE I

Molar absorptivities of Fe(II) and Cu(I) chelates in I–II and I–III mixed reagent systems $c(II, III) = 0\cdot2$ mmol l^{-1}, $c(I) = 0\cdot6$ and $2\cdot0$ mmol l^{-1}, respectively, $c(Cu) = 4-50$ μmol . l^{-3}, $c(Fe) = 4-40$ μmol l^{-1}, c(ascorbic acid) = 10 mmol l^{-1}, $0\cdot1$M formate buffer, pH 3·5

| λ nm | $\varepsilon \pm d(\varepsilon)^a$, cm^2 mmol^{-1} | | | |
| | I–III | | I–II | |
	Cu	Fe	Cu	Fe
440	8 216 ± 105	6 136 ± 245	9 276 ± 160	11 865 ± 215
460	10 366 ± 130	8 068 ± 160	11 525 ± 130	14 685 ± 255
480	11 738 ± 115	9 944 ± 185	12 556 ± 142	17 026 ± 304
500	10 500 ± 145	12 998 ± 235	10 603 ± 153	19 133 ± 327
510	—	—	7 929 ± 125	19 737 ± 373
520	5 822 ± 95	17 026 ± 350	5 635 ± 101	20 781 ± 400
530	—	—	4 224 ± 97	22 153 ± 408
535	—	—	3 716 ± 72	22 524 ± 377
540	3 306 ± 65	21 860 ± 375	—	—
550	2 216 ± 74	26 180 ± 412	2 205 ± 70	14 566 ± 162
580	1 446 ± 68	22 320 ± 328	1 261 ± 64	5 305 ± 72
600	826 ± 45	12 708 ± 227	—	—

^{a} Determined from calibration dependences $A = A_0 + \varepsilon c_M$, $n = 6$, $r_{xy} \geqq 0\cdot999$; $d(\varepsilon) = 3s_{xy}$.

TABLE II

Comparison of actual and calculated concentrations of Fe and Cu in their mixtures using I–III mixed reagent in static conditions; $c(I) =$ = 2·0 mmol l⁻¹, $c(III)$ = 0·2 mmol l⁻¹, c(ascorbic acid) = 10 mmol l⁻¹, 0·1M formate buffer, pH 3·5

No.	c(Cu), µmol l⁻¹					c(Fe), µmol l⁻¹				
	added	found[a]	Δ[a], %	found[b]	Δ[b], %	added	found[a]	Δ[a], %	found[b]	Δ[b], %
1	5·00	5·28	5·60	5·39	7·80	50·00	50·42	0·80	50·38	0·76
2	10·00	10·70	7·00	10·76	7·60	50·00	50·36	0·72	50·37	0·74
3	25·00	26·30	5·20	26·31	5·24	50·00	50·40	0·80	50·47	0·94
4	50·00	51·25	2·50	51·24	2·48	50·00	49·49	−1·02	49·59	−0·82
5	50·00	52·12	4·24	52·14	4·28	50·00	49·86	−0·28	49·98	−0·04
6	50·00	52·80	5·60	52·81	5·62	50·00	50·18	0·36	50·28	0·56
7	50·00	50·63	1·26	50·70	1·40	25·00	26·28	5·12	26·30	5·20
8	50·00	49·75	−0·75	49·82	−0·36	5·00	5·08	1·60	5·05	1·00
9	5·00	5·01	0·20	5·01	0·20	5·00	5·08	1·60	5·09	1·80
10	10·00	10·59	5·90	10·60	6·00	10·00	10·27	2·70	9·63	−3·70
11	25·00	25·61	2·44	25·63	2·52	25·00	25·44	1·76	25·44	2·44

[a] λ 440, 460, 480, 500, 520, 540, 560, 580, and 600 nm; [b] λ 480, 500, 520, 560, 580, and 600 nm.

TABLE III

Comparison of actual and calculated concentrations of Fe and Cu in their mixtures using I–II mixed reagent in static conditions; $c(I) = 2.0$ mmol l^{-1}, $c(II) = 0.6$ mmol l^{-1}, c(ascorbic acid) $= 10$ mmol l^{-1}, 0.1M formate buffer, pH 3.5

No.	c(Fe), μmol l^{-1}					c(Cu), μmol l^{-1}				
	added	found[a]	Δ^a, %	found[b]	Δ^b, %	added	found[a]	Δ^a, %	found[b]	Δ^b, %
1	25.00	23.05	−7.80	23.90	−4.40	25.00	26.10	4.40	25.33	1.32
2	25.00	24.61	−1.56	25.79	3.16	10.00	10.33	3.30	10.30	3.00
3	25.00	24.28	−2.88	24.16	−3.36	25.00	25.46	1.84	24.50	−2.00
4	10.00	8.99	−10.1	9.56	−4.40	25.00	27.02	8.08	24.91	−0.36
5	5.00	4.65	−7.00	4.95	−1.00	25.00	26.46	5.84	25.36	1.44
6	50.00	47.96	−4.08	49.23	−1.54	50.00	52.95	5.90	50.91	1.82
7	50.00	48.59	−2.82	49.39	−1.82	25.00	26.08	4.32	24.75	−1.00
8	30.00	28.93	−3.57	29.82	−0.60	50.00	51.35	2.70	49.87	−0.20
9	10.00	9.14	−8.60	9.77	−2.30	50.00	50.24	0.48	49.18	−1.64
10	10.00	9.52	−4.80	9.74	−2.60	10.00	9.86	−1.40	9.48	−5.20

[a] λ 440, 460, 490, 500, 510, 520, 530, 535, 550, 560 and 580 nm; [b] λ 480, 500 and 560 nm.

results obtained with the use of 9 wavelengths being virtually identical with that obtained using 4 wavelengths of the absorption maxima of the chelates (Table II).

The use of molar absorptivities obtained from metal ion-single reagent systems results in higher relative deviations and poorer accuracy of determination. The least accurate results were obtained for solutions with low concentrations of Fe (error as high as 30%). In all cases, the use of a lower number of wavelengths (λ 480, 500, 560 and 580 nm) is associated with lower relative deviations of the calculated concentrations of Fe. The determination of low concentrations of Cu in the presence of a high excess of Fe, with relative deviations up to $2-4\%$, gives largely more accurate results than calculation using the ε value for Fe–Cu mixed solutions.

With the *I–II* mixed reagent, more accurate Fe and Cu concentrations (errors up to 5 and 9%, respectively) were obtained (see Table III) using a lower number of wavelengths lying near the absorption maxima of the corresponding chelates (480, 500 and 535 nm, and 480, 500 and 560 nm, respectively). The accuracy of the calculated concentrations was unaffected by the kind of the molar absorptivities set used.

If the wavelengths are suitably chosen, the relative deviation of concentration data for the analytes with the two-component mixed reagents does not exceed 5% except for the least favourable mixtures with a high concentration excess of one metal ion where the error can be $10-15\%$.

Simultaneous Determination of Cu and Fe in Waters
by Multicomponent Flow Injection Analysis Using I–II *or* I–III *Mixed Reagent*

Molar absorptivities of Fe and Cu chelates with the individual reagents *I, II, III* or the *I–II* mixed reagent in the dynamic FIA mode (at $F_m = 1\cdot0$ ml min^{-1}, $L_r = 40$ cm, i.d. $= 0\cdot6$ mm (PTFE), $V_1 = 30$ µl) are $8-10$ times lower than those obtained in the static mode, which corresponds to a dispersion coefficient of $D \approx 8-10$. Owing to the parallel lowering in the standard deviation of dispersion of points about the regression straight line s_{xy} or in the standard deviation of determination of the blank s_0, the determination limits[13] remain unaltered (Table IV).

In the reverse FIA method, where 25 µl of a solution of the *I–II* or *I–III* mixed reagent with $c(I) = 0\cdot6$ or $2\cdot0$ mmol l^{-1}, respectively, and $c(II$ or $III) = 0\cdot2$ mmol . . l^{-1} in $0\cdot1$M formate buffer, pH $3\cdot5$, is injected into a pulseless continous stream of an acid solution containing a model mixture of Fe and Cu ions and ascorbic acid $(F_m = 1\cdot0$ ml min^{-1}, $L_r = 55$ cm, i.d. $= 0\cdot6$ mm (PTFE), $V_1 = 25$ µl), the molar absorptivities of the chelates are $3-5$ times lower than in the static mode but about 2 times higher than in the conventional FIA mode. Owing to this and the slight lowering in the s_{xy} or s_0 values, the determination limit decreases.

Calibration curves $\Delta A = f(c_M)$ for reverse FIA using the *I–II* mixed reagent are linear over the concentration regions $c(\text{Cu}) = 4-40$ µmol l^{-1} or $c(\text{Fe}) = 1$ to 43 µmol l^{-1}; at $c(\text{Cu}) = 5$ µmol l^{-1} or $c(\text{Fe}) = 30$ µmol l^{-1}, the reproducibility

TABLE IV

Molar absorptivities and determination limits of Fe and Cu chelates with I-III and I-II mixed reagents in the conventional and reverse FIA modes; $c(II, III) = 0.2$ mmol l^{-1}, $c(I) = 0.6$ and 2.0 mmol l^{-1}, respectively, $c(Fe) = 4-40$ μmol l^{-1}, $c(Cu) = 1-45$ μmol l^{-1}, $F_m = 1$ ml min^{-1}, $L_r = 40$ and 55 cm, respectively, $V_l = 30$ and 25 μl, respectively, i. d. $= 0.6$ mm (PTFE)

| λ nm | FIA with I-II system | | | | Reverse FIA with I-II system | | | | Reverse FIA with I-III system | | | |
| | Fe | | Cu | | Fe | | Cu | | Fe | | Cu | |
	ε^a	DL^b	ε^a	DL^b	ε^a	DL^b	ε^a	DL^b	ε^a	DL^b	ε^a	DL^b
475	1 575 ± 39	2·6	896 ± 36	4·3	3 351 ± 27	1·0	2 688 ± 42	1·5	1 806 ± 42	4·7	2 293 ± 26	3·2
490	1 725 ± 34	2·1	904 ± 40	4·8	3 920 ± 29	2·1	2 802 ± 32	1·7	2 151 ± 37	3·2	2 152 ± 29	3·2
505	1 801 ± 80	4·7	658 ± 44	7·1	4 086 ± 37	2·2	2 571 ± 22	2·6	2 593 ± 56	3·6	1 569 ± 62	2·7
520	1 951 ± 71	3·9	424 ± 25	6·3	4 468 ± 51	1·6	1 814 ± 18	3·0	3 295 ± 28	3·0	1 145 ± 39	3·5
535	2 074 ± 6	3·9	291 ± 19	7·0	4 853 ± 50	1·8	1 199 ± 26	3·3	4 099 ± 39	2·9	815 ± 41	2·9
550	1 805 ± 41	5·4	228 ± 19	9·0	4 169 ± 41	2·3	735 ± 19	2·9	4 833 ± 44	2·3	444 ± 23	1·9
565	1 025 ± 75	8·0	182 ± 25	14·7	2 358 ± 27	3·0	436 ± 30	3·5	4 546 ± 51	1·9	312 ± 17	3·1

^{a} Molar absorptivity $\varepsilon \pm d(\varepsilon)$ in cm^2 mmol^{-1}, $d(\varepsilon) = 3s_{xy}$ ($n = 6$, $r_{xy} \geqq 0.9992$); ^{b} determination limit expressed in terms $DL = A_{lim} = 10s_0$, where s_0 is the standard deviation of the blank ($n = 10$).

of measurement $(n = 10)$ is characterized by $s_r = 0\cdot92$ and $0\cdot87\%$, respectively.

The FIA method of simultaneous determination of Fe and Cu in model mixtures at various Fe : Cu ratios (Table V) enables the analytes to be determined with relative deviations of 5% (conventional FIA) or 2% (reverse FIA). This is in a good agreement with the results of simultaneous determination of the two metal ions in the static mode. The most accurate data were again obtained using molar absorptivities for Cu–Fe–*I–II* mixtures, both for the higher and lower numbers of wavelengths used (Table V). Reverse FIA using the *I–III* mixed reagent gave somewhat poorer results.

Based on the above results, methods of simultaneous determination of Fe and Cu in drinking and surface waters by conventional and reverse FIA were worked out. The procedure is as follows. Water sample 25 ml volume with 5 drops of HNO_3 $(1 : 1)$ is evaporated to 5 ml and transferred to a 25 ml volumetric flask, and (for the

TABLE V

Results of simultaneous FIA determination of Fe and Cu in model mixtures using *I–II* mixed reagent; $c(I) = 0\cdot6$ mmol l^{-1}, $c(II) = 0\cdot2$ mmol l^{-1}, c(ascorbic acid) $= 10$ mmol l^{-1}, $0\cdot1$M formate buffer pH 3·5, $F_m = 1$ ml min^{-1}, $L_r = 40$ and 55 cm, respectively, i.d. $= 0\cdot6$ mm (PTFE), $V_1 = 25$ or 30 µl

No.	$c(Fe)$, µmol l^{-1}			$c(Cu)$, µmol l^{-1}		
	added	found	$\Delta, \%$	added	found	$\Delta, \%$
			FIA			
1	4·00	4·19	4·7	20·00	19·09	−4·5
2	8·00	8·32	4·0	8·00	8·33	4·1
3	16·00	16·55	3·4	16·00	15·88	−0·8
4	14·00	15·08	4·7	4·fl0	4·61	−4·0
5	24·00	23·17	−3·5	24·00	24·45	1·9
6	12·00	11·66	−2·8	22·00	22·28	1·3
			Reverse FIA			
1	2·00	1·92	−4·0	20·00	20·17	0·8
2	10·00	10·07	0·7	40·00	40·86	2·1
3	40·00	39·24	−1·9	10·00	9·86	−1·4
4	40·00	41·02	2·5	4·00	4·09	2·2
5	30·00	30·31	1·0	30·00	30·07	0·2
6	2·00	1·86	−7·0	40·00	40·22	0·6
7	40·00	39·98	−0·1	2·00	1·76	−12·0
8	30·00	30·72	2·4	2·00	1·85	−7·5
9	2·00	1·92	−4·0	30·00	30·35	1·2

standard addition method) a standard amount of one or the other ion is added. After addition of 2·5 ml of 0·1M ascorbic acid and 2·5 ml of 1·0M formate buffer at pH 3·5, the solution is brought to volume and allowed to stand for 5 min; then, 30 μl is injected into a continuous stream of the *I–II* mixed reagent $(c(I) = 0·6$ mmol . . l^{-1}, $c(II) = 0·2$ mmol l^{-1}, 0·1M formate buffer pH 3·5, $F_m = 1·0$ ml min^{-1}). The peak height is measured at 475, 490, 505, 520, 535, 550, and 565 nm.

The concentrations of Cu and Fe in sample were determined either using ε values in Table IV, or by the standard addition method omitting data for $\lambda = 565$ nm. The obtained concentrations of copper in drinking water (Table VI) should be regarded as crude data, the corresponding s_r value approaching 40%. In the standard addition method the data were processed by the LLSQM program from the $\Delta A =$ $= f(c^*(Cu))$ and $\Delta A = f(c^*(Fe))$ dependences where the asterisk labels concentrations from the standard additions, and the slopes of the calibration curves served as the apparent molar absorptivities of the two chelates in conditions approaching closely those in real water samples. The concentrations of the two ions obtained by the standard addition method are very close to those found using the molar absorptivities set for pure and mixed solutions of reagents and metal ions. The dispersion of the results, however, is lower in the standard addition method.

In the reverse FIA mode, 25 μl of solution of mixed reagent is injected into a continuous stream of the analyzed water, acidified with several drops of concentrated HNO_3. The method affords more accurate and precise results (Table VI) owing to

TABLE VI

Results of determination of Fe and Cu in drinking (A) and surface waters (B—E) using *I–II* mixed reagent; $c(I) = 0·6$ mmol l^{-1}, $c(II) = 0·2$ mmol l^{-1}, c(ascorbic acid) $= 10$ mmol l^{-1}, 0·1M formate buffer pH 3·5, $F_m = 1$ ml min^{-1}, $L_r = 40$ and 55 cm, respectively, i.d. $= 0·6$ mm (PTFE), $V_1 = 25$ or 30 μl

Sample	Method	$\varrho(Fe)$ mg l^{-1}	s_r %	$\varrho(Cu)$ mg l^{-1}	s_r %	n
A	FIA	0·235 ± 0·032	15·0	0·091 ± 0·040	46·0	7
A	FIA[a]	0·272 ± 0·015	6·0	0·089 ± 0·023	28·0	7
B	Reverse FIA	0·396 ± 0·012	3·0	0·271 ± 0·020	7·3	10
C	Reverse FIA	0·451 ± 0·010	2·2	0·375 ± 0·016	4·3	10
D	Reverse FIA	0·253 ± 0·011	4·0	0·439 ± 0·015	3·4	10
D	Spectrophotometry	0·267 ± 0·015	5·5	0·448 ± 0·022	5·0	5
E	Reverse FIA	0·668 ± 0·017	2·5	0·724 ± 0·031	4·2	10
E	Spectrophotometry	0·691 ± 0·033	4·8	0·709 ± 0·020	2·8	5

[a] Standard addition method.

the lower determination limit[13] and wider concentration span, and the data agree well with those obtained by the conventional spectrophotometric method using the two organic analytical reagents.

The two modifications of the FIA determination of Fe and Cu in model samples as well as in actual drinking and surface waters and waste waters using the *I–II* or *I–III* mixed reagents are sufficiently sensitive, accurate and precise, the sensitivity, however, is 3 or 8 times lower than in the conventional static mode.

Owing to the very slight effect of the number of wavelengths used in the experimental data treatment, it will be possible to use standard detectors with the dual wavelength measurement facility without impairing the accuracy and precision of determination, as has been demonstrated on a case of simultaneous determination of Cu ions in biological material[14] with the *I–II* or *I − III* mixed reagent.

As compared to the conventional approach to indirect FIA analysis employing a single organic reagent exhibiting a low selectivity and reacting with all components in sample, multiligand multicomponent FIA affords more accurate and precise results, owing to the higher difference between the spectral characteristics of reaction products, which in conventional multicomponent flow injection analysis (MC-FIA) are determined basically by the properties of analyte. The own absorption of the free organic reagent in the nearby spectral range, particularly for organic dyes with extensive π-electron systems, also plays an important part. Since the reagent has to be used in a higher concentration excess with respect to the summary concentration of the analytes, the own absorption of the reagent in the conventional MC-FIA is often commensurable with or even higher than the total absorption of the reaction components. This has a serious negative effect on the accuracy and precision of the determination.

Another important factor in MC-FIA can be mutually competitive equilibria of analytes and other components, which can affect considerably the quantitativeness of formation of the analytically usable reaction products and thereby give rise to apparent deviations from additivity of absorbances[6,7].

In this respect, the multiligand MC-FIA approach employing two or several highly selective reagents, each reacting predominantly with one of the components to be determined, is very convenient. The reaction products formed offer, by a suitable choice of reagent, a higher variability than as is possible in the conventional MC-FIA. In this manner, a higher selectivity of the simultaneous determination and improved accuracy and precision of data can be achieved by a shift of the reaction equilibria in favour of the analytically usable reaction products.

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