COMPARISON OF THE REACTIONS OF FERRIC IONS WITH CHROMAZUROL S, ERIOCHROMAZUROL B AND ERIOCHROMCYANINE R IN THE PRESENCE OF SURFACE ACTIVE SUBSTANCES AND SPECTROPHOTOMETRIC DETERMINATION OF Fe(III)-IONS WITH CHROMAZUROL S–CETYLPYRIDINIUM BROMIDE SYSTEM

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The spectrophotometric methods of determining Fe(III) ions with Chromazurol S, Eriochromazurol B and Eriochromcyanine R in the presence of cationic, anionic and nonionic tensides were compared using the basic optical and statistical parameters. A method was proposed and developed for the determination of Fe(III) ions using Chromazurol S in blood serum and plasma at pH 4·7—5·0 in a medium of $2 . 10^{-4}$M cetylpyridinium bromide and 0·1M pyridine buffer and at pH 6·0—6·5 in a medium of $2 . 10^{-3}$M cetylpyridinium bromide and 0·1M hexamethylenetetramine buffer with molar absorption coefficients of $1·31 . 10^5$ and $1·40 . 10^5$, respectively. The determination is sufficiently specific even at pathological concentrations of the other serum components.

In spite of the ever increasing importance of the atomic absorption determination method, the spectrophotometric determination of iron in biological materials is of great importance in a number of clinical laboratories. Comparison of the reaction of Fe(III) ions with chromogenic agents from the group of N-heterocyclic azodyes (pyridylazoresorcinol — PAR, thiazolylazoresorcinol — TAR) and hydroxytriphenylmethane dyes (Chromazurol S—CAS, Eriochromazurol B — CAB, Eriochromcyanine R — ECR) in aqueous medium or in aqueous–ethanolic medium indicates that PAR, in contrast to TAR, is sufficiently sensitive and a very promising reagent for analytical practice[1]. Reagents from the hydroxytriphenylmethane group of dyes are somewhat more sensitive in aqueous or ethanolic–aqueous media ($\varepsilon = 7·0—8·1 . 10^4$ mmol^{-1} cm^2) than N-heterocyclic azodyes; however, the reproducibility of the determination is lower. The sensitivity of the reaction of Fe(III) ions with hydroxytriphenylmethane dyes is much higher[2-6] in the presence of surface active substances — tensides ($\varepsilon = 1·2—1·7 . 10^5$ mmol^{-1} cm^2), which allows analysis of a minimum amount of initial sample, the volume of which is especially important in clinical examinations. The precision and reproducibility of the determination using the metal–hydroxytriphenylmethane dye–tenside ternary system are affected by a number of factors (component concentration, procedure and means of component mixing, solution temperature, *etc.*). Thus a more basic study of this ternary system was undertaken considering the possibility of using it in clinical practice.

This work deals with comparison of the ternary systems of the most important chromogenic agents from the group of hydroxytriphenylmethane dyes – Chromazurol S, Eriochromazurol B and Eriochromcyanine R, whose structural formula is given in Fig. 1. Comparison of the optical characteristics, sensitivity, precision, selectivity and further parameters of their reactions with Fe(III) ions in the presence of cationic, anionic and nonionic tensides was carried out on the basis of analysis of the absorbance curves measured under various experimental conditions and treatment of the calibration curves by the least squares method using the STAT program (ref.[7-10]).

EXPERIMENTAL

Chemicals and Instruments

The stock solution of 0·1008M-Fe(III) was prepared by dissolving $Fe(NO_3)_3.9\,H_2O$, *p.a.* purity (Lachema, Czechoslovakia) in 1M-HNO_3, recrystallizing the commercial substance first from 1M-HNO_3 medium. The concentration of Fe(III) was found gravimetrically.

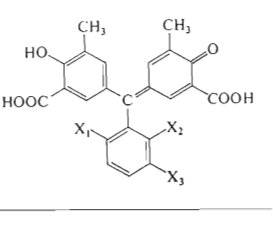
Chromazurol S (3″-sulpho-2″,6″-dichloro-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, CAS), Eriochromazurol B (2″,6″-dichloro-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, CAB) and Eriochromcyanine R (2″-sulpho-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, ECR) were purified by a combination of precipitation and extraction methods[8] using the commercial substances from Geigy (Basle, Switzerland) or from ICN Pharmaceuticals (New York, U.S.A.). The stock solutions with a concentration of $3 . 10^{-4}$—$1 . 10^{-3}$M were prepared by dissolving the solid substance of the free acid LH_4^0 or LH_3^0 in a small amount of 1M-KOH; after complete dissolving the solution was diluted to the mark with redistilled water (CAS, ECR) or ethanol (CAB). The solutions were stable for at least one week.

Zephiramine (benzyldimethyltetradecylammonium chloride, $C_{23}H_{42}ClN$, M_r 368·04) from Dojindo Co., Japan, stock solution $4 . 10^{-2}$M in ethanol. Cetylpyridinium bromide ($C_{21}H_{38}BrN$, M_r 384·44, designated CPB) was the product of Lachema, Czechoslovakia, recrystallized from ethanol. The stock solution contained $2 . 10^{-2}$M in 20% v/v ethanol. Cetyltrimethylammonium bromide ($C_{19}H_{42}BrN$, M_r 364·45, designated CTMA) was the product of Lachema, Czechoslovakia and was recrystallized from ethanol. The stock solution was $2 . 10^{-2}$M in 20% v/v ethanol. Septonex (1-ethoxycarbonylpentadecyltrimethylammonium bromide, Czechoslovak Pharmacopoeia 3: $C_{21}H_{44}BrN.O_2$, M_r 422·5) was the product of Slovakofarma Hlohovec, Czechoslovakia. The stock solution was $4 . 10^{-2}$M in ethanol.

FIG. 1

Structural Formula of Chromazurol S, Eriochromazurol B and Eriochromcyanine R

CAS: $X_1 = X_2 = Cl$, $X_3 = SO_3H$; CAB: $X_1 = X_2 = Cl$, $X_3 = H$; ECR: $X_1 = SO_3H$ $X_2 = X_3 = H$.



Sodium laurylsulphate ($C_{12}H_{25}O—SO_3Na$, M_r 288·38, designated SDS) was a product of BDH Ltd., Poole, Great Britain. The stock solution contained 2% w/v of the substance in water. Brij 3 5(polyoxyethylenemonolauryl ether, $n \sim 20$, $M_r \sim 900$) was the product of Merck, GFR. The stock solution was 2% w/v in water. Triton X-100 (octylphenolpolyethylene glycol ether, analytical grade) was the product of Koch-Light Lab. Ltd, Great Britain. The stock solution was 2% w/v in water.

For adjustment of the optimum pH value, the following buffers were tested: 1M solutions of acetate, formate, pyridine, tris-hydroxymethylaminomethane, hexamethylenetetramine, triethanolamine and maleinic buffers. Hexamethylenetetramine (HMT) was recrystallized from water or precipitated from ethanolic solution by excess ether; the other components were of *p.a.* purity.

Solutions of 0·6M trichloroacetic acid in 2M-HCl and thioglycolic acid from the Bio-La-Test system (Lachema, Czechoslovakia) were used for determining Fe(II) The precision and accuracy of the method were controlled by determining the concentration of Fe(III) ions in a standard Fe(II) solution from the same system and in samples of control serum from Hyland Travenol G.m.B.h. (Hyland Ferro — Check P 01 or N 01).

The effects of the other components of blood serum were studied by adding the nitrate solutions with concentrations of 1·22 mg Ca(II)/ml, 2·0 μg Mg(II)/ml, 12·7 μg Cu(II)/ml, 0·043 μg Cd(II)/ml, 0·415 μg Pb(II)/ml. 0·52 μg Mn(II)/ml and 12·8 μg Zn(II)/ml. The remaining chemicals were commercial products of *p.a.* purity (EDTA, KOH, NaOH) or *p.p.* purity (NH_4OH, HNO_3, HCl) from Lachema, Czechoslovakia. The ethanol employed contained 5% v/v methanol. The KOH solution was purified by coprecipitation of metallic impurities with barium(II) carbonate.

The acidity of the solutions was measured using a PHM 64 pH meter with a G202B glass electrode and K 401 saturated calomel electrode from Radiometer, Denmark. The instrument was calibrated regularly using a set of standard buffers (oxalate pH 1·68, phthalate pH 4·01, phosphate pH 6·48 and borate pH 9·18 at 25°C). The absorption spectra in graphical and digital form and the other remaining absorbance curves were measured on a Superscan 3 double beam digital recording spectrophotometer, controlled by a HP 9815A on-line desk-top computer (Varian, Switzerland and Hewlett–Packard, USA), at a constant temperature of 25°C. The individual absorbance curves, $A = f(\lambda)$, $A = f(\mathrm{pH})$, $A = f(c_L)$ and $A = f(c_M)$, *etc.* were measured in the classical way in 50 ml volumetric flasks and using a titration technique in an apparatus for spectrophotometric measurements in a closed cycle[9]. The recording and direct treatment of the experimental data were carried out using a program for direct recording and evaluation of absorbance curves using the Superscan 3 spectrophotometer and HP 9815A on-line computer (ref.[4]).

The basic parameters of the Fe(III)–hydroxytriphenylmethane dye–tenside ternary system were compared on the basis of a study of the absorption curves measured over a broad interval of varied experimental conditions by a procedure described in an earlier work[1].

RESULTS AND DISCUSSION

The absorbance of the ternary Fe(III)–hydroxytriphenylmethane dye–tenside system depends, among other factors, on the order and method of mixing the individual components of the reaction mixture. Of the tested mixing procedures: *a*) tenside, ethanol, HNO_3, reagent, Fe(III) solution, pH adjustment; *b*) Fe(III) solution, reagent, HNO_3, tenside, ethanol, pH adjustment; *c*) Fe(III) solution, tenside, ethanol,

reagent, HNO_3, pH adjustment; *d*) reagent, HNO_3, tenside, ethanol, Fe(III) solution, pH adjustment and *e*) Fe(III) solution to which was added the mixed reagent (tenside. ethanol, HNO_3, reagent, buffer for pH adjustment), procedures *a*) and *e*) were found to be best. To attain maximum reproducibility of the measurement, it was always necessary to begin with an acidic medium and shift the pH to higher values. When this procedure is maintained, the maximum absorbance value is attained in solutions with pH 2–5·5 after 2–3 minutes and remains constant for at least two hours. In the pH interval 6–8, it is necessary to measure the absorbance of the solution within 15 minutes after pH adjustment; after longer times the pH shifts to higher values with a simultaneous change in the position of the absorption maximum to longer wavelengths and a decrease in the absorbance value at the absorbance maximum of the ternary species.

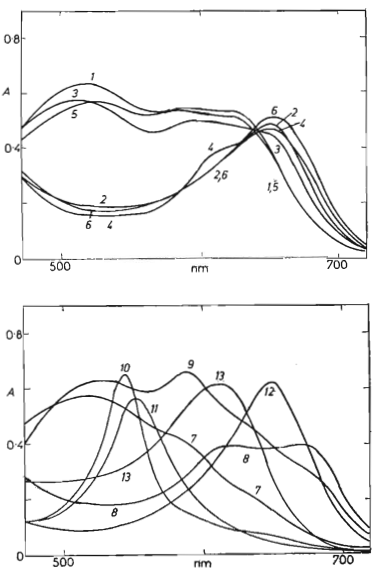


FIG. 2

Absorption Spectra of Hydroxytriphenylmethane Dyes with Fe(III) Ions in the Presence of Cationic, Anionic and Nonionic Tensides $c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_L = 3{\cdot}75 \cdot 10^{-5}$M, 8% v/v ethanol, *l* 0·01 (HNO_3 + + KOH), 25°C, *l* = 10 mm

curve pH tenside c_T: *1* 3·90 CPB $2 \cdot 10^{-3}$M; *2* 5·86 CPB $2 \cdot 10^{-3}$M; *3* 4·54 Septonex $4 \cdot 10^{-3}$M; *4* 6·45 Septonex $4 \cdot 10^{-3}$M; *5* 4·55 CTMA $2 \cdot 10^{-3}$M; *6* 6·29 CTMA $2 \cdot 10^{-3}$M; *7* 5·58 Triton X-100 0·1% w/v, *8* 6·30 Zephiramine $4 \cdot 10^{-3}$M; *9* 4·63 Zephiramine $4 \cdot 10^{-3}$M; *10* 4·99 Brij 35 0·1% w/v; $c_M = 3 \cdot 10^{-6}$M, $c_L = 3 \cdot 10^{-5}$M; *11* 5·31 SDS 0·1% w/v, $c_M = 3 \cdot 10^{-6}$M, $c_L = 3 \cdot 10^{-5}$M, *12* 6·50 CTMA $2 \cdot 10^{-3}$M, $c_M = 2 \cdot 10^{-6}$M, $c_L = 2{\cdot}5 \cdot 10^{-5}$M; *13* 6·34 CTMA $2 \cdot 10^{-3}$M, $c_M = 2{\cdot}5 \cdot 10^{-6}$M, $c_L = 2{\cdot}5 \cdot 10^{-5}$M; curves *1*—*11* Chromazurol S, curve *12* Eriochromcyanine R, curve *13* Eriochromazurol B.

The absorption spectra of solutions of Fe(III) ions with Chromazurol S, $A = f(\mathrm{pH}, \lambda)$ were measured in the presence of cationic, anionic and nonionic tensides (CTMA, CPB, Septonex, Zephiramine, sodium laurylsulphate, Brij 35, Triton X-100) in submicellar and micellar concentrations in dependence on the acidity of the medium (for experimental conditions, see Table I and Fig. 2). The absorption spectra indicate gradual formation of one to two absorbing ternary species in solution, whose optical characteristics depend primarily on the acidity of the medium and on the type and concentration of tenside (Fig. 2 and Table I).

The absorbance–pH curves (Fig. 3) measured under similar experimental conditions indicate the presence of one or two overlapping formation regions in the pH intervals 2–4 and 4·5–6·0. The absorbance maximum with a variously broad plateau on the absorbance-pH curves $\Delta A = f(\mathrm{pH})$ is generally attained in weakly acid or neutral medium. The horizontal parts of the absorbance-pH curves defining the optimal acidity conditions for development of the spectrophotometric method of determining Fe(III) ions with Chromazurol S in the presence of various tensides are listed in Table II.

The absorption spectra (Table I and Fig. 2) and absorbance-pH curves for solutions of Fe(III) ions with Eriochromazurol B and Eriochromcyanine R in the presence of cationic tensides (CTMA, CPB) measured under similar experimental conditions also indicate gradual formation of several intensely absorbing species with absorbance maxima in the wavelength region 600–650 nm. These species are formed quantitatively in weakly acidic or neutral pH regions.

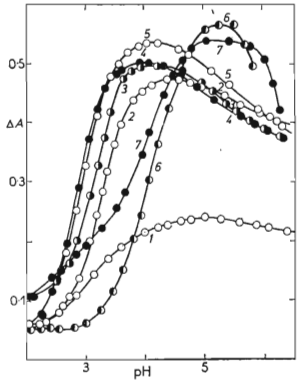


FIG. 3

Absorbance-pH Curves for Fe(III) Ions with Hydroxytriphenylmethane Dyes in the Presence of Tenside

$c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_L = 3{\cdot}75 \cdot 10^{-5}$M, 8% v/v ethanol, for the other conditions, see Fig. 2, 625 nm. Curve tenside c_T: *1* Triton X-100 0·1% w/v, *2* Septonex $4 \cdot 10^{-3}$M, *3* Zephiramine $4 \cdot 10^{-3}$M, *4* CPB $2 \cdot 10^{-3}$M, *5* CTMA $2 \cdot 10^{-3}$M, *6* Brij 35 0·1% w/v, *7* SDS 0·1% w/v, 554 nm, curves *6* and *7* $c_M = 4{\cdot}785 \cdot 10^{-6}$M, $c_L = 4{\cdot}785 \cdot 10^{-5}$M.

It follows from the given results of comparison of the interactions of Fe(III) ions with Chromazurol S in the presence of cationic, anionic and nonionic tensides that the cationic tensides (CTMA, CPB, Zephiramine, Septonex) have the greatest effect on the colour contrast in the reaction ($\Delta\lambda_{max}$ 140–170 nm), while anionic (sodium laurylsulphate, SDS) and nonionic (Brij 35, Triton X-100) tensides affect the colour contrast of the reaction much less ($\Delta\lambda_{max}$ 30–50 nm). The sensitivity of the reaction is most favourably affected by the cationic tensides cetyltrimethylammonium bromide and cetylpyridinium bromide (molar absorptivities $1{\cdot}38-1{\cdot}40 \,.\, 10^{-5}$ and 1·36 to $1{\cdot}39 \,.\, 10^{-5}$ $mmol^{-1}$ cm^2). Cationic cetyltrimethylammonium bromide is most favourable for the interaction of Fe(III) ions with Eriochromazurol B and Eriochromcyanine R.

Comparison of the Reaction of Fe(III) *Ions with Hydroxytriphenylmethane Dyes in the Presence of* CTMA

The absorption spectra of solutions of Fe(III) with hydroxytriphenylmethane dyes in the presence of the critical micellar concentration $c_T = 2 \,.\, 10^{-3}$M-CTMA was measured in pH intervals of 1–9 and 2–6 under the experimental conditions given in Table I. A summary of the optical characteristics is also given in this Table.

The absorbance-pH curves measured under the given concentration conditions for wavelengths of 630, 645 and 680 nm (CAS), 605, 615 and 645 nm (ECR) and 625, 654 and 657 nm (CAB) indicate quantitative formation of the absorbing species in the pH intervals 6·0–6·75 (CAS), 6–7 (ECR) and 6–6·5 (CAB). The measured dependence of the absorbance on the reagent concentration $\Delta A = f(c_L)$ for $c_M = 5{\cdot}0 \,.\, 10^{-6}$M (CAS) or $c_M = 2{\cdot}5 \,.\, 10^{-6}$M (CAB, ECR, CAS) in the presence of the critical micellar concentration $c_T = 2 \,.\, 10^{-3}$M-CTMA for the above pH intervals indicate that a concentration ratio of $c_L/c_M \sim 6-10$ is sufficient for quantitative formation. The dependence of the absorbance on the concentration of tenside $A = f(c_T)$, measured for the same concentration of Fe(III) ions with a ten-fold concentration excess of reagent indicates that the absorbance is independent of the tenside concentration in this pH interval from $c_T = 4 \,.\, 10^{-4}$M to 5.10^{-3}M. At higher tenside concentrations the absorbance decreases as a result of decomposition of ternary species to the individual components or binary species.

The effect of the following buffers (TRIS, HMT, pyridine) in concentrations of $c_B = 0{\cdot}02-0{\cdot}5$M was studied under the above concentration conditions over the whole pH interval, pH 4·5–7·0. A concentration of 0·1M pyridine buffer was found to be most suitable; TRIS yields a high blank absorbance value and HMT slightly decreases the absorbance from concentrations of $c_B \sim 0{\cdot}1$M.

The calibration curves $\Delta A = f(c_M)$ measured under conditions given in Table III are linear in the concentration interval $c_{Fe} = 0{\cdot}70-5{\cdot}0 \,.\, 10^{-6}$M. The values of the molar absorptivity and other calibration curve parameters, evaluated by linear

TABLE I

Survey of the Optical Characteristics of the Fe(III)–HTFMB–Tenside Ternary System $c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_L = 3{\cdot}75 \cdot 10^{-5}$M, 8% v/v ethanol, pH = 1—9, λ = 330—730 nm, $I = 0{\cdot}10$ ($HNO_3 + KNO_3$), t = 25°C.

System	pH	λ_{max}, nm	λ_{ip}, nm	λ, nm[a]
CAS–CTMA[b]	2·2—3·4	626, 588, 510[c]	540, 354	630, 645,
	3·5—4·0	626, 588	354	680
	4·2—6·2	650, 418[c]	642, 454	
CAS–CTMA[d]	1	467, 587		620, 630,
	2·1—3·1	632, 587, 510[c]	498, 483, 362	645, 680
	3·3—4·8	650, 375	398	
	5·2—6·0	645—650, 420[c]	497	
CAS–CPB[b]	2·2—4·0	625, 588, 506[c]	538—554, 353	620, 625, 630,
	4·2—6·8	653, 423[c]	639, 458, 353	645, 650, 655
CAS–CPB[e]	2·2—4·5	655, 625,	dtto	dtto
	4·7—6·0	655, 680		
CAS–Septonex[f]	2·1—2·7	585, 510[c]	541—553, 354	625, 650
	2·8—4·8	650, 589	541—553, 354	
	4·9—7·0	650, 420[c]	456, 354	
CAS–Zephiramine[f]	2·1—3·5	630, 588, 524	553, 353	625, 650
	3·7—4·3	672, 424[c]	353	
	4·6—7·4	672, 424[c]	462, 353	
CAS–SDS[g,h]	2·3—3·2	574	500, 487	625, 554
	3·6—5·3	554, 387[c]	521, 350	
	5·7	553, 390[c]	465, 350	
CAS–Triton	2·0—4·2	635, 590, 468	484, 353	625, 650
X-100[g]	4·2—5·5	635, 590, 520	353	
	5·5—6·9	635, 425[c]	462, 353	
CAS–Brij 35[g,h]	2·2—3·3	555	492, 370, 482	545, 630
	3·7—4·9	544, 630	515—520, 561	
	5·0—6·7	545, 630	463—478, 593	
CAB–CTMA[i]	1·0—2·9	585, 508[c], 468	482, 356	654, 657
	3·6—6·3	655, 380	577, 356, 422	
	6·6—9·0	650, 384	500	
ECR–CTMA[j]	2·0—3·2	505·[c]		605, 615, 635
	3·6—5·4	581	536, 355	
	5·7—6·9	591—615	554—590, 355	
	6·9—8·7	615—635, 430[c]	640—650, 480, 564	
ECR–CPB[b]	2·1—4·5	610, 510[c]	362	605, 610, 640
	4·8	640, 530[c]	464, 362	

regression using the least squares method and the STAT program (ref.[10]) with an HP 9815A desk-top computer are listed in Table III.

Determination of Fe(III) *Ions with Chromazurol* S *in the Presence of* CPB

Fig. 4 depicts the shape of the absorption spectra in dependence on the acidity of the solution and a summary of the optical parameters is given in Table I. The absorption

TABLE II

Molar Absorption Coefficient Values for the HTFMB–Fe(III)–Tenside Ternary System and Optimal pH Intervals for Spectrophotometric Determination of Fe(III) Ions

$c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_L = 3{\cdot}75 \cdot 10^{-5}$M, 8% v/v ethanol, I 0·10, $t = 25°C$, $c_T = 2 \cdot 10^{-3}$M.

System	pH_{opt}	λ, nm	$10^5 \cdot \varepsilon$, mmol^{-1} cm^{2}[a]
CAS–CTMA	6·0—6·75	626, 650	1·40, 1·28
CAS–CTMA[b,c]	5·2—6·0	645, 650	1·34, 1·36
CAS–CPB	4·7—5·0	625	1·39
	6·0—6·5	653	1·36
CAS–CPB[b,d]	4·7—5·0	625	1·36
	6·0—6·5	650	1·31
CAS–Septonex[c]	4·1—5·0	650	1·23
CAS–Zephiramine[c]	4·0—4·8	630, 672	1·26, 1·00
CAS–Triton X-100[e]	4·8—5·2	635	0·55
CAS–SDS[e,f]	5·0—5·75	554	
CAS–Brij 35[e,f]	5·0—5·5	544	
CAB–CTMA[g,e]	6·0—6·5	654	0·99
ECR–CTMA[g]	6·0—7·0	615, 635	1·26, 1·24
ECR–CPB	4·5—5·5	610	1·22
	5·9—6·5	640, 610	1·23, 1·23

[a] Values found from the absorbance-pH curves for the appropriate wavelengths, [b] $c_M = 4{\cdot}785 \cdot 10^{-6}$M, $c_L = 4{\cdot}785 \cdot 10^{-5}$M, [c] $c_T = 4 \cdot 10^{-3}$M, [d] $c_T = 2 \cdot 10^{-4}$M, [e] $c_T = 0{\cdot}10\%$ w/v, [f] $c_M = 3{\cdot}0 \cdot 10^{-6}$M, $c_L = 3{\cdot}0 \cdot 10^{-5}$M, [g] $c_M = 2{\cdot}5 \cdot 10^{-6}$M, $c_L = 2{\cdot}5 \cdot 10^{-6}$M.

←

[a] Wavelengths used in measurement of the absorption curves, [b] tenside concentration $c_T = 2 \cdot 10^{-3}$M, [c] absorption maximum of the reagent-tenside binary species, [d] $c_T = 4 \cdot 10^{-3}$M, $c_M = 4{\cdot}785 \cdot 10^{-6}$M, $c_L = 4{\cdot}785 \cdot 10^{-5}$M, [e] $c_T = 2 \cdot 10^{-4}$M, $c_M = 4{\cdot}875 \cdot 10^{-6}$M, $c_L = 4{\cdot}785 \cdot 10^{-5}$M, [g] $c_T = 0{\cdot}10\%$ w/v, [f] $c_T = 4 \cdot 10^{-3}$M, [h] $c_M = 3{\cdot}0 \cdot 10^{-6}$M, $c_L = 3{\cdot}0 \cdot 10^{-5}$M, [i] $c_M = 2{\cdot}5 \cdot 10^{-6}$M, $c_L = 2{\cdot}5 \cdot 10^{-5}$M, $c_T = 0{\cdot}1\%$ v/v, [j] $c_M = 2{\cdot}5 \cdot 10^{-6}$M, $c_L = 2{\cdot}5 \cdot 10^{-5}$M, $c_T = 2 \cdot 10^{-3}$M.

TABLE III

Comparison of the Basic Parameters of the Calibration Curves and Conditions for the Determination of Fe(III) Ions with Hydroxytriphenylmethane Dyes in the Presence of Cationic Tensides

Conditions, parameter	ECR–CTMA[a]	CAB–CTMA[a]	CAS–CTMA[b]	CAS–CPB[c]	CAS–CPB[e]
pH interval	6·0—7·0	6·0—6·5	6·0—6·75	4·7—5·0	6·0—6·5
pH_{opt}	6·5	6·2	6·5	4·8	6·2
λ_{max}, nm ML	615	654	645	625	655
λ_{max}, nm L	425	426	420	422	422
$\Delta\lambda_{max}$, nm ML—L	190	228	225	203	233
c_L/c_M	6	5	10	10	6
Absorbance stability ML	3—120′	3—120′	3—120′	3—120′	3—15′
$\varepsilon \pm d(\varepsilon)$ mol^{-1} cm^2	123 750 ± 2 165[d]	101 500 ± 4 856[d]	107 500 ± 4 327	130 411 ± 2 053	139 637 ± 1 849
	123 313 ± 6 724	99 021 ± 3 960	122 300 ± 3 986[d]		
c_M (linearity) . 10^6 (M)	0·70—5·0	0·70—4·0	0·70—5·8	0·75—3·75	0·45—5·625
$A_{b1} \pm d(A_{b1}) . 10^3$	84 ± 74[d]	111 ± 23[d]	168 ± 16[d]	94 ± 11	11 ± 2
	228 ± 21	191 ± 13	133 ± 13		
Correlation coefficient r_{xy}	0·99985[d]	0·99673[d]	0·99700[d]	0·99880	0·99720
	0·98359	0·99863	0·99855		
L_D (μg/ml) . 10^3	11·73[d], 79·3	45·1[d], 30·3	33·85[d], 23·4	11·27	24·1
$S_I . 10^3$ ($A = 0·010$)	4·5[d], 7·6	5·2[d], 4·55	7·35[d], 5·64	4·28	3·99
Precision, % rel.	0·19	0·08	0·17	0·08	0·26
$s_{xy}(A) . 10^3$	6·2[d], 24·55	15·4[d], 12·1	14·5[d], 12·6	6·2	14·2

[a] 0·1M Pyridine buffer, $c_L = 2·5 \cdot 10^{-5}$M, $c_T = 2 . 10^{-3}$M, [b] 0·1M pyridine buffer, $c_L = 5·0 . 10^{-5}$M, $c_T = 2 . 10^{-3}$M, [c] 0·1M pyridine buffer, $c_L = 3·75 . 10^{-5}$M, $x_T = 2 . 10^{-4}$M, [d] unbuffered solutions, [e] 0·1M-HMT buffer, $c_L = 2·25 . 10^{-5}$M, $c_T = 2 . 10^{-3}$M.

spectra and absorbance-pH curves (Fig. 5) confirm that at least two absorbing species are formed in solution, of which that absorbing in the wavelength region 620–635 nm attains maximum absorbance in the pH interval 4·75–5·00 and that with absorption maximum at 645–655 nm has maximum absorbance in the pH region 6·0–6·5. The dependence of the absorbance on the reagent concentration $\Delta A = f(c_L)$ for $c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_{CPB} = 2 \cdot 10^{-4}$M, pH 4·75–5·00 and wavelengths of 620, 625 and 630 nm indicates that a concentration ratio of $c_L/c_M \sim 10$ is sufficient for quantitative formation of the absorbing species. In the pH interval 6·0–6·5, $c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_{CPB} = 2.10^{-3}$M and wavelengths 645,650 and 655 nm the concentration ratio of $c_L/c_M \sim 6$ is sufficient. In the pH interval 4·75–5·00 or 6·0–6·5 and for the above wavelengths and c_M, the dependence of the absorbance on the tenside concentration $\Delta A = f(c_{CPB})$ was measured. Determination of a concentration of $2 \cdot 10^{-4}$M at pH 4·75–5·00 or $2 \cdot 10^{-3}$M at pH 6·0–6·5 was found most suitable.

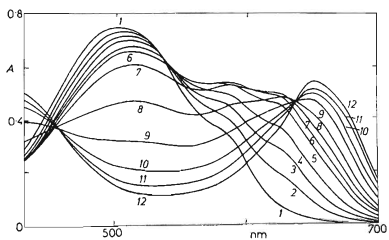


FIG. 4

The Curves of the Absorption Spectra of Fe(III) ions with Chromazurol S in the Presence of CPB

$c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_L = 3{\cdot}75 \cdot 10^{-5}$M, 8% v/v ethanol, $c_{CPB} = 2 \cdot 10^{-3}$M, $I = 0{\cdot}01$ (HNO_3 + KOH), 25°C, *1* 10 mm; Curve, pH: *1* 2·20, *2* 2·65, *3* 2·86, *4* 3·04, *5* 3·32, *6* 3·59, *7* 4·05, *8* 4·61, *9* 5·16, *10* 5·61, *11* 6·06, *12* 6·78.

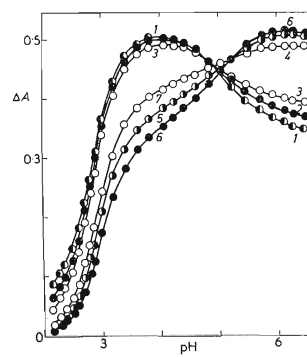


FIG. 5

Absorbance-pH Curves for the Fe(III)–CAS–CPB System for Solutions with a Concentration Excess of Reagent

For the experimental conditions, see Fig. 4; curve λ (nm): *1* 620, *2* 625, *3* 630, *4* 645, *5* 650, *6* 655.

Under optimum experimental conditions ($c_L = 3{\cdot}75 \cdot 10^{-5}$M, $c_M = 3{\cdot}75 \cdot 10^{-6}$M, $c_{CPB} = 2 \cdot 10^{-4}$M, 620, 625 and 630 nm) in the pH interval 4·75–5·00 or $c_{CPB} = 2 \cdot 10^{-3}$M, wavelengths of 645, 650 and 655 nm in the pH interval 6·0–6·5, the absorbance-pH curves were measured in the presence of 0·1M solutions of formate, acetate, pyridine, maleinate, HMT and TRIS buffers In the pH interval 4·75–5·00, pyridine buffer is most suitable at a pH of 4·8; in the pH interval 6·0–6·5, HMT buffer with pH 6·2 is most suitable, It follows from measurement of the dependence of the absorbance on the concentration of these buffers that they do not affect the absorbance of the ternary Fe(III)–CAS–CPB system in the given pH interval at concentrations of $c_B = 0{\cdot}02-0{\cdot}20$M. A concentration of $c_B = 0{\cdot}10$M was chosen as optimal.

A series of absorbance-pH curves $\Delta A = f(\mathrm{pH})$ for $c_L = 3{\cdot}75 \cdot 10^{-5}$M and $c_M = 3{\cdot}75 \cdot 10^{-6}$M, measured in a 0·1M pyridine medium (620, 625 and 630 nm) or 0·1M-HMT (645, 650 and 655 nm) for several CPB concentrations in the range $c_{CPB} = 1 \cdot 10^{-4}-2 \cdot 10^{-2}$M confirm the optimal concentration $c_{CPB} = 2 \cdot 10^{-4}$M found earlier for pH 4·75–5·0 or $2 \cdot 10^{-3}$M for pH 6·0–6·5. In the pH range 4·75 to 5·0 turbidity is formed at concentration $c_{CPB} \leqq 1 \cdot 10^{-4}$M; at $c_{CPB} > 5 \cdot 10^{-4}$M the pH interval in which the absorbance is independent of the pH narrows considerably and the absorbance decreases at higher pH values. In the pH interval 6·0–6·5 the solution absorbance increases with increasing CPB concentration over the whole concentration interval $c_{CPB} = 2 \cdot 10^{-4}-1 \cdot 10^{-3}$M; in the concentration interval $c_{CPB} = 1{\cdot}5-5{\cdot}0 \cdot 10^{-3}$M the absorbance is independent of the c_{CPB} value and a higher CPB concentrations the absorbance decreases.

A series of absorbance curves for $c_M = 3{\cdot}75 \cdot 10^{-6}$M Fe(III) and $c_{CPB} = 2 \cdot 10^{-4}$M (0·1M pyridine, 620, 625 and 630 nm) or $c_{CPB} = 2 \cdot 10^{-3}$M (0·1M-HMT, 640, 645, 650 and 655 nm) and reagent concentration of $c_L = 2{\cdot}25-4{\cdot}50 \cdot 10^{-5}$M-CAS indicate

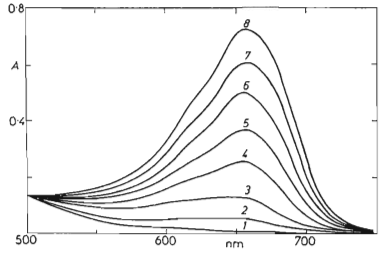


FIG. 6

Curves of the Absorption Spectra of the Fe(III)–CAS–CPB System in Dependence on the Concentration of Fe(III) Ions, $A = f(c_M, \lambda)$

$c_L = 2{\cdot}25 \cdot 10^{-5}$M CAS, $c_{CPB} = 2 \cdot 10^{-3}$M, 8% v/v ethanol, $c_{HMT} = 0{\cdot}10$M, pH 6·2, 25°C, *1* 10 mm; curve $10^7 \cdot c_M$(M): *1* 0·0, *2* 4·5, *3* 9·75, *4* 18·75, *5* 26·25, *6* 37·50, *7* 45·00, *8* 56·25.

that, in the pH interval 4·75–5·0 the optimal reagent concentration is $c_L = 3{\cdot}75 \cdot 10^{-5}$M, which ensures quantitative formation of the coloured species and simultaneously has a relatively low blank value. In the pH interval 6·0–6·5 a concentration of $c_L = 2{\cdot}25 \cdot 10^{-5}$M is sufficient, yielding an overall blank absorbance value of $A \leqq 0{\cdot}002$ in the given wavelength interval, which renders this pH interval advantageous for its low blank value.

The absorption spectra for solutions with $c_L = 3{\cdot}75 \cdot 10^{-5}$M; $c_{CPB} = 2 \cdot 10^{-4}$M in a medium of 0·1M pyridine buffer with pH 4·8 or $c_L = 2{\cdot}25 \cdot 10^{-5}$M, $c_{CPB} = 2 \cdot 10^{-3}$M in a medium of 0·1M-HMT buffer with pH 6·2 (Fig. 6) indicate formation of a single coloured product in the whole concentration range $c_M = 7{\cdot}5 \cdot 10^{-7}$ to $7{\cdot}5 \cdot 10^{-6}$M-Fe(III). The calibration curves measured under the same experimental conditions at wavelengths of 620, 625, 630 nm or 645, 650 and 655 nm are linear for the concentration range $c_M = 0{\cdot}75-3{\cdot}75 \cdot 10^{-6}$M in pyridine buffer medium with pH 4·8 and for $c_M = 0{\cdot}45-5{\cdot}625 \cdot 10^{-6}$M-Fe(III) in HMT buffer medium at pH 6·2. The molar absorptivity values and other calibration curve parameters are listed in Table III.

The method of determining Fe(III) with Chromazurol S in the presence of CPB was used for both pH intervals for control of serum from the Hyland Co., whose composition corresponds to human blood serum with a defined content of Fe(III) ions. The Fe concentration for individual determination methods is given by the manufacturer in the range 190–240 μg Fe/ml. The precision and accuracy of the method were also verified using standard Fe(II) solutions from the Bio-La-Test system from Lachema after oxidation of the Fe(II) ions with peroxodisulphate. A survey of the determination results for pure solutions and for solution in which proteins were denatured, freeing the iron ions from the protein bonds and in which coloured ternary species were formed, is given in Table IV.

Both methods with successive addition of the individual components of the reaction mixture and addition of the mixed reagent all at once (procedures *a*) and *e*)) enable measurement of the final absorbance value over 5–15 minutes after mixing the sample components, leading to stabilization of the pH value and the solution absorbance becoming independent of time and attaining a maximal value. The precision of the determination is generally better than 5%, ensuring sufficiently precise and reliable results in practice provided that the procedure is observed. The method with weak acid medium in the presence of pyridine yields somewhat more precise results than the method with HMT buffer, but the differences are not statistically significant.

Other metals present in pathological concentrations do not interfere in the determination of Fe(III), except for higher Cu(II) contents. The interference from Cu(II) ions can be suppressed by adding 10 mg of thiourea or sodium citrate without decreasing the sensitivity of the determination of Fe(III) ions. The effect

of both masking agents was verified on samples of control serum from the Hyland Co., to which a solution of of Cu(II) ions was added, corresponding to a 2–5-fold excess over the normal concentrations of Cu(II) in blood serum.

TABLE IV

Determination of the Fe(III) Ion Concentration in Control Samples of Blood Serum (Hyland Ferro Check) and Standard Fe(III) Solutions

Sample	Concentration of Fe(III), μM				
	stated	found[a]	CV[c]	found[b]	CV[c]
P 01[h]	36·9	36·3 ± 0·07	1·93	37·2 ± 0·09	2·42
N 01[h]	13·7	13·5 ± 0·02	1·48	13·4 ± 0·05	3·73
N 01[d,i]	13·7	13·7 ± 0·04	2·91	13·4 ± 0·04	2·99
N 01[e,i]	13·7	13·5 ± 0·03	2·22	13·2 ± 0·05	3·80
Fe(III)[f,h]	8·0	8·1 ± 0·01	1·23	8·2 ± 0·02	2·44
Fe(III)[g,h]	8·0	8·0 ± 0·01	1·08	8·2 ± 0·02	2·44
Fe(III)[g,h]	24·0	24·0 ± 0·06	2·50	24·2 ± 0·08	3·30

[a] 0·1M Pyridine buffer medium, pH 4·8, [b] 0·1M-HMT buffer, pH 6·2, [c] variation coefficient, [d] determination in the presence of Cu(II), masking with 100 mg of thiourea, [e] masking of Cu(II) with 100 mg of sodium citrate, [f] determination with gradual addition of the reaction mixture components, [g] determination with addition of the reagent all at once, [h] 10 determinations, [i] 5 determinations.

TABLE V

Procedure for the Determination of Fe(III) in Blood Serum

Conditions	Serum blank	Standard	Sample
Serum, ml	—	—	0·2
Standard solution, Fe, ml	—	0·2	—
Redistilled water, ml	0·2	—	—
Deproteinated reagent, ml[a]	0·2	0·2	0·2
mix, centrifuge for 10—15 min at 3000 rpm[b]			

[a] 0·6M Trichloroacetic acid in 2·0M-HCl, [b] 0·2 ml of supernatant are pipetted into a test tube and 4·8 ml of solution of mixed reagent are pipetted into all solutions ($c_{CPB} = 2 . 10^{-4}$M, 8% v/v ethanol, $c_L = 3·75 . 10^{-5}$M Chromazurol S, 0·1M solution of pyridine buffer of pH 4·8, 100 mg thiourea); measured after 5—15 min at 620 nm.

Procedure: The solutions are mixed according to the scheme in Table V, are centrifuged at 3000 r.p.m. for 10—15 minutes, 0·2 ml portions of supernatant are pipetted into clean test--tubes and 4·8 ml of mixed reagent ($c_L = 3{\cdot}75 \cdot 10^{-5}$M-CAS, $c_T = 2 \cdot 10^{-4}$M CPB, 8% v/v ethanol, 0·1M pyridine buffer of pH 4·8) and 100 mg thiourea are added to all solutions. The sample and standard absorbances are measured against a blank in cuvettes with optical path-lengths of 10 mm at 620 nm within 5—15 minutes after preparation. The method can also be applied on a microscale using 0·2 ml of supernatant and 0·8 ml of mixed reagent.

REFERENCES

1. Gotzmannová D., Kubáň V.: This Journal *45*, 1793 (1980).
2. Garčic A.: Clin. Chim. Acta *94*, 115 (1979).
3. Nishida H.: Japan Analyst *19*, 221 (1970).
4. Nishida H.: Japan Analyst *18*, 1451 (1969).
5. Nishida H., Horiuchi Y.: Japan Analyst *17*, 1519 (1968).
6. Savvin S. B., Chernova R. K., Kudryavtseva L. M.: Zh. Anal. Khim. *34*, 66 (1979).
7. Kubáň V.: Chem. Listy *74*, 862 (1980).
8. Mouková N., Kubáň V., Sommer L.: Chem. Listy *73*, 1106 (1979).
9. Kubáň, Sommer L., Havel J.: This Journal *40*, 604 (1975).
10. Sommer L., Langová M., Kubáň V.: Scripta Fac. Sci. Nat. Univ. J. E. Purkyně Brně, Chemia 1, *8*, 13 (1978).

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