THE OPTICAL AND ACID-BASE PROPERTIES OF CHROMATOGRAPHICALLY PURE CHROMAZUROL S, ERIOCHROMAZUROL B AND ERIOCHROMCYANINE R

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The positions of the absorption maxima and the values of the molar absorption coefficients of all the acid–base forms and the dissociation constants of the individual acid–base transitions of chromatographically pure Chromazurol S, Eriochromazurol B and Eriochromcyanine R were found by graphical and numerical interpretation of the absorbance curves measured in aqueous medium and mixed water–ethanol and water–DMF medium. These values were compared with the values given in the literature.

Hydroxytriphenylmethane dyes with salicylic acid functional analytical groups have been found useful in analytical practice as sensitive spectrophotometric reagents for a large number of metal ions. The importance of this group of organic reagents has increased considerably as a result of the notable effect of surface active substances — tensides — on the acid–base behaviour of these reagents and on the optical properties of their metal chelates.

Most commercial preparations of hydroxytriphenylmethane dyes contain, in addition to the active component of the reagent, a number of initial substances, intermediates, final products of the synthesis and inorganic salts (NaCl, Na_2SO_4, *etc.*). The individual preparations are very different in their physical, chemical, optical and complexing properties. Various degrees of reagent contamination can thus explain the differences in the acid–base characteristics of the reagents, in the composition, optical properties and stability constants of their chelates with metal ions and in the basic parameters of the spectrophotometric methods.

The requirement of high purity of the substances used for spectrophotometric study of complexing equilibria and exhaustive knowledge of the optical and acid–base properties of the reagent in the pure form has led to a search for sufficiently efficient purification methods and for good purity control methods. Elemental analysis, potentiometric titrations[1] and paper[1,2] or thin-layer chromatography[2,3] were used to determine the content of the active component and to check the purity of the hydroxytriphenylmethane dyes. The purification was most frequently carried out employing the different solubilities of the reagents and impurities in various organic solvents[4–6], the low solubility of the molecular form of the reagent in acid medium[2–15] or preparative chromatography on paper or on a cellulose column[2].

Next to preparative chromatography, the most effective method of purification is repeated extraction of the molecular form of the reagent into organic solvents combined with its precipitation in acid medium[3], while simple methods using only the decreased solubility of the mole-

cular forms of the reagent or different solubility of the reagent and impurities are not suitable for preparations containing a greater number of derivatives (Chromazurol S preparations containing Eriochromazurol B).

This work describes the purification of Eriochromcyanine R, Chromazurol S and Eriochromazurol B and the separation of Chromazurol S and Eriochromazurol B by preparative thin-layer chromatography or by combination of precipitation of the molecular form of the reagent and its subsequent extraction into alkyl ethers. The basic optical and acid–base properties were determined for the pure substances prepared in this way.

EXPERIMENTAL AND RESULTS

Chemicals and Instruments

The substances Chromazurol S (2″,6″-dichloro-3″-sulpho-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, $C_{23}H_{16}Cl_2O_9.2\,H_2O$, M_r 575·382, designated CAS) from Geigy (Basel, Switzerland), Lachema (Brno, Czechoslovakia), Merck (Darmstadt, GFR) and ICN Pharmaceuticals Inc. (New York, USA), Eriochromazurol B (2″,6″-dichloro-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, $C_{23}H_{16}Cl_2O_6$, M_r 459·042, designated CAB) from Geigy Dyestuffs Div. (Canada), ICN Pharmaceuticals Inc. (New York, USA) and ACNA (Italy), Eriochromcyanine R (2″-sulpho-3,3′-dimethyl-4-hydroxyfuchson-5,5′-dicarboxylic acid, $C_{23}H_{18}O_9S.2\,H_2O$, M_r 506·492, designated ECR) from Geigy (Basel, Switzerland) and Lachema (Brno, Czechoslovakia) and Eriochromgeranol (3,3′,3″-trimethyl-4′,4″-dihydroxyfuchson-5,5′,5″-tricarboxylic acid, $C_{25}H_{20}O_9$, M_r 464·43, designated ECG) from Geigy (Basel, Switzerland) were studied. The stock reagent solutions with a concentration of $1 \cdot 10^{-4}$—$1 \cdot 10^{-3}$M were prepared by dissolving a weighed amount of the substance, equilibrated in the air, in 0·1M-KOH or NH_4OH, ethanol or dimethylformamide (DMF).

The remaining chemicals were of *p.a.* purity (EDTA, KOH, KNO_3) or *p.p.* purity (HNO_3, HCl, NH_4OH); the solvents of *p.a.* purity (ethanol containing 5% v/v methanol, DMF) were purified by distillation. Potassium hydroxide *p.a.* was purified by coprecipitation of the non-amphoteric hydroxide of heavy metals on barium(II) carbonate. The ionic strength of the solution was maintained at a constant value of I 0·10 by mixing suitable volumes of HNO_3, KNO_3 and KOH. Water was distilled twice in a Bi-18 Destamat® quartz apparatus (Heraus Quarzschmelze, GFR).

The chromatographic purity of the reagents was controlled on Silufol® (Kavalier, Czechoslovakia) strips (15 × 2·5 cm) or foils (20 × 20 cm) impregnated by ascending development with 0·01—0·05M-EDTA, after prior washing out of organic impurities by ascending development with chloroform to overflow. The commercial CAS, CAB, ECR and ECG substances as the sodium salts were applied at the start in amounts of 15—20 μg as 0·5% solutions. The purified free acids were applied at the start in amounts of 5—10 μg ECR or 15—20 μg CAS, CAB or ECG as 0·25% alkaline aqueous (CAS, ECR) or ethanolic (CAB, ECG, ECR) solutions or as solutions in 50% v/v ethanol and in pure ethanol (ECR). The solutions were applied by repeated application in *c.* 1 cm strips.

Purified[16] silica gel SG 41 (Whatman, Great Britain) with a grain size of 5—20 μm (26 g silica gel in 50 ml of water per plate) or Silpearl Extra Pure (Kavalier, Czechoslovakia) was used to prepare poured or sprinkled layers without a binder for preparative TLC chromatography

on glass plates (20 × 20 × 0·15 cm). The poured layers were dried freely in the air, activated for 60 minutes at 110°C and deactivated for 4 h in the air. The sprinkled preparative layers and Silufol[R] foils, 30 min activation only, were treated similarly.

The chromatograms were developed in Desaga cuvettes (Heidelberg, GFR) in a saturated or unsaturated chamber with 70 ml (preparative layers), 50 ml or 35 ml (Silufol[R] foils) of the elution system or in extraction ground-glass test tubes 29/32 with 5 ml (Silufol[R] strips) of n-butanol–glacial acetic acid–water (7 : 1 : 3, 7 : 1 : 5, 6 : 1 : 2 or 4 : 1 : 5), isopropanol–ammonia–water (8 : 1 : 2) or diethyl ether–glacial acetic acid (30 : 1) (ref.[14]).

The spectrophotometric measurements were carried out by a titration technique at 25°C in 10—40 mm cuvettes on a digital double-beam Superscan[R] 3 spectrophotometer (Varian, Switzerland) controlled by an HP 9815A desk-top calculator (Hewlett–Packard, USA) using programs for direct collection and treatment of the experimental data[17]. The solution acidity was measured using a digital PHM 64 meter (Radiometer, Denmark) with a glass G 202 B electrode and saturated calomel K 401 electrode. The instrument was regularly calibrated with phosphate buffer (pH 6·48 at 25°C) before and after the titration and was standardized once a week with a set of tetraoxalate (pH 1·68), phthalate (pH 4·01) and tetraborate (pH 9·18) buffers. The values for mixed water–ethanol or water–DMF media were not corrected and are designated by the symbol pH for simplicity.

Methods

The absorption spectra in dependence on the acidity of the medium $A = f(\lambda, \mathrm{pH})$ were recorded graphically in steps of $\Delta\mathrm{pH} \sim 0{\cdot}3$—$0{\cdot}5$ or $\Delta(-\log c_H) \sim 0{\cdot}5$ over a wavelength interval of 350 to 750 nm. The position of the isosbestic points, characterizing the individual acid–base transitions of the reagent and the position of the absorption maxima of the absorption bands of all the acid–base forms of the reagent were found from the recording and the optimal wavelength values for digital spectra recording and for measurement of the absorbance-pH curves were chosen from the graphical recording of the absorption spectra.

The absorption spectra in digital form were recorded in the acidity region for the existence of a single acid-base form of the reagent for 50—150 discrete wavelength values in the interval 350—650 nm with steps of $\Delta\lambda = 2$—10 nm. The exact position of the absorption band maxima, the position of the inflection points on the curves and the overlapping of the absorption bands were found from the digital recording using a program for calculation of the first and second derivative $\mathrm{d}A/\mathrm{d}\lambda$ and $\mathrm{d}^2A/\mathrm{d}\lambda^2$ (ref.[17]).

The values of the dissociation constants and molar absorption coefficients were calculated from the absorbance-pH curves measured for 20—25 discrete wavelength values in the region of maximum absorbance of the individual acid-base forms of the reagent in steps of $\Delta\ \mathrm{pH} \sim 0{\cdot}2$ to $0{\cdot}3$ or $A - \log c_H \sim 0{\cdot}5$, by graphical interpretation using the slope-intercept transformations[18] and numerical treatment by the adjusted PRCEK I program (ref.[19,20]). The results were complemented by values found by the general KANKARE minimization program[21,22]. The number of species absorbing in a given pH interval was found by matrix analysis of the spectra for 15—20 wavelengths and 10—15 acidity values, uniformly distributed over the studied pH interval, using the RANKANAL program[23].

TLC *Control of the Reagent Purity*

All commercial Chromazurol S preparations contain, in addition to the principal active component CAS, various concentrations of the unsulphonated analogue, Eriochromazurol B, in concentrations of 2—11% and at least one zone corresponding

to synthesis side products (Fig. 1). Commercial CAB preparations are generally chromatographically pure; only the product of Geigy Dyestuffs contained a blue-green oxidation product of the synthesis which was identical with the substance causing the blue-green colour of an alkaline solution of CAB in 30% DMF after prolonged standing in the air.

The commercial Eriochromcyanine R preparations were separated in acidic and ammoniacal systems into two differently intense spots with the same colour. Separation into two zones was observed using Silufol^{R} and Silufol Extra Pure^{R} (Kavalier, Czechoslovakia) foils impregnated with 0·05M-EDTA and unimpregnated Silufol Extra Pure^{R} foils in an ammoniacal system, while no separation was observed or it was very incomplete using unimpregnated Silufol Extra Pure^{R} foils in acidic elution systems (Fig. 2). The upper zone is coloured more slowly than the lower zone,

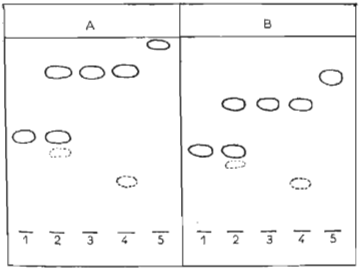

FIG. 1

The TLC Chromatograms for Chromazurol S, Eriochromazurol B and Eriochromgeranol on Silufol^{R} Foils Impregnated with 0·05M-EDTA

A: Unsaturated chamber, B: saturated chamber, elution system: n-butanol–glacial acetid–water 7 : 1 : 3. *1* Purified CAS, 2 commercial CAS substance, *3* purified CAB, *4* commercial CAB, *5* commercial ECG; the purple CAS and blue-green (CAB) zones of impurities in the commercial CAS and CAB are designated by a dotted line.

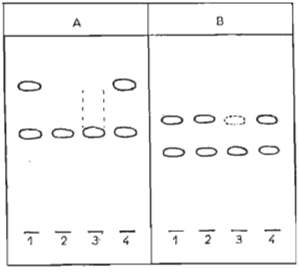

FIG. 2

TLC Chromatograms of Eriochromcyanine R on Silufol^{R} Foils Impregnated with 0·05M-EDTA and Developed in an Unsaturated Chamber in an n-Butanol–Glacial Acetic Acid–Water System 7 : 1 : 3 (A) or Isopropanol–Ammonia–Water 8 : 1 : 2 (B) System

1 Commercial ECR (Lachema), aqueous solution, 2 commercial ECR (Lachema), alkaline aqueous solution, *3* purified ECR (Geigy), alkaline aqueous solution, *4* purified ECR (Geigy), solution in 50% v/v or 96% v/v ethanol.

especially in ammoniacal medium, where zone colouration takes 24 h or more. After elution of the zone and repeated chromatographic development, even with two-dimensional chromatography immediately after development in one direction or after drying and exposure of the chromatogram to the air for several days, the two spots always separated into two new zones with the same R_F values (Table I).

The purified free acid ECR (Geigy) applied in an aqueous alkaline solution separates after chromatographic development in an ammoniacal elution system into a very intense lower zone and a less intense upper zone. The zone intensity of the purified substance dissolved in 50% or 96% ethanol is opposite in the ammoniacal elution system.

In acid elution systems the lower zone is more intense and the upper zone is a poorly defined smear, when an alkaline aqueous solution is applied to the start. For substances dissolved in 50% or 96% ethanol, the intensity of the upper zone is the same or greater than that of the lower zone (Fig. 2*A*).

The results obtained on Silufol Extra Pure^R foils impregnated with 0·05M-EDTA are in agreement with the separation on Silufol^R impregnated with EDTA. Either separation of ECR into two zones did not take place or was incomplete, forming smears, on Silufol Extra Pure^R foils not impregnated with EDTA in acid elution systems. In an ammoniacal system on unimpregnated Silufol Extra Pure^R foils, the separation into two zones was the same as the separation on Silufol^R.

The presence of two spots does not reflect the presence of impurities in the reagent, but corresponds to the presence of two structural forms of the reagent – the planar form with an open ring and a deformed form with a closed sulphone ring. The sulphone form of the reagent is formed primarily in media buffered with EDTA (pH ~5) and in weakly alkaline media. This less polar sulphone form has a higher R_F value. The concentration ratio of the two forms changes in dependence on the character of the elution system and method of preparing the solution applied at the start. The sulphone form is stabilized by the presence of ethanol. On drying the silica gel, the colourless sulphone form changes into the intensely coloured LH_2^{2-} form of the reagent, in dependence on time, so that after a certain time two intensely coloured zones appear on the chromatogram. The sulphone form is not formed in acid or alkaline medium.

The commercial Eriochromgeranol preparation is chromatographically pure (Fig. 1, position 5).

Preparation of the Chromatographically Pure Reagents

In the preparation of very pure CAS, CAB, ECR and ECG substances by preparative chromatography, an amount of 10–12 mg of the reagent in the form of a 0·5 to 1·0% aqueous solution was applied at the start of the poured layer. The chromatograms were developed in an n-butanol–glacial acetic acid–water 7 : 1 : 3 or 7 : 1 : 5

system to a front distance of 18 cm. At the start of the sprinkled layers was applied 10–12 mg of CAB as a 0·5% aqueous solution and the chromatograms were developed in a diethyl ether–glacial acetic acid 30 : 1 system to a front distance of 18 cm.

CAS or ECR was extracted from the separated zone into water, the silica gel was centrifuged off at 5000 rpm and the chromatographically pure CAS was precipitated with hydrochloric acid, dried in the air and over solid KOH and equilibrated in the air. The content of the active component in the $LH_4^0.2\,H_2O$ form was 80·02% (elemental analysis) or 74·83% (spectrophotometry[3]). The yield was about 20% of the commercial substance.

CAB was extracted from the separated zone with diisopropylether, precipitated from the organic layer by excess water (1 : 5), filtered off on a S3 frit and the chromatographically pure CAB substance was dried over solid KOH and equilibrated in the air. The CAB content in the LH_3^0 form was 98·25% CAB (elemental analysis) or 100·00% CAB (spectrophotometry[3]). The yield was about 20% of the commercial substance.

In chemical purification of the commercial CAS substance containing 2–11% CAB, the different solubilities and extractabilities of the molecular forms of CAS and CAB at pH 3 and pH ~0 were employed. The molecular form of CAB was isolated from an aqueous solution of the sodium salt of CAS (5 g in 30 ml) acidified with HCl to pH ~3 by repeated extraction of LH_3^0 with 50 ml ether (5–7 times) or ether saturated with HCl to a pale yellow colour of the organic phase. The pure

TABLE I

R_F Values for the CAS, CAB, ECR and ECR Substances

Substance	R_F	
	acid elution system	ammoniacal system
CAS	0·40—0·46[a] 0·45—0·59[b]	0·40[a] 0·51[b]
CAS[c]	0·30—0·42[a] 0·40—0·50[b]	0·35[a] 0·39[b]
CAB	0·65—0·72[a] 0·82—0·91[b]	0·50[a] 0·69[b]
CAB[d]	0·24—0·28[a] 0·26—0·34[b]	0·72[a] 0·82[b]
ECR, upper zone	0·55—0·57[a] 0·74—0·76[b]	0·42[a] 0·56[b]
ECR, lower zone	0·39—0·42[a] 0·50—0·55[b]	0·35[a] 0·40[b]
ECG	0·79—0·81[a] 0·97—1·00[b]	0·45[a] 0·59[b]

[a] Saturated chamber; [b] unsaturated chamber, [c] purple zone of commercial CAS, [d] blue-green zone of the oxidation products of the synthesis (only for the product from Geigy Dyestuffs Div. Canada)

CAS which remains in the aqueous phase in the form of the LH_3^- anion was precipitated from the solution as the molecular LH_4^0 form by acidification of the solution with HCl to a final HCl concentration of 1·5–2M. The CAS precipitate was filtered off on an S3 frit, washed several times with 25 ml of 2M-HCl and 10 ml water. The chromatographically pure crystalline CAS was dried over solid KOH and equilibrated in the air. The content of the active component in the $LH_4.2\,H_2O$ form was 92·41% CAS (elemental analysis) or 100·00 ± 0·22% CAS (spectrophotometry[3]) and the yield of the purification method was 30–40% based on the commercial substance.

The aqueous solution of the sodium salt of CAB (5 g CAB in 30 ml water) was filtered through an S3 frit and the molecular form of CAB was precipitated by acidifying the solution with hydrochloric acid to pH ~3. The CAB precipitate was extracted with the same volume of diethyl ether and the coprecipitated impurities were back extracted 3–4 times with the same volume of water. Chromatographically pure CAB was precipitated from the organic phase in the crystalline form by excess water (1 : 5) and filtered off on an S3 frit or isolated by slow evaporation of the diethyl ether, rinsed with water of pH ~3, dried over solid KOH and equilibrated in the air. The content of the active component as LH_3 was 97·46% CAB (elemental analysis) or 100·00 ± 1·13% (spectrophotometry[3]) with a yield of 30–40% (related to the commercial substance).

The aqueous solution of the ECR salt (2 g in 30 ml) was filtered through an S3 frit and the molecular LH_4^0 form was precipitated by acidification of the solution with hydrochloric acid to a final concentration of 1·5–2·0M-HCl, the precipitate was separated on an S3 frit and washed twice with 25 ml of 2M-HCl and 10 ml water. The precipitate was dissolved in 100 ml chloroform and the pure ECR was back-extracted twice with 100 ml water. The ECR solution was again acidified with HCl to a final concentration of 2M-HCl and the precipitate was again extracted into 100 ml chloroform. The chromatographically pure substance was isolated in the crystalline form by slow evaporation of $CHCl_3$, dried over solid KOH and equilibrated in the air. The content of the active component as $LH_4^0.2\,H_2O$ was 98·79% ECR (elemental analysis) with a yield of 40% (related to the commercial substance).

Acid–Base and Optical Properties of CAS, CAB *and* ECR

The optical characteristics (absorption maximum, molar absorption coefficient, position of the isosbestic points, *etc.*) and the acid–base characteristics (pK_{ai}) were found by measuring a series of absorbance-pH curves and absorption spectral curves of approx. $1 . 10^{-5}$M aqueous solutions of CAS, CAB and ECR in variously concentrated acid media, $c_{H_2SO_4} = 1-17$M without considering the ionic strength of the solutions. The absorption spectra and absorbance-pH curves of $1·0-5·0 . 10^{-5}$M aqueous solutions of Chromazurol S and Eriochromcyanine R and $1·0-4·0 . 10^{-5}$M

solutions of the less soluble unsulphonated Eriochromazurol B in media of 50% v/v ethanol and 30% v/v DMF were measured in the pH interval 1–14 at constant ionic strength I 0·10. In the region of transition of the anionic and sulphone forms of the reagent, in the pH interval 3·5–6·0, the absorbance-pH curves of Eriochromcyanine R were measured in the classical way in volumetric flasks. The absorbance for the individual pH values was measured at time intervals of 3, 5, 10 and 15 minutes and the final absorbance was found by graphical extrapolation of the $A = f(\tau)$ dependence for $\tau = 0$. To mask possible traces of metallic impurities, all the measurements were carried out in the presence of $0{\cdot}5-1{\cdot}0 \cdot 10^{-3}$M-EDTA.

A survey of the optical characteristics of the individual acid–base forms of CAS, CAB and ECR and the dissociation constants for the individual acid–base equilibria together with the literature data is given in Tables II and III.

The sharp isosbestic points in the individual acidity regions of the medium, numerical interpretation of the absorption spectra and matrix analysis of the absorption spectra by the RANKANAL program indicate that, in dependence on the acidity, there are 6 or 5 acid–base forms of the reagent in solutions of CAS and ECR or CAB. A scheme of the individual acid–base equilibria is given in Fig. 3.

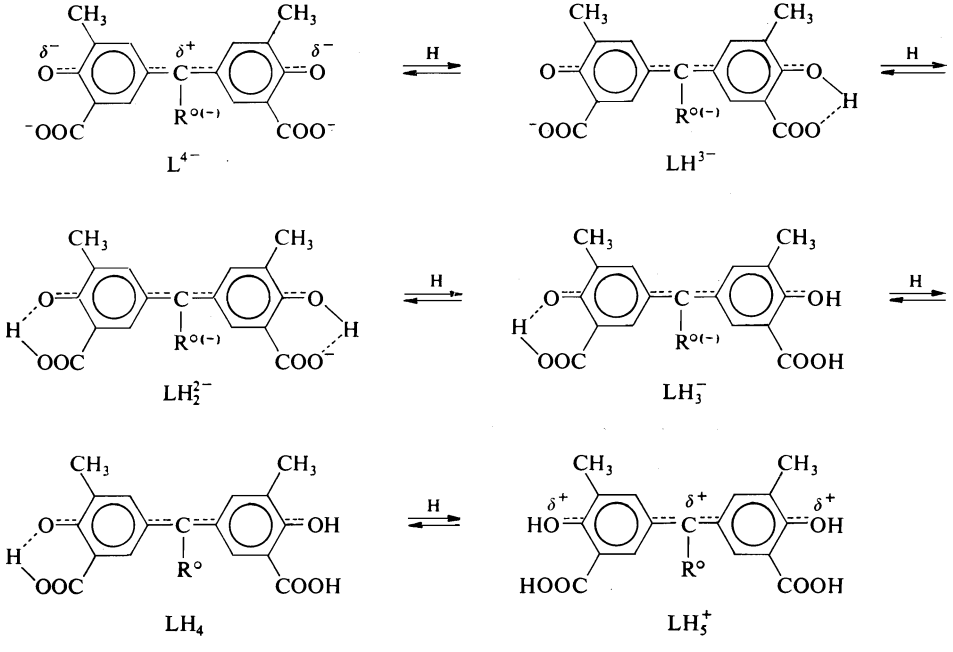

FIG. 3

Scheme of the Dissociation Equilibria of Chromazurol S and Eriochromcyanine R (R^-) and Eriochromazurol B (R°)

TABLE II

Optical Characteristics of Chromazurol S, Eriochromazurol B and Eriochromcyanine R
$c_L = 1{\cdot}0—5{\cdot}0 \,.\, 10^{-5}$M CAS a ECR, $c_L = 1{\cdot}0—4{\cdot}0 \,.\, 10^{-5}$M CAB, $c_{EDTA} = 0{\cdot}5—1{\cdot}0 \,.\, 10^{-3}$M, $I = 0{\cdot}10(HNO_3 + KOH)$, $t = 25{\cdot}00 \pm 0{\cdot}05$°C

Form	L^{4-}	LH^{3-}	LH_2^{2-}	LH_3^-	LH_4^0	LH_5^+	?	ref.[b]
				Chromazurol B				
pH	>12·5	6·5—10·0	3·0—3·7	0—1	−1 to +1	1—15M	>15M	
λ_{max} (nm)[a]	599 (7·79)	427 (2·09)	493 (2·04)	468 (2·17)	468 (2·17)	541 (7·99), 399	545, 399	this work
	598 (5·53)	429 (—)	499 (1·49)	469 (—)	541 (5·30)	545 (5·30), 399	—	5
	598 (7·56)	430 (2·24)	490 (2·24)	466 (2·30)	466 (2·30)	542 (7·80)	—	6
	600 (2·06)	430 (1·00)	490 (1·02)	465 (1·04)	—	545 (2·66)	—	26
	600 (2·30)	430 (1·50)	490 (1·30)	465 (1·25)	—	545 (2·50)	—	28
	598	427	492	463	542	542	—	4
	590	430	500	480	480	—	—	24
	595	425	490	465	—	—	—	3
	600 (1·86)	430 (0·66)	500 (0·79)	470 (0·80)	—	—	—	25
		427 (2·10)						14
				Eriochromcyanine R				
pH	>12·5	6—10	2·8—4·0	0—1·2	−1 to +1	1—15M	>15M	
λ_{max} (nm)[a]	586 (5·96)	439 (2·05)	519 (2·33)	478 (1·94)	478 (1·98)	510 (7·87), 398	522, 398	this work
	584 (3·85)	444 (—)	517 (1·33)	476 (—)	476 (—)	510 (5·99), 398	522, 398	5
	585 (1·92)	445 (—)	520 (1·14)	480	480	510		10
				Eriochromazurol B[f]				
pH	>12·5	5·6—10·0	3·4—3·9	0—1	1—15M	15M		this work[c]
λ_{max} (nm)[a]	599 (6·15)	428 (2·10)	508 (2·19)	460 (2·19)	530 (6·14)			this work[c]
			580 (0·94)					this work[c]
			518 (2·05)	462 (2·19)	533 (6·24)			this work[d]
			575 (1·48)					this work[d]
	595 (4·60)	430 (1·35)	490 (1·35)	470 (1·44)	530 (4·60)			26[e]
		430 (2·06)						10

[a] The values of the molar absorption coefficient at the absorption maximum λ_{max} $mmol^{-1}$ cm^2 are given in brackets, [b] for experimental conditions, see Table III, [c] 20% v/v ethanol medium, [d] 30% v/v DMF medium, [e] aqueous medium, [f] mixed water–ethanol or water-DMF medium.

TABLE III

Dissociation Constants $pK_{ai} \pm 3\sigma$ for Chromazurol S, Eriochromcyanine R and Eriochromazurol B
$c_L = 1{\cdot}0$—$5{\cdot}0 \,.\, 10^{-5}$M CAS and ECR, $c_L = 1{\cdot}0$—$4{\cdot}0 \,.\, 10^{-5}$M CAB, $I = 0{\cdot}10$ (HNO_3 + KOH), $t = 25{\cdot}00 \pm 0{\cdot}05$°C

Reagent	pK_{a1} (LH/L)	pK_{a2} (LH_2/LH)	pK_{a3} (LH_3/LH_2)	pK_{a4} (LH_4/LH_3)	pK_{a5} (LH_5/L_4H)	I	t, °C	ref.
CAS	11·79 ± 0·03	4·87 ± 0·01	2·27 ± 0·01	0·07 ± 0·10	—1·9	0·10 KNO_3	25	this work[a,f]
	11·78 ± 0·02	4·89 ± 0·02	2·27 ± 0·01	—	—2·0	0·10 KNO_3	25	this work[b,f]
	11·79 ± 0·02	4·88 ± 0·02	2·37 ± 0·01	—	—4·8	0·10 $NaClO_4$	18—22	5
	11·81 ± 0·03	4·71 ± 0·03	2·25 ± 0·05			0·10 KCl	20	4, 27
	11·75 ± 0·05	4·88 ± 0·05	2·25 ± 0·10	—1·2 ± 0·4	—	0·10 $NaClO_4$	25	13
	11·52	4·73	2·28	0·4	—2·2	0·10		6
	11·86, 11·88	4·73	2·24	—	—	0·02 $NaNO_3$	?	31[c]
	11·93, 11·94	4·92	2·07	—	—	0·02 $NaNO_3$	?	31[d]
	11·47	4·86	2·45	—	—	0·2		24
	12·21	4·92	2·27	—	—	0·10	20	25
	12·4	5·0	2·3		—2			26
	11·8	4·7	2·3					29
	12·34	4·68	2·9			0·5		28
ECR	11·80 ± 0·06	5·44 ± 0·05	2·26 ± 0·03	0·10 ± 0·10	—2·0	0·10 KNO_3	25	this work[a,f]
	11·83 ± 0·05	5·46 ± 0·05	2·25 ± 0·03	—	—2·0	0·10 KNO_3	25	this work[b,f]
	11·85 ± 0·01	5·47 ± 0·05	2·23 ± 0·01	—	—4·9	0·10 $NaClO_4$	18—22	5
	11·83 ± 0·04	5·74 ± 0·04	1·83 ± 0·02	—	—			10
CAB[e]	12·00 ± 0·10	6·07 ± 0·03	2·68 ± 0·04	—	—2·0	0·10 KNO_3	25	this work[a,f]
	11·95 ± 0·10	6·10 ± 0·05	2·62 ± 0·05	—	—2·0	0·10 KNO_3	25	this work[b,f]

[a] Numerical treatment by the KANKARE program for 20—25 discrete wavelength values in the interval 350—650 nm, [b] numerical treatment by the PRCEK HP program for 8—10 wavelength values in the interval 450—550 nm (pH 1—8) or 400—600 nm (pH 8—13), [c] commercial substance from Fluka, [d] substance from Fluka purified by a combination of precipitation and extraction methods see the procedure for purifying CAS), [e] 50% v/v ethanol medium, [f] $\sigma = 1/2 \,.\, \log((K + \sigma K)/(K - \sigma K))$.

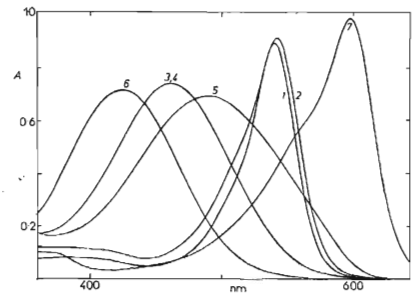

FIG. 4

Curves of the Absorption Spectra of the Individual Acid–Base Forms of Chromazurol S

$c_L = 5{\cdot}52 \cdot 10^{-6}$M, $c_{EDTA} = 5{\cdot}0 \cdot 10^{-4}$M, $I = 0{\cdot}10$ (HNO_3, KOH), $l = 40$ mm, $t = 2500{\cdot}°C$. Curve form pH: *1* LH_5^+ conc. H_2SO_4, *2* LH_5^+ 15M-H_2SO_4, *3* LH_4^0 1·12, *4* LH_3^- 1·12, *5* LH_2^{2-} 3·72, *6* LH^{3-} 8·60, *7* L^{4-} 1M-KOH.

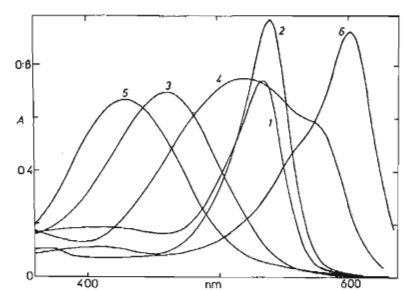

FIG. 5

Curves of the Absorption Spectra of the Individual Acid–Base Forms of Eriochromazurol B

$c_L = 7{\cdot}95 \cdot 10^{-6}$M, $c_{EDTA} = 5{\cdot}0 \cdot 10^{-4}$M, $I = 0{\cdot}10$ (HNO_3, KOH), $l = 40$ mm, $t = 25{\cdot}00°C$. Curve form pH: *1* LH_4^+ conc. H_2SO_4, *2* LH_4^+ 15M-H_2SO_4, *3* LH_3^0 1·07, *4* LH_2^- 4·02, *5* LH^{2-} 9·10, *6* LH^{3-} 1M-KOH.

The shapes of the absorption spectra of the individual acid–base forms are evident from Figs 4–6.

Gradual association of protons on the hydroxyl group with partial negative charge results in transition of the completely symmetrical anionic blue-purple $L^{4-}(L^{3-})$ form into the yellow unsymmetrical anionic $LH^{3-}(LH^{2-})$ form with marked quinoid and phenolic character of the oxygen atoms. Bonding of a further proton to the carboxyl group of the benzene nucleus with the quinoid oxygen produces the symmetrical red anionic form LH_2^{2-} (LH_2^-) with two equivalent hydrogen bonds. This acid–base form is converted by association of a further proton with the second carboxyl group to the orange unsymmetrical $LH_3^-(LH_3^0)$ form, which then contains a grouping of carboxyl group with a quinoid oxygen on one benzene ring and a grouping of undissociated carboxyl group next to a phenolic oxygen on the other benzene ring. These two analytically important groupings then determine whether the metal ion–reagent interaction results in dissociation of one or two protons.

Protonation of the quinoid oxygen atom in concentrated acid medium leads to formation of orange-red cationic species LH_5^+ (LH_4^+). Protonation of the sul-

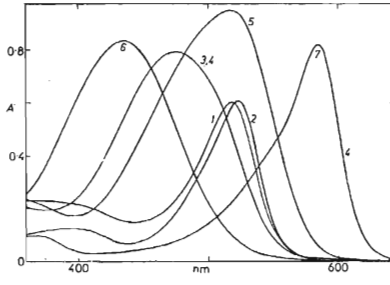

FIG. 6

Curves of the Absorption Spectra of the Individual Acid–Base Forms of Eriochromcyanine R after Elution from the TLC Chromatogram (lower zone)

$c_{EDTA} = 5{\cdot}0 \cdot 10^{-4}$M, for the other conditions, see Fig. 4, $l = 10$ mm. Curve, form pH: *1* LH_5^+ conc. H_2SO_4, *2* LH_5^+ 15M-H_2SO_4, *3* LH_4^0 2M-H_2SO_4, *4* LH_3^- 1·10, *5* LH_2^{2-} 3·72, *6* LH^{3-} 9·24, *7* L^{4-} 1M-KOH.

pho groups of Chromazurol S and Eriochromcyanine R proceeds without a marked colour effect and without a change in the value of the molar absorption coefficients in the pH interval pH = −1 to +1. This acid–base equilibrium partially overlaps with deprotonation of the carboxyl group, which occurs in acid media at pH 1–3.

For Eriochromcyanine R, these acid–base equilibria are connected with a decrease in the absorbance in the pH interval 3·5–6·0 in the region of maximum absorption of the LH_2 or LH form. The changes in the absorbance are probably connected with transition of the LH_2^{2-} form to a structure with a closed sultone ring, which is colourless or absorbs only in the near UV region. These two structural forms also behave differently during chromatographic separation on thin layers of silica gel in various elution systems. With Chromazurol S the sulfone ring is not closed because of the steric screening of the sulpho group by the two chlorine atoms in the 2 and 6 positions. The structural transition between the anionic LH_2^{2-} species and the sultone form of ECR in this pH region is slowed down by the presence of cationic tensides, which block the sulpho group by formation of the $LH_3^-.T^+$ or $LH_2^{2-}.2T^+$ ion associate at submicelle concentrations of tenside or by bonding of the reagent molecule to the tenside micelles, $LH_3^-.nT^+$ or $LH_2^{2-}.nT^+$ in the region of critical and supercritical micelle concentrations of the tenside[32].

Eriochromazurol B in the LH_3^0 form is poorly soluble in acid media and requires the presence of at least 50% ethanol or 30% v/v DMF. Mixed media can successfully be replaced by the presence of submicelle or micelle concentrations of tensides[32], both in the study of acid–base equilibria and in the study of complexing equilibria of this and the above-mentioned reagents.

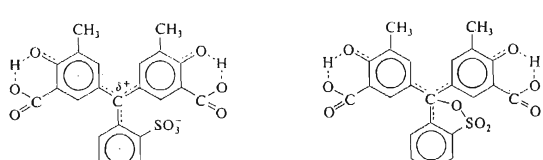

FIG. 7

Transition of the Anionic Forms LH_2^{2-} and Sulphone Forms of Eriochromcyanine R in the pH Interval 3·5—6·0

REFERENCES

1. Sommer L., Kubáň V.: This Journal *32*, 4355 (1967).
2. Malaník V., Malát M.: Anal. Chim. Acta *76*, 464 (1975).
3. Mouková N., Kubáň V., Sommer L.: Chem. Listy *73*, 1106 (1979).
4. Funasaki W., Ando T., Fujimura K., Janai T.: Jap. Analyst *17*, 482 (1968); ref. in Fresenius Z. Anal. Chem. *245*, 124 (1969).
5. Dixon E. J., Grisley L. M., Sawayer R.: Analyst (London) *25*, 945 (1970).
6. Baldwin W. G., Stranks D. R.: Austr. J. Chem. *21*, 603 (1968).
7. Martynov A. P., Novak V. P., Reznik B. E.: Zh. Anal. Khim. *32*, 519 (1977).
8. Kohara H., Ishibashi N., Fukamashi K.: Jap. Analyst *17*, 1400 (1969).
9. Langmyhr F. J., Stumpe T.: Anal. Chim. Acta *32*, 535 (1965).
10. Suk V., Mikeťuková V.: This Journal *24*, 3629 (1959).
11. Chernova R. K., Bolshakova E. G., Petrova I. K.: Zh. Anal. Khim. *29*, 214 (1974).
12. Adamovich L. P., Timofeyeva I. I., Jucis B. V.: Zh. Obshch. Khim. *30*, 1325 (1960).
13. Banerjee A., Dey A. K.: J. Inorg. Nucl. Chem. *30*, 3134 (1968).
14. Langmyhr F. J., Klausen K. S.: Anal. Chim. Acta *29*, 149 (1963).
15. Garčic A.: Clin. Chim. Acta *94*, 115 (1979).
16. Seiler H., Seiler M.: Helv. Chim. Acta *43*, 1939 (1960).
17. Kubáň V.: Chem. Listy *74*, 862 (1980).
18. Sommer L., Kubáň V., Havel J.: Folia Fac. Sci. Nat. Univ. J. E. Purkyně (Chemia 7) *11*, 1 (1970).
19. Havel J., Kubáň V.: Scripta Fac. Sci. Nat. Univ. J. E. Purkyně (Chemia 2) *1*, 87 (1971).
20. Kubáň V.: Scripta Fac. Sci. Nat. Univ. J. E. Purkyně, (Chemia 2) *2*, 81 (1972).
21. Kankare J. J.: Anal. Chem. *42*, 1322 (1972).
22. Šteflová Z., Havel J.: Unpublished results.
23. Wallace R. H.: J. Phys. Chem. *64*, 899 (1960).
24. Malát M.: Anal. Chim. Acta *25*, 289 (1961).
25. Adamovich L. P.: Morgul-Meshkova O. V., Jucis B. V.: Zh. Anal. Khim. *17*, 678 (1962).
26. Mustafin I. S., Ivanova A. N., Lisenko N. F.: Zh. Anal. Khim. *20*, 17 (1965).
27. Semb A., Langmyhr F. J.: Anal. Chim. Acta *35*, 286 (1966).
28. Tatayev O. A., Bagdasarov K. N.: *Primenenie Organicheskikh Reaktivov v Spektrofotometrii*, p. 35. Izd. Dagestan. Gos. Univ. 1972.
29. Nishida H.: Jap. Analyst *19*, 34 (1970), *20*, 1080 (1971).
30. Lisenko N. F., Mustafin I. S.: Zh. Anal. Khim. *22*, 25 (1967).
31. Evtimova B. E.: Dokl. Bulg. Akad. Nauk *31*, 559 (1978).
32. Burešová I., Kubáň V., Sommer L.: This Journal, in press.

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