DETERMINATION OF DISSOCIATION CONSTANTS OF 7-(CARBOXYPHENYLAZO)-8-HYDROXYQUINOLINE-5-SULPHONIC ACIDS BY NONLINEAR REGRESSION OF SPECTROPHOTOMETRIC DATA*

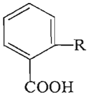
Milan MELOUN and Jaromíra CHÝLKOVÁ

Analytical Chemistry Department,
Institute of Chemical Technology, 532 10 Pardubice

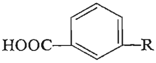
Dissociation constants of three metallochromic indicators 7-(*o*-, *m*-, *p*-carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acids and values of molar absorption coefficients of the forms L^{3-}, HL^{2-}, H_2L^-, H_3L have been determined in 0·1M-$NaClO_4$ medium by non-linear regression of pH-absorbance curves by the program DCLET and by regression matrix analysis of the spectra by the program FA608 + EY608, respectively. All the three derivatives have been synthetized, purified, and their purity checked by chromatography.

The first three communications of this series dealt with protonation[1] and complexation equilibria[2,3] of two 7-arylazo derivatives of 8-hydroxyquinoline-5-sulphonic acid. Busev and Talipova[4] prepared three 7-(carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acids differing in position of the carboxyl groups (*ortho*, *meta*, *para*) and tested these three derivatives as metallochromic indicators for determination of In^{3+} ions. Smolin and coworkers[5] proposed these reagents for use in determination of Ca^{2+} and Sr^{2+} ions. In our laboratory attention was focused to study of complexation equilibria and analytical applications of these three derivatives to a number of metals[6].

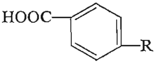
This communication describes determination of dissociation constants and values of molar absorption coefficients of all coloured variously protonated forms of the three 7-(carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acids denoted further as *o*-derivative (*O*), *m*-derivative (M) and *p*-derivative (*P*):



O



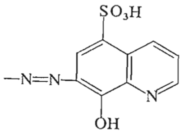
M



P

---

* Part IV in the series Complexation Equilibria of Some Azo Derivatives of 8-Hydroxyquinoline-5-sulphonic Acid; Part III: This Journal *44*, 2032 (1979).



R

EXPERIMENTAL

Chemicals and Solutions

7-(2-Carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acid (*o*-derivative) was prepared by diazotization of *o*-aminobenzoic acid with sodium nitrite in hydrochloric acid medium at 0°C and subsequent coupling of the diazonium salt with 8-hydroxyquinoline-5-sulphonic acid in sodium carbonate medium at 0°C. Purity of the used aminobenzoic acids was checked by liquid chromatography. The prepared indicator was isolated from the reaction mixture by precipitation with acetone and purified by reprecipitation from aqueous solution with acetone. Its purity was checked chromatographically on Silufol plates with pyridine–ethanol (2 : 1) as eluent. The indicator was dissolved in 1M sodium hydroxide and precipitated by acidification with hydrochloric acid. The solutions were standardized by photometric titration with 0·002M-Cu^{2+} salt solution in acetate buffer using the titration device TAL (ref.[7]) connected with a Spekol (Zeiss, Jena) photometer.

7-(3-Carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acid (*m*-derivative) and 7-(4-carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acid (*p*-derivative) were prepared by reverse diazotization of *m*- and *p*-aminobenzoic acids, respectively, in hydrochloric acid medium at 0°C (sodium nitrite) and by subsequent coupling with 8-hydroxyquinoline-5-sulphonic acid in sodium carbonate medium at 0°C. The two aminobenzoic acids are less soluble in water, and their direct diazotization was incomplete and was followed by subsequent reaction with the non-diazotized aminobenzoic acid. Isolation and purification of the indicators was the same as with the *o*-derivative. Their purity was checked by paper chromatography (Whatman 2 impregnated with Chelaton 3, descending arrangement, water–pyridine–ethanol 2 : 1 : 4). Photometric titration with Cu^{2+} salt could not be used for standardization of the *m*- and *p*-derivatives, because the complexes formed are little stable. Therefore, their concentration was determined by acid-base titration using the cell glass electrode–saturated calomel electrode. The indicators contained about 10% impurities mostly of inorganic nature. Solutions of the *o*-, *m*-, and *p*-derivatives in alkaline and neutral media are stable within about 200 h. Acid solutions of the *p*-derivative begin to decolourize after about 20 h due to slow precipitation of the indicator salted out. Copper(II) sulphate, sodium hydroxide, perchloric acid, sodium perchlorate, acetate buffer, and phosphate buffer were prepared from *p.a.* chemicals (Lachema) as it is described in the previous reports[1,2].

Spectrophotometric Measurements

Apparatus, experimental arrangement and all the spectrophotometric procedures used were described in the first communication of this series[1].

Evaluation Methods of Experimental Data

The evaluation procedure of spectral curves by regression matrix analysis with FA608 + EY608 program[8] is described in the first report of this series[1]. The absorbance-pH curves were evaluated by non-linear regression using the DCLET program[9] which represents an extended and improved version of the original JDC-LETAG program[10]. The program DCLET is used simultaneously for testing of the suggested chemical model (*i.e.* number of equilibria and stoichiometry of the coloured particles). The criterion of differentiation is a good fit of the calculated regression curve to the given experimental points. In contrast to the original version of JDC-LETAG, where the fit was only judged from the obtained value of standard deviation of absorbance $\sigma(A) = (U/(n - m))^{1/2}$, where U is the value of sum of squares of residues in the found minimum, n stands for number of points, and m is number of the parameters determined, or it was judged from frequency of sign changes in the residues, in the DCLET program objective statistical analysis of residues is involved. In case of a well proposed chemical model statistical characteristics indicate that the set of residues shows normal Gauss distribution. The normal Gauss distribution is encountered with the set whose first statistical moment $m_{r,1}$ approaches numerically zero, the third statistical moment $m_{r,3}$ equals zero, and the fourth statistical moment $m_{r,4}$ equals 3. Square root of the second statistical moment $m_{r,2}$ is the standard deviation of the set, here it is the standard deviation of absorbance $\sigma(A)$. The definition equations are given elsewhere (Eqs (*25*) to (*28*) of ref.[2]). The Pearson χ^2 test of good agreement is applied according to ref.[11]. It cannot be presumed that with genuine experimental data a distinct Gauss normal distribution of residues will be found after finding the best estimate of parameters, in any case, however, this analysis of residues represents an efficient aid for suggestion of the chemical model.

Computation Technique

The computations were carried out with an EC 1040 (500 k) computer (using the FA608 + + EY608 (82 k after segmentation) program by Kankare[8] and a DCLET (80 k after segmentation) by Meloun and Čermák[9]) in The Computation Centre of UNICHEM Pardubice.

RESULTS AND DISCUSSION

Depending on pH aqueous solutions of 7-(carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acids contain successively the following four absorbing species: L^{3-}, HL^{2-}, H_2L^-, and H_3L. Dissociation of the sulphonic acid group cannot be studied, as all the three derivatives are slightly soluble in strongly acid media. Table I gives positions of maxima of the absorption bands and those of isosbestic points of the variously protonated species.

The protonation equilibria were studied by two experimental techniques. The non-linear regression of absorbance-pH curves combined with pH-photometric titration (technique (A), ref.[1]) was especially used for evaluation of the dissociation constants. Values of molar absorption coefficients depending on wavelengths were obtained for various protonated species especially by matrix analysis of the whole spectra measured by pH-photometric titrations carried out outside the cell (technique (B), ref.[1]).

Distinct differentiation between all the three equilibria is only possible with the *m*-derivative both in the spectral curves and in the absorbance-pH curves (Fig. 1).

The spectral curves show three distinct isosbestic points at 400 nm, 483 nm and 521 nm. At first sight the *o*-derivative behaves spectrophotometrically as a dibasic acid, in the *A*–pH curves there are only two distinct jumps, and the spectra contain two sharp isosbestic points at 450 and 501 nm. The *p*-derivative shows two isosbestic points at 473 nm and 496 nm, but spectra of the solutions of pH 3·2 to 4 do not cross either of these isosbestic points. Similarly *A*–pH curves also show a beginning of a further equilibrium in this pH region.

Current evaluation of the fit involves comparison of magnitude of the residue with standard deviation of absorbance of the used spectrophotometer (s_k), frequency of changes of the sign of the residues. Differentiation between two chemical models is

TABLE I

Positions of Maxima of Absorption Bands and Positions of Isosbestic Points in Spectra of Various Protonated Species of *o*-, *m*- and *p*-Derivatives Read from Spectral Curves Measured at Various pH Values

ortho: c_L 4·41 . 10^{-5}M, pH 2·73 to 11·42, *d* 9·98 mm; *meta*: c_L 4·18 . 10^{-5}M, pH 2·07 to 9·85, *d* 9·98 mm; *para*: c_L 6·60 . 10^{-6}M, pH 1·59 to 8·96, *d* 49·98 mm; *I* 0·1 ($NaClO_4$ + Na_3PO_4 + + $HClO_4$); 25°C.

Form	Positions of absorption bands				Isosbestic points	
	λ_{max} nm	ε_{max}	λ_{max} nm	ε_{max}	λ_{isosb} nm	ε_{isosb}
			ortho			
H_3L	502	18 122	534	17 640		
H_2L^-	510	17 550			>501	17 640
HL^{2-}	490	18 763				
L^{3-}	470	11 105			>460	10 997
			meta			
H_3L	500	19 223	530	20 232	>400	6 364
H_2L^-	500	14 810			>521	14 832
HL^{2-}	490	19 475			>483	19 139
L^{3-}	480	19 321				
			para			
H_3L	505	23 840	535	24 820	>496	22 364
H_2L^-	500	23 204				
HL^{2-}	495	21 500			>473	16 545
L^{3-}	485	17 601				

facilitated by objective analysis of the residues. Table II gives an example of the tested model of three protonation equilibria with all the three studied derivatives. Reliability of the seven calculated parameters ($\mathrm{p}K_{a4}$, $\mathrm{p}K_{a3}$, $\mathrm{p}K_{a2}$, ε_L, ε_{HL}, ε_{H_2L}, ε_{H_3L}) is documented, first of all, by the obtained standard deviation of absorbance $\sigma(A)$ or $m_{r,2}^{1/2}$, which is close in value to the quantity of the used spectrophotometer s_k 0·003 for Spekol and measurements at a constant wavelength. The statistical moments and the Pearson χ^2 test also indicate the Gauss distribution of the residues.

Table III gives a survey of the dissociation constants evaluated by non-linear regression of A–pH curves. With all the three derivatives the model of three dissociation equilibria was tested, and from the statistical characteristics it follows that the model was proved in every case.

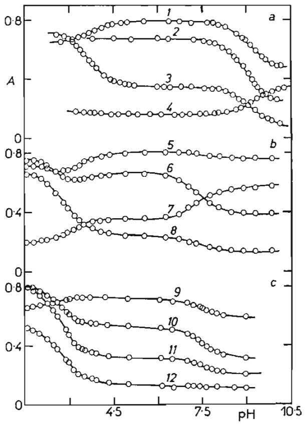


FIG. 1

Absorbance-pH Curves of Solutions of the *o*-, *a m*- *b* and *p*- *c* Derivatives for Various Wavelengths (nm)

1 o,490, *2 o*,515, *3 o*,540, *4 o*,410, *5 m*,490, *6 m*,520, *7 m*, 430, *8 m*,540, *9 p*,490, *10 p*,530, *11 p*,540, *12 p*,550; c_L 4·41 . 10^{-4}M (*o*); c_L 4·18 . 10^{-5}M (*m*); c_L 6·6 . 10^{-6}M (*p*); *I* 0·1 ($NaClO_4$, Na_3PO_4, HClO); *d* 9·98 (*o*, *m*); *d* 49·98 (*p*); 25°C.

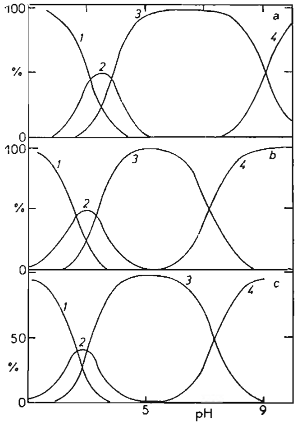


FIG. 2

Distribution Diagrams of Relative Population of Various Protonated Species of the Acid–Base Equilibrium of the *ortho*-Derivative (*a*), *meta*-Derivative (*b*) and *para*-Derivative (*c*) Depending on pH

1 H_3L, *2* H_2L^-, *3* HL^{2-}, *4* L^{3-}.

TABLE II

Evaluation of Dissociation Constants and Molar Absorption Coefficients of Selected *A*-pH Curves of *o*-, *m*- and *p*-Derivatives by DCLET Program

ortho: c_L 6·9 . 10^{-6}M, λ 540 nm, *d* 49·98 mm; *meta*: c_L 4·18 . 10^{-5}M, λ 540 nm, *d* 9·98 mm; *para*: c_L 7·3 . 10^{-6}M, λ 450 nm, *d* 49·98 mm; 25°C, *I* 0·1 ($NaClO_4 + Na_3PO_4 + HClO_4$); χ^2_{theor} (6, 0·95) 12·60; $\Delta A = 10^3(A_{exp} - A_{calc})$.

Position	*ortho*			*meta*			*para*		
No of points	37			36			34		
i	pH	*A*	Δ*A*	pH	*A*	Δ*A*	pH	*A*	Δ*A*
1	2·892	0·687	—5	1·565	0·660	—13	1·633	0·224	—1
2	2·998	0·678	1	1·750	0·666	6	1·734	0·225	0
3	3·065	0·675	9	1·817	0·653	—12	1·872	0·232	1
4	3·150	0·652	1	2·000	0·640	6	1·986	0·235	1
5	3·270	0·622	—5	2·058	0·631	5	2·181	0·244	—1
6	3·373	0·602	—2	2·244	0·593	— 2	2·385	0·260	1
7	3·417	0·595	1	2·500	0·547	4	2·474	0·268	0
8	3·508	0·571	—2	2·550	0·530	— 1	2·599	0·279	1
9	3·685	0·530	0	2·750	0·483	0	2·707	0·288	—2
10	3·773	0·510	0	2·788	0·471	— 3	2·805	0·297	—1
11	3·923	0·480	2	2·956	0·428	— 5	2·909	0·308	0
12	4·188	0·432	1	3·000	0·420	— 2	2·947	0·308	2
13	4·387	0·408	2	3·185	0·382	0	3·089	0·321	0
14	4·507	0·392	—2	3·250	0·366	— 2	3·256	0·334	0
15	4·700	0·378	—2	3·364	0·350	— 3	3·361	0·338	—2
16	4·875	0·372	0	3·518	0·327	4	3·488	0·344	0
17	4·994	0·366	—1	3·772	0·295	3	3·631	0·350	4
18	5·406	0·360	1	4·000	0·273	— 1	3·990	0·357	2
19	6·796	0·350	—2	4·082	0·272	3	5·603	0·357	0
20	7·138	0·350	0	4·369	0·257	1	6·153	0·358	—2
21	7·572	0·342	—3	4·872	0·245	— 1	6·512	0·365	—1
22	7·851	0·338	—1	5·569	0·238	— 3	6·679	0·368	—2
23	8·076	0·335	5	6·266	0·232	— 2	6·866	0·375	—3
24	8·289	0·320	3	6·691	0·222	0	6·999	0·378	—2
25	8·547	0·296	3	6·750	0·220	0	7·170	0·384	0
26	8·761	0·266	—2	7·056	0·205	0	7·239	0·386	1
27	8·886	0·248	—2	7·295	0·192	1	7·303	0·392	3
28	9·075	0·222	1	7·500	0·180	2	7·477	0·400	3
29	9·296	0·187	0	7·740	0·164	— 2	7·609	0·408	3
30	9·429	0·167	0	8·000	0·155	0	7·777	0·414	0
31	9·564	0·150	0	8·072	0·153	0	7·910	0·422	0
32	9·667	0·135	—3	8·250	0·147	— 2	8·145	0·430	1
33	9·779	0·130	3	8·464	0·144	— 1	8·640	0·440	—3
34	9·850	0·120	—1	8·915	0·140	— 1	8·968	0·442	—2
35	9·928	0·115	0	9·316	0·140	1			
36	10·013	0·108	—1	9·855	0·140	— 1			
37	10·101	0·104	0						

TABLE II

Continued

Position	*ortho*	*meta*	*para*
pK_{a4}	9·11 ± 0·00	7·30 ± 0·03	7·49 ± 0·00
pK_{a3}	3·93 ± 0·00	3·31 ± 0·03	2·91 ± 0·00
pK_{a2}	3·24 ± 0·00	2·55 ± 0·00	2·78 ± 0·00
ε_L	2 275 ± 16	3 301 ± 12	12 212 ± 73
ε_{HL}	10 232 ± 30	5 796 ± 23	9 753 ± 27
ε_{H_2L}	15 220 ± 10	9 812 ± 19	8 953 ± 41
ε_{H_3L}	22 090 ± 2	16 785 ± 46	5 973 ± 6
$\sigma(A)$	0·003	0·004	0·002
$m_{r,1}$	$-3{\cdot}13 \,.\, 10^{-5}$	$-2{\cdot}50 \,.\, 10^{-5}$	$1{\cdot}53 \,.\, 10^{-5}$
$m_{r,2}$	0·003	0·003	0·002
$m_{r,3}$	0·819	—1·271	0·297
$m_{r,4}$	5·164	7·594	2·260
$\chi^2_{(6,0{\cdot}95)}$	3·86	4·00	3·62

Table IV gives values of molar absorption coefficients of the species L^{3-}, HL^{2-}, H_2L^- and H_3L at some dominant wavelengths. The values were evaluated by non-linear regression of A–pH curves (DCLET program) and by regression matrix analysis of spectra (FA608 + EY608 program).

Absorbance spectra at various pH were evaluated by the regression matrix analysis (Table V), and, at the same time, the most probable chemical model was also tested. In case of the *m*-derivative, where three equilibria are obvious from mere visual inspection of the spectra, the two-equilibria model needed not to be tested to save time. With the *o*- and *p*-derivatives the model of three equilibria was not so obvious at first sight and, therefore, was tested with the computer. The absorbance standard deviations $\sigma(A)$ are lower for the model of three equilibria. Table IV gives values of molar absorption coefficients depending on wavelength for all the coloured variously protonated species determined by the FA608 + EY608 program.

The distribution diagram in Fig. 2 shows relative population of the various protonated species of the acid–base equilibrium depending on pH.

Table III

Dissociation Constants of *o*-, *m*- and *p*-Derivatives Determined by Non-Linear Regression of *A*-pH Curves with DCLET Program at Various Wavelengths

ortho: c_L 4·41 . 10^{-5}M, *d* 9·98 mm; *meta*: c_L 4·18 . 10^{-5}M, *d* 9·98 mm; *para*: c_L 6·60 . 10^{-6}M, *d* 49·98 mm; *I* 0·1 ($NaClO_4$ + Na_3PO_4 + + $HClO_4$); 25°C, χ^2_{theor} (6; 0·95) 12·60. In case of the Gauss normal distribution the statistical moments have the following values: $m_{r,1} = 0$; $m_{r,2}^{1/2} = 0{\cdot}003$ (for Spekol); $m_{r,2}^{1/2} = 0{\cdot}004$ (for VSU 2G); $m_{r,3} = 0$; $m_{r,4} = 3$.

nm	n	pK_{a4}	pK_{a3}	pK_{a2}	Statistical characteristics					
					$\sigma(A)$	$m_{r,1}$	$m_{r,2}$	$m_{r,3}$	$m_{r,4}$	$\chi^2(6; 0{\cdot}95)$
				ortho						
480	10	9·14 ± 0·03	^{a}	^{a}	0·005	2·86 . 10^{-7}	0·004	−0·308	1·899	4·40
510	10	9·12 ± 0·00	^{a}	^{a}	0·002	5·96 . 10^{-8}	0·002	0·133	1·614	4·40
540	10	9·07 ± 0·04	^{a}	^{a}	0·005	−1·24 . 10^{-6}	0·004	0·709	3·089	2·80
530	10	9·12 ± 0·01	^{a}	^{a}	0·003	1·07 . 10^{-7}	0·002	−0·387	1·430	7·60
420	7	^{a}	3·94 ± 0·00	3·16 ± 0·00	0·006	1·98 . 10^{-6}	0·003	−4·570	2·091	3·29
460	7	^{a}	3·95 ± 0·00	3·26 ± 0·00	0·002	5·15 . 10^{-4}	0·001	1·000	3·040	5·57
480	7	^{a}	3·93 ± 0·00	3·04 ± 0·00	0·004	−7·82 . 10^{-5}	0·002	0·459	1·617	10·1
average		*9·11 ± 0·03*	*3·94 ± 0·01*	*3·15 ± 0·11*						

				meta						
440	45	7·52 ± 0·00	2·99 ± 0·00	2·53 ± 0·00	0·002	—6·49 . 10^{-6}	0·002	—0·095	2·530	4·60
490	36	7·52 ± 0·00	3·34 ± 0·01	2·38 ± 0·00	0·005	1·52 . 10^{-4}	0·005	0·008	4·470	4·44
520	20	7·52 ± 0·01			0·002	—2·71 . 10^{-7}	0·002	—0·076	1·650	5·60
530	45	7·57 ± 0·00	3·29 ± 0·00	2·69 ± 0·00	0·003	5·60 . 10^{-6}	0·003	0·280	2·421	3·53
540	45	7·46 ± 0·00	3·18 ± 0·00	2·37 ± 0·00	0·002	—4·03 . 10^{-5}	0·002	0·219	2·550	5·31
550	27		3·26 ± 0·00	2·28 ± 0·00	0·002	—4·41 . 10^{-6}	0·002	0·057	2·330	5·30
average		*7·52 ± 0·04*	*3·27 ± 0·07*	*2·34 ± 0·06*						
				para						
450	34	7·49 ± 0·00	2·91 ± 0·00	2·78 ± 0·00	0·002	1·53 . 10^{-5}	0·002	+0·298	2·660	3·65
470	14	ε	3·14 ± 0·04	2·79 ± 0·00	0·005	3·78 . 10^{-4}	0·004	—2·739	2·319	3·14
480	19	[a]	2·99 ± 0·00	2·96 ± 0·00	0·002	—2·27 . 10^{-5}	0·002	—0·174	2·116	0·79
490	13	7·46 ± 0·03	[a]	[a]	0·003	—1·47 . 10^{-7}	0·002	0·352	2·199	6·08
530	27	7·48 ± 0·00	3·16 ± 0·00	2·78 ± 0·00	0·005	—1·62 . 10^{-4}	0·005	0·365	2·848	1·74
540	27	7·40 ± 0·00	3·01 ± 0·00	2·79 ± 0·00	0·005	—4·96 . 10^{-4}	0·004	0·004	3·658	1·74
average		*7·46 ± 0·04*	*3·04 ± 0·10*	*2·82 ± 0·08*						

---

[a] The equilibrium does not make itself felt at the wavelength.

TABLE IV

Molar Absorption Coefficients of the Forms L^{3-}, HL^{2-}, H_2L^- and H_3L of the *o*-, *m*- and *p*-Derivatives at Selected Wavelengths Determined by DCLET Program and at Wavelengths of the Whole Spectrum Determined by FA608 + E1608 Program

ortho: c_L 4·41 . 10^{-5}M, d 9·98 mm; *meta*: c_L 4·18 . 10^{-5}M, d 9·98 mm; *para*: c_L 6·60 . 10^{-6}M, d 49·98 mm; I 0·1 ($NaClO_4 + Na_3PO_4 + HClO_4$); 25°C.

λ nm	DCLET				FA608 + EY608			
	ε_L	ε_{HL}	ε_{H_2L}	ε_{H_3L}	ε_L	ε_{HL}	ε_{H_2L}	ε_{H_3L}
				ortho				
390					4 250	1 897	2 954	3 079
400					5 392	1 669	2 354	2 170
410					6 765	2 101	2 288	1 808
420					8 106	3 059	2 473	1 873
430					9 362	4 461	3 407	2 393
440					10 240	6 011	4 467	3 277
450					10 750	8 312	6 099	4 613
460	[a]	11 720 ± 7	8 945 ± 26	7 580 ± 1	11 080	10 960	8 388	6 814
470					11 100	13 380	10 870	9 324
480	10 605 ± 79	16 305 ± 77	13 286 ± 0	11 654 ± 0	10 600	16 440	13 310	11 810
490					9 449	18 760	16 020	14 810
500					7 599	18 020	16 610	18 120
510	5 491 ± 26	17 111 ± 26	[a]	[a]	5 493	17 090	17 550	17 740
520					3 456	16 990	16 900	17 000
530	1 789 ± 43	13 690 ± 43	[a]	[a]	1 819	13 590	16 260	17 640
540					809	6 764	14 290	17 390
550					220	2 182	9 620	12 490
560					46	459	4 002	5 376
				meta				
390					8 013	7 691	6 717	7 664
400					9 096	7 194	6 219	6 374
410					10 490	7 103	5 976	5 239
420					12 050	7 563	6 090	4 648
430					13 570	8 436	6 440	4 648
440					14 870	9 721	7 235	5 283
450	16 327 ± 5	11 786 ± 8	6 838 ± 14	6 752 ± 0	16 270	11 740	8 531	6 757
460					17 780	13 940	10 070	9 051
470	19 027 ± 19	15 944 ± 19	11 633 ± 32	11 267 ± 5	19 000	15 970	11 820	11 240
480					19 320	18 340	13 450	13 800
490	18 318 ± 15	19 398 ± 12	14 964 ± 28	17 623 ± 5	18 420	19 470	14 550	17 420
500					16 230	18 320	14 810	19 220
510					13 060	17 380	13 950	18 120
520					9 440	16 050	12 700	18 970

TABLE IV

(*Continued*)

λ nm	DCLET				FA608 + EY608			
	ε_{L}	ε_{HL}	ε_{H_2L}	ε_{H_3L}	ε_{L}	ε_{HL}	ε_{H_2L}	ε_{H_3L}
				meta				
530					6 017	11 720	11 510	20 230
540	3 301 ± 12	5 796 ± 23	9 812 ± 19	16 785 ± 46	3 332	5 878	9 354	16 350
550					1 734	2 317	5 104	7 657
560					855	909	2 139	2 787
570					482	446	943	1 248
				para				
390					5 790	7 410	7 826	7 908
400					6 574	6 670	8 617	6 332
410					7 804	6 506	6 780	5 246
420					9 202	6 980	7 362	4 908
430					10 686	7 940	8 562	5 114
440					12 080	9 288	10 090	5 834
450					13 346	11 416	12 332	7 408
460					14 720	14 084	15 166	10 012
470					16 006	16 430	18 044	13 002
480					17 214	19 144	20 780	15 736
490					17 600	21 500	22 860	19 828
500	16 837 ± 28	20 960 ± 27	[a]	[a]	16 846	21 120	23 300	23 840
510					15 048	19 620	22 400	23 680
520	12 378 ± 27	19 025 ± 7	18 801 ± 1	22 469 ± 0	12 536	19 008	20 420	22 740
530	9 270 ± 15	16 238 ± 14	16 558 ± 39	24 579 ± 5	9 490	16 254	17 760	24 740
540	6 159 ± 14	9 970 ± 10	16 302 ± 11	24 582 ± 0	6 158	9 844	13 676	24 820
550	[a]	4 624 ± 36	7 097 ± 35	16 577 ± 34	3 376	4 160	8 828	16 222
560					1 684	1 538	3 924	6 284
570					795	647	1 698	2 214

[a] The equilibrium does not make itself felt spectrophotometrically at the wavelength.

Whereas the pH-photometric measurements were carried out (in case of the *o*- and *m*-derivatives) in a 1 cm cell with concentrations about 4 . 10^{-4}M, for the *p*-derivative it was necessary to use a substantially lower concentration 6·6 . 10^{-6}M and a 5 cm cell. In acid region pH 2 to 4 the *p*-derivative shows an anomalous course of the *A*–pH curves due probably to formation of associates (intermolecular hydrogen bond of carboxylic acid groups). These associates obviously are not formed at concentrations about 10^{-6}M, and course of the *A*–pH curves is analogous to that of the *m*-derivative.

TABLE V

Search for the Most Probable Chemical Model by Regression Matrix Analysis of Spectral Curves of Acid–Base Equilibria of the *o*-, *m*- and *p*-Derivatives by FA608 + EY608 Program

ortho: c_L 4·41 . 10^{-5}M, *d* 9·98 mm; *meta*: c_L 4·18 . 10^{-5}M, *d* 9·98 mm; *para*: c_L 6·60 . 10^{-6}M, *d* 49·98 mm; *I* 0·1 ($NaClO_4 + Na_3PO_4 + HClO_4$); 25°C.

Position	*ortho*	*meta*	*para*
Solutions, n_s	16	23	26
Wavelengths, n_w	18	19	19
Coloured species, n_c	4	4	4
Residual error abs., s_k	0·003	0·001	0·001
Model H_3L, H_2L^-, HL^{2-}, L^{3-}			
pK_{a2}	3·17 ± 0·19	2·60 ± 0·02	2·67 ± 0·03
pK_{a3}	3·76 ± 0·24	3·28 ± 0·03	2·93 ± 0·06
pK_{a4}	9·19 ± 0·24	7·32 ± 0·03	7·43 ± 0·06
Degrees of freedom, n_v	234	380	437
$\sigma(A)$	0·005	0·003	0·004
Model H_2L, HL^-, L^{2-}			
pK_{a3}	3·59 ± 0·01	[a]	2·65 ± 0·01
pK_{a4}	9·07 ± 0·01		7·45 ± 0·03
Degrees of freedom, n_v	252		456
$\sigma(A)$	0·006		0·007

[a] The model was not tested, because preliminary analysis of spectra indicates that the model H_3L, H_2L^-, HL^{2-}, L^{3-} is indisputable.

TABLE VI

Survey of Dissociation Constants of 7-(*o*-, *m*-, *p*-Carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic Acids

I 0·1 (Na_3PO_4, $NaClO_4$, *K* III, $HClO_4$) 25°C.

Equilibrium	—log K_a		
	ortho	*meta*	*para*
$HL^{2-} \rightleftharpoons L^{3-} + H^+$	9·11 ± 0·03	7·52 ± 0·04	7·46 ± 0·04
$H_2L^- \rightleftharpoons HL^{2-} + H^+$	3·94 ± 0·01	3·27 ± 0·07	3·04 ± 0·10
$H_3L \rightleftharpoons H_2L^- + H^+$	3·15 ± 0·11	2·34 ± 0·06	2·82 ± 0·08

From the survey of dissociation constants (Table VI) of all the three derivatives it follows, that the dissociation constant of the *o*-derivative is somewhat higher than those of the *m*- and *p*-derivatives, which is obviously due to intramolecular hydrogen bond between the carboxyl and hydroxyl groups.

REFERENCES

1. Meloun M., Pancl J.: This Journal *41*, 2365 (1976).
2. Meloun M., Chýlková J., Pancl J.: This Journal *43*, 1027 (1978).
3. Meloun M., Pancl J.: This Journal *44*, 2032 (1979).
4. Busev A. I., Talipova L. L.: Zh. Anal. Khim. *17*, 447 (1962).
5. Smolin D. D., Tichonova L. I., Rytova A. A.: Zh. Obsch. Khim. *32*, 2418 (1962).
6. Chýlková J., Meloun M.: Unpublished results.
7. Říha V., Meloun M.: Chem. Listy *68*, 1289 (1974); Czech. 149 408 (1972); Ger. (GDR) 99 008 (1972).
8. Kankare J. J.: Anal. Chem. *42*, 1322 (1970).
9. Meloun M., Čermák J.: Talanta *26*, 569 (1979).
10. Meloun M., Čermák J.: Sb. Věd. Pr., Vys. Šk. Chemickotechnol. Pardubice, *30*, 49 (1973).
11. Spiegel M. R.: *Theory and Problems of Statistics*. SI (metric), Chapter 7. McGraw-Hill, New York 1972.

Translated by J. Panchartek.