

# THE LEAST SQUARES COMPUTER ESTIMATION OF DISSOCIATION CONSTANTS OF SNAZOXS AND NAPHTHYLAZOXINE 6S FROM SPECTROPHOTOMETRIC DATA\*

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The dissociation constants of two metallochromic indicators, 7-(4-sulpho-1-naphthylazo)-8-hydroxyquinoline-5-sulphonic acid (SNAZOXS) and 7-(6-sulpho-2-naphthylazo)-8-hydroxyquinoline-5-sulphonic acid (Naphthylazoxine 6S), were determined spectrophotometrically in a medium of 0.1M-NaCl or NaClO<sub>4</sub> at 25°C. The absorbance *vs* pH curves were treated by graphical linearization and by numerical non-linear regression using the JDC-LETAG computer program. The molar absorption coefficient *vs* wavelength curves were computed for all five variously protonized forms of the two metallochromic indicators, employing Kankare's program FA 608 + EY 608.

The large group of 7-arylazoderivatives of 8-hydroxyquinoline-5-sulphonic acid represent excellent metallochromic indicators employed in chelometric titrations of many metal cations<sup>1,2</sup>. The formation of coloured chelates is accompanied by a substantial change in the colouration ( $\Delta\lambda = \lambda_{\max}^{\text{ML}} - \lambda_{\max}^{\text{L}}$ ), typical of N-heterocyclic azocompounds substituted in the *p*-position with respect to the phenolic hydroxyl. In the first papers in this series, two azoxines differing only in the steric arrangement of the molecule will be discussed: SNAZOXS, *i.e.* 7-(4-Sulpho-1-naphthylazo)-8-hydroxyquinoline-5-sulphonic acid, and Naphthylazoxine 6S, *i.e.* 7-(6-Sulpho-2-naphthylazo)-8-hydroxyquinoline-5-sulphonic acid were first synthesized by Guerrin, Sheldon and Reilley<sup>3</sup>, who also utilized SNAZOXS for the complexometric determination of ten metal cations by EDTA titration. Twenty five metal cations were titrated by Fritz, Abbink and Payne<sup>4</sup> using Naphthylazoxine 6 S. Busev and coworkers carried out a study of the chelometric determinations of indium<sup>2,5</sup> and gallium<sup>6</sup> using the two indicators. Goyal and Tandon determined the composition of complex V(IV): SNAZOXS, 1 : 2, spectrophotometrically<sup>7</sup> and the stability constants of the SNAZOXS complexes with Cr(VI), Mo(VI) and W(VI) potentiometrically<sup>8</sup>. Tsumeo Shimizu<sup>9</sup> employed Naphthylazoxine 6S as an indicator for the spectrophotometric determination of scandium. Püschel and coworkers have developed a microdetermination method for arsenates and arsenic in organic substances<sup>11,12</sup>, employing titration with a lead(II) salt and SNAZOXS indicator. Vytřas and coworkers<sup>13,14</sup> studied colour changes in direct chelometric titrations of Zn with SNAZOXS and Naphthylazoxine 6S using the C.I.E. colour system. Meloun and Čermák<sup>15</sup> evaluated the chelometric titrations of lead and zinc with SNAZOXS and

\* The first part in the series: Complexation Equilibria of Some Azo Derivatives of 8-Hydroxyquinoline-5-sulphonic Acid.

Naphthylazoxine 6S indicators by non-linear regression employing a computer program<sup>17</sup>. Meites<sup>16</sup> gives the dissociation constants for SNAZOXS as  $pK_1$  7.0 and  $pK_2$  3.0 at 10.1 and 25°C and for Naphthylazoxine 6S as  $pK_1$  7.4 and  $pK_2$  3.1 at 10.1 and 25°C.

In this paper, the protonation equilibrium of SNAZOXS and Naphthylazoxine 6S are studied spectrophotometrically. The results of graphical linearization of the pH-absorbance curves measured by two different techniques are compared with the results of numerical non-linear regression of these curves employing the JDC-LETAG program<sup>18,19</sup>. The values of the molar absorption coefficients for all the protonated forms of the two azoxines as a function of the wavelength were determined using Kanakare's FA 608 + EY 608 program<sup>20</sup>.

## EXPERIMENTAL

### Chemicals and Solutions

SNAZOXS, *p.a.*, and Naphthylazoxine 6S, commercial products from Spolana, Neratovice, contain coloured impurities interfering in the spectrophotometric measurements. The two indicators were purified by precipitation from a concentrated solution with acetone<sup>17</sup>. In the first portion of the precipitate, coloured impurities were detected chromatographically and, in the remainder of the precipitate, the content of the pure indicator was found. The indicator purity was controlled by chromatography on Whatman No 2 paper impregnated with EDTA, in a descending arrangement using a water-pyridine-ethyl acetate (1 : 2 : 4) system. The purified indicator was washed with acetone and dried at 60°C. The content of pure indicator was determined by elemental analysis for azo-nitrogen, corresponding to 85.6% for SNAZOXS and 92.9% for Naphthylazoxine 6S. Sodium hydroxide (1M) was prepared by dilution of 50% NaOH prepared according to Sørensen<sup>21</sup>. The titre was determined by potentiometric titration of oxalic acid under an inert argon atmosphere. The solution was stored in a polyethylene bottle fitted with an ascariete tube. Perchloric acid (1M) was obtained by dilution of 70% HClO<sub>4</sub> (*p.a.*, Merck) with redistilled water. The titre was determined by potentiometric titration of HgO in KI medium<sup>22</sup>. Sodium perchlorate (1M) was prepared by neutralization of recrystallized sodium hydrocarbonate (*p.a.* Lachema) with concentrated perchloric acid (70% *p.a.*, Merck) and was recrystallized twice from redistilled water<sup>10,23</sup>. A phosphate buffer (0.001425M-Na<sub>3</sub>PO<sub>4</sub> + 0.09145M-NaClO<sub>4</sub>) was prepared by weighing doubly recrystallized sodium phosphate and the ionic strength was adjusted to 1.0 with sodium perchlorate or chloride. Standard buffers with declared pH (S) values were prepared according to Bates<sup>24</sup> from chemicals purified by repeated recrystallization from redistilled water freed of carbon dioxide. Sodium chloride (1M) was prepared by dissolving 58.44 g of the substance (*p.a.* Lachema) in redistilled water and diluting to 1000 ml. Distilled water was purified on a central ion-exchange apparatus and then distilled in a ground-glass apparatus. The water quality was controlled by conductivity measurement.

The prepared solutions were freed of traces of heavy metals by extraction into a dithizone solution in tetrachloromethane.

### Measurement of pH-Absorbance Curves

Spectral curves were orientatively measured at various pH values on a Specord recording spectrophotometer (Zeiss, Jena) with a TAD titration adapter (ref.<sup>25</sup>), employing procedure (A). The spectra for quantitative evaluation were obtained by procedure (B) on a compensation single-beam

spectrophotometer, VSU-2-G (Zeiss, Jena), provided with an external titration apparatus connected with the measuring cuvette. *A vs pH* curves at constant wavelength were obtained by pH-photometric titration (procedure (A)) on a single-beam Spekol spectrophotometer with an amplifier (Zeiss, Jena), using a TAL adapter (ref.<sup>17,26-28</sup>).

*Method A: pH-photometric titration performed directly in the cuvette.* The *A vs pH* curves in a pH range of 2–12 are carried out advantageously by combined pH-photometric microtitration with a TAL or TAD adaptor on a Spekol spectrophotometer with an amplifier or on a Specord instrument; for details see refs<sup>17,19</sup>. In the solution titrated in the cuvette (type C, Zeiss, Jena), with a volume of 22 ml and a length of 50 mm, are immersed the microburette orifice, a glass electrode and a reference electrode, a thermometer and a stirrer. The solution is stirred with a Teflon stirrer driven by a small electromotor. Capillaries for introducing argon into the solution and above it are also placed in the cuvette. The pH value is adjusted by addition of several microlitres of 1M-HClO<sub>4</sub> to the solution of an alkali salt of a weak acid containing the studied coloured substance; the absorbance value is measured simultaneously.

*Method B: pH-photometric titration performed outside the cuvette.* pH-photometric titrations can be carried out in the titration apparatus according to Havel<sup>29,30</sup>. In a titration vessel with a total volume of c. 120 ml are immersed the tip of the microburette, one branch of the Wilhelm bridge<sup>31</sup>, a thermometer, a stirrer and an argon inlet. The vessel was connected with the measuring cuvette by a thin polyethylene tube through the Teflon stopper of the cuvette. Another tube connected the bottom of the cuvette with a 100 ml polyethylene syringe. When the solution was drawn into the cuvette, it flowed from the vessel through the polyethylene tube into the cuvette and from the cuvette into the polyethylene tube connected to the syringe. Care was exercised to prevent the solution from flowing into the syringe. When expelling the solution from the cuvette, the air in the syringe pressed the solution back into the titration vessel. In this way the cuvette and the connecting polyethylene tubes were rinsed several times with the measured solution. 10.01 and 50.01 mm long replaceable cuvettes were employed. The pH of 50 ml of the measured solution was varied by microlitre additions of 1M-HClO<sub>4</sub> using a home-made microburette<sup>26</sup>.

After equilibration in the titration vessel, the pH was measured and the solution was transferred using the syringe into the cuvette. The cuvette was first rinsed several times with the solution from the titration vessel and then the solution absorbance was measured. The solution was then returned into the titration vessel and the pH was again measured. The whole procedure was repeated after another addition of perchloric acid.

#### pH-Measurement

In method (A) the pH was measured using pH-meters PHM-4d (Radiometer, Copenhagen) and OP 205 (Radelkis, Budapest) with a G202B glass electrode (Radiometer, Copenhagen) and a silver chloride electrode<sup>32</sup> with 1M-NaCl, separated from the measured solution by a frit. All measurements were carried out at a temperature of (25 ± 0.1)°C maintained by a V3 water thermostat (Prüfgeräte-Werk, Medingen). The values of the pH obtained were corrected to the Nernstian slope value, 57.82 mV/pH, determined using five standard buffers, and for the varying values of the liquid-junction potential between the standard buffer and the measured solution employing the relationship

$$p\alpha_{H^+} = [pH(X) - pH(S)] \frac{59.16}{57.82} + pH(S) + \frac{1}{59.16} [E_j(S) - E_j(X)], \quad (1)$$

where pH(S) is the  $p\alpha_{H^+}$  value for the standard buffer which was used for the pH-meter calibration

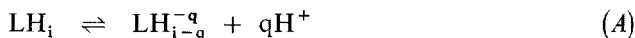
and  $E_j(S)$  is the liquid-junction potential developed in the standard buffer,  $\text{pH}(X)$  is the  $\text{p}a_{\text{H}}$  value determined and  $E_j(X)$  is the liquid-junction potential value in the measured solution, whose ionic strength is chiefly affected by the NaCl content. The  $E_j(X)$  values for various ionic strengths were measured in a concentration cell consisting of two silver chloride electrodes. The correction of the pH using Eq. (1) is significant when the pH is measured in solutions whose ionic strength differs considerably from that of the standard buffer. If the ionic strengths of the standard buffer and the measured solution are very similar, then difference  $E_j(S) - E_j(X)$  is negligible.

In method (B) the pH was measured with an OP 204/1 pH-meter and an OP 7171-1/A glass electrode (Radelkis) and a silver chloride electrode<sup>32</sup> separated by the Wilhelm bridge<sup>31</sup> from the measured solution. The measurements were carried out at  $(25 \pm 0.1)^\circ\text{C}$ , maintained by the same thermostat as in method (A). The pH-values determined were corrected only for the Nernstian slope of the glass electrode using Eq. (1), assuming that  $E_j(X) \approx E_j(S)$ .

## METHODS OF EVALUATION OF EXPERIMENTAL DATA

### Graphical Handling of the $A$ vs pH Curves

The principles of the graphical method employed in this paper can be found elsewhere<sup>30,33,34</sup>. Only the final equations, obtained by transformation of the equilibrium constant equations, mass balances and the equations for the overall solution absorbance (assuming the validity of the Lambert-Beer law), will be given here. For equilibrium (A)



$$c_L A^{-1} = \varepsilon_1^{-1} - (c_L \varepsilon_2 - A) a_{\text{H}^+}^{-q} A^{-1} \varepsilon^{-1} K_{\text{ai}} = \varepsilon_1^{-1} - Q_1 a_{\text{H}^+}^{-q}, \quad (2)$$

$$c_L A^{-1} = \varepsilon_2^{-1} - (A - c_L \varepsilon_1) a_{\text{H}^+}^q A^{-1} \varepsilon_2^{-1} K_{\text{ai}}^{-1} = \varepsilon_2^{-1} + Q_2 a_{\text{H}^+}^q, \quad (3)$$

where  $A$  is the absorbance,  $c_L$  is the total substance concentration,  $\varepsilon_1$  and  $\varepsilon_2$  are the molar absorption coefficients,  $K_{\text{ai}}$  is the mixed equilibrium constant of reaction (A) and the other symbols have their usual significance. The value of  $q$  is obtained from the plot,  $c_L/A = f(Q_1[\text{H}^+]^{-q} \text{ or } Q_2[\text{H}^+]^q)$ . The values of the molar absorption coefficients and the equilibrium constant are obtained from the intercept ( $\varepsilon_1$  and  $\varepsilon_2$ ) and from the slope ( $K_{\text{ai}}$ ).

### Non-Linear Regression of the $A$ vs pH Curves

Using non-linear regression of the  $A$  vs pH curves, the molar absorption coefficients and protonation constants for all light-absorbing species in the protonation equilibrium can be obtained. If a polybasic acid,  $\text{LH}_j$ , yields a mixture of variously protonated species  $\text{LH}_j$  (where  $j = 0, \dots, J$ ) and the charges are omitted for the sake of simplicity), the overall mixed protonation constant of species  $\text{LH}_j$ ,  $\beta_{01j}$ , or its

mixed dissociation constant,  $K_{ai}$ , will be given by

$$\beta_{01j} = \frac{[\text{LH}_j]}{[\text{L}] a_{\text{H}^+}^j} = 1 / \prod_{i=j+1}^J K_{ai} \quad (4)$$

Assuming that all variously protonated species  $\text{LH}_j$  absorb radiation at the given wavelength, absorbance  $A$  of the solution for a unit cuvette length will equal

$$A = c_L (\varepsilon_L + \sum_{j=1}^J \varepsilon_{\text{LH}_j} \cdot 10^{j \cdot \log a_{\text{H}^+} + \log \beta_{01j}}) / (1 + \sum_{j=1}^J 10^{j \cdot \log a_{\text{H}^+} + \log \beta_{01j}}) \quad (5)$$

In order to numerically evaluate the  $A$  vs pH curve described by equation (5), *i.e.* by the functional relationship,  $A = f(\log \beta_{01j}, \varepsilon_L, \varepsilon_{\text{LH}_j}; \text{pH})$  (where  $j = 1, \dots, J$ ), the least squares method can be employed and the unknown parameters adjusted so that the function

$$U = \sum_i w_i (A_{\text{calc},i} - A_{\text{exp},i})^2, \quad (6)$$

where  $w_i$  is the statistical weight, usually equal to unity, attains a minimum value. The procedure is given in the JDC-LETAG program (refs<sup>17,18,19</sup>). The zeroth approximation is assessed from the  $A$  vs pH plot. The fit of the calculated regression  $A$  vs pH curve to the experimental points, *i.e.* as small a value of residuals ( $A_{\text{exp}} - A_{\text{calc}}$ ) as possible and as frequent a change in their sign as possible, is a criterion of the reliability of the determined parameters.

#### Matrix Analysis of the $A$ vs pH Dependence

The experimental and computation scheme of the determination of the protonation constants of a multicomponent system, employing Kankare's<sup>20</sup> FA 608 and EY 608 programs, can be summarized in the following points: *a*) The residual error in the absorbance,  $s_k$ , is evaluated using Wernimont's procedure<sup>35</sup>. *b*) Wavelengths at which changes in the spectra are as large as possible are selected. *c*) The spectra are measured on solutions prepared so that the concentration of the basic component ( $\text{H}^+$ ) is varied in the widest possible interval. *d*) Using the second moment absorbance matrix, the number of light-absorbing species in the system is determined from the rank of the matrix and the spectra are refined by removal of breakdown points. *e*) Equilibrium stoichiometry of coloured complex species is assumed and the equilibrium constants of the assumed reactions are determined by minimization of function  $U$ . After location of the minimum of function  $U$ , the curves of the molar absorption coefficient vs the wavelength are computed for all coloured species present in the solution and finally their equilibrium concentrations are calculated. *f*) The closest possible fit of the computed spectra to the experimental points is a measure of the reliability of the computed protonation constants, molar absorption coefficients and the selected equilibrium stoichiometry of the complex species. This fit is evaluated by comparing the standard deviation of the absorbance,  $\sigma(A)$ , calculated from the value of function  $U$  at the minimum, with the absorbance residual error,  $s_k$ .

## RESULTS AND DISCUSSION

Five optically active species,  $H_4L^+$ ,  $H_3L$ ,  $H_2L^-$ ,  $HL^{2-}$  and  $L^{3-}$ , are present at equilibrium in aqueous solutions of azoxines S, in dependence on the pH (Fig. 1–4). The first two, deep red-purple species,  $H_4L^+$  and  $H_3L$ , predominate in strongly acidic media and exhibit absorption maxima at 548 and 550 nm for SNAZOXS and at 529 and 543 nm for Naphthylazoxine 6S (Table I). Two poorly pronounced isobestic points appear on the absorption curves of Naphthylazoxine 6S: one corresponds to deprotonation of the  $H_4L^+$  form and the other to deprotonation of the

TABLE I  
Survey of Optical Data for Various Protonated Forms of SNAZOXS and Naphthylazoxine 6S

Form	Medium	pH Region (or log $C_{HClO_4}$ )	$\lambda_{max}$ nm	$\epsilon_{max}$	$\lambda_{isob.}$ nm	$\epsilon_{isob.}$
SNAZOXS						
$H_4L^+$	$HClO_4$	(>1.3M)	550	24 900	} 475	7 680
$H_3L$	$HClO_4$	(0.1–1.0M)	548	17 620		
$H_2L^-$	} I 0.1 NaClO <sub>4</sub>	1–2	534	11 420	} 475	6 370
$HL^{2-}$		4–6	513	15 020		
$L^{3-}$		>8	500	16 950	} { 421 527	} 7 400 14 170
Naphthylazoxine 6S						
$H_4L^+$	$HClO_4$	(>1.0M)	529	30 300	} 466	8 400
$H_3L$	$HClO_4$	(0.1–0.7M)	543 530 550	24 370 24 400		
$H_2L^-$	} I 0.1 NaClO <sub>4</sub>	1–2	516	16 600	} 459	7 950
$HL^{2-}$		4–6	500	20 280		
$L^{3-}$		>8	492	22 750	} { 415 520	} 9 500 16 9.0

TABLE II  
Survey of Evaluated Sets of Spectra and pH-Absorbance Curves for SNAZOXS and Naphthylazoxine 6S

Set	Medium	$\lambda$ , nm	$c_L$ , mol/l	$d$ , mm	No of points	pH range	Exper. technique
SNAZOXS							
1	I 0.1 ( $\text{Na}_3\text{PO}_4$ , $\text{NaClO}_4$ , $\text{HClO}_4$ , K III)	400–640	$8.00 \cdot 10^{-5}$	10.01	24	1.84–10.58	B
2		470			45	1.51–11.27	A
3		470			59	1.55–11.22	A
4	I 0.1	470			41	1.52–11.15	A
5	( $\text{Na}_3\text{PO}_4$ ,	470	$8.60 \cdot 10^{-6}$	50.01	45	1.48–11.27	A
6	$\text{NaCl}$ ,	470			54	1.49–11.21	A
7	$\text{HClO}_4$ ,	450			46	1.52–11.21	A
8	K III)	560			43	1.48–11.16	A
9		580			37	1.47–11.20	A
10	$\text{HClO}_4$	400–640	$2.00 \cdot 10^{-5}$	50.00	13	–1.00 to +0.95	B
Naphthylazoxine 6S							
1	I 0.1 ( $\text{Na}_3\text{PO}_4$ , $\text{NaClO}_4$ , $\text{HClO}_4$ , K III)	400–640	$4.00 \cdot 10^{-5}$	10.01	26	1.39–10.38	B
2		470			48	1.57–10.64	A
3		470			64	1.33–10.58	A
4	I 0.1	470	$6.80 \cdot 10^{-6}$	50.01	41	1.50–10.80	A
5	( $\text{Na}_3\text{PO}_4$ ,	470			50	1.31–10.65	A
6	$\text{NaCl}$ ,	470			42	1.52–10.73	A
7	$\text{HClO}_4$ ,	450			36	1.28–10.45	A
8	K III)	490			40	1.40–10.59	A
9		540			43	1.36–10.56	A
10		550			54	1.36–10.37	A
11		560			18	1.40–10.21	A
12	$\text{HClO}_4$	400–640	$1.05 \cdot 10^{-5}$	50.00	16	–1.52 to +0.95	B

$H_3L$  form. However, a single sharp isosbestic point appears with SNAZOXS, indicating that dissociation of the two hydrogens could occur simultaneously.

In a pH region of 1–3, a red-purple species,  $H_2L^-$ , exists, with an absorption maximum at 534 nm for SNAZOXS and at 516 nm for Naphthylazoxine 6 S. The red  $HL^{2-}$  form with an absorption maximum 513 nm and 500 nm for SNAZOXS and Naphthylazoxine 6S, respectively, predominates at pH 4–7. Finally, aqueous solutions are orange at pH > 7.8 due to the presence of the  $L^{3-}$  form with an absorption maximum at 500 nm for SNAZOXS and 492 nm for Naphthylazoxine 6S.

Table II surveys the combined pH-photometric titrations of the two azoxines. The data for evaluation of the dissociation constants were obtained using procedure (A). The measurement of the whole spectral curves employing procedure (B) was carried out in order to determine the curves of the molar absorption coefficients as functions of the wavelength for individual variously protonated forms. The measurement in the Hammett region was performable only using procedure (B).

The optical data given in Table I were determined from the absorbance curves of the two indicators measured at various pH values, depicted in Figs 1–4. The absorbance matrices for these spectra were evaluated employing the FA 608 +

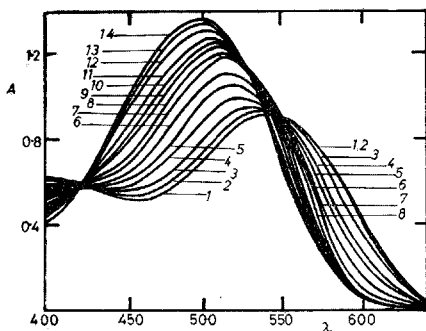


FIG. 1

Absorption Spectra of SNAZOXS in Aqueous Solution

$c_L = 8.0 \cdot 10^{-5} M$ ;  $l = 0.10$  (NaClO<sub>4</sub> + Na<sub>3</sub>PO<sub>4</sub> + KIII);  $t = 25.0^\circ C$ ;  $d = 10.01$  mm; pH adjusted with HClO<sub>4</sub>. Curves for pH: 1 1.84; 2 2.15; 3 2.46; 4 2.79; 5 3.07; 6 3.40; 7 3.94; 8 5.65; 9 6.50; 10 6.75; 11 7.03; 12 7.50; 13 8.01; 14 10.58.

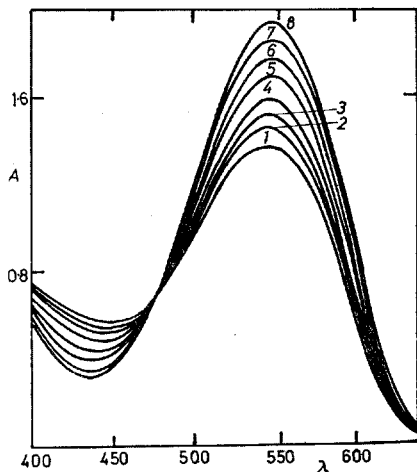


FIG. 2

Absorption Spectra of SNAZOXS in Concentrated Perchloric Acid Medium

$c_L = 2.0 \cdot 10^{-5} M$ ;  $t = 25.0^\circ C$ ;  $d = 50.00$  mm. Curves for concentrations of HClO<sub>4</sub> (M): 1 0.1; 2 0.3; 3 0.5; 4 1.0; 5 2.0; 6 3.5; 7 6.5; 8 9.0.



+ EY 608 program; the protonation constants thus determined are given in Table III and the dependences of the molar absorption coefficients on the wavelength in Figs 5 and 6.

Kankare's program FA 608 + EY 608 was employed for testing whether dissociation of the  $H_4L^+$  for to  $H_3L$  and finally to  $H_2L^-$  takes place in a single step or in two steps. The sum of the least squares of the deviations between the calculated and measured absorbance values for Naphthylazoxine 6S amounted to  $U = 5.69 \cdot 10^{-3}$  for chemical model  $H_4L^+ \rightarrow H_3L \rightarrow H_2L^-$  involving stepwise equilibrium and  $U = 7.62 \cdot 10^{-1}$  for dissociation of the two protons in a single step. The fit of the computed spectra to the experimental points was also much better when stepwise equilibrium was assumed. The standard deviations of the absorbance computed for the individual solutions in the given wavelength interval are given in Fig. 7.

In Table IV, the results of non-linear regression of the  $A$  vs pH curves are compared for measurement by the titration inside (procedure (A)) or outside (procedure (B)) the cuvette. The absorbance differences,  $\Delta A = 10^3(A_{\text{exp}} - A_{\text{calc}})$ , are a criterion of the reliability of the determined parameters, obtained by non-linear regression

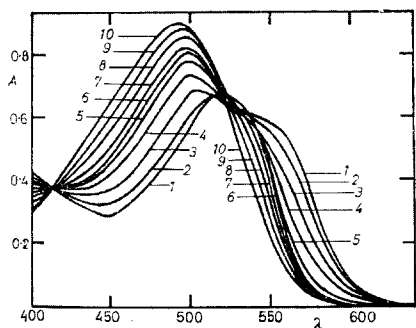


FIG. 3

Absorption Spectra of Naphthylazoxine 6S in Aqueous Solution

$c_L = 4.0 \cdot 10^{-5} M$ ;  $I = 0.10$  ( $NaClO_4 + Na_3PO_4 + KIII$ );  $t = 25.0^\circ C$ ;  $d = 10.01$  mm; pH adjusted with  $HClO_4$ . Curves for pH: 1 1.84; 2 2.48; 3 3.02; 4 3.48; 5 4.11; 6 5.37; 7 6.78; 8 7.26; 9 7.76; 10 10.38.

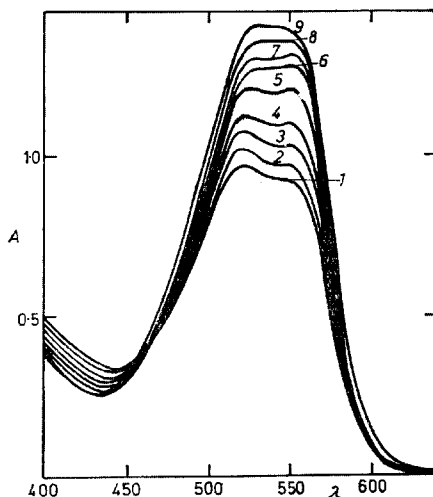


FIG. 4

Absorption Spectra of Naphthylazoxine 6S in Concentrated Perchloric Acid Medium

$c_L = 1.05 \cdot 10^{-5} M$ ;  $t = 25.0^\circ C$ ;  $d = 50.00$  mm. Curves for concentrations of  $HClO_4$  (M): 1 0.03; 2 0.1; 3 0.3; 4 0.7; 5 1.5; 6 2.5; 7 4.5; 8 6.5; 9 9.0.

of the  $A$  vs pH curve using the JDC-LETAG program. The technique of combined  $A$ -pH titration directly in the cuvette at a constant wavelength (procedure (A)) is faster and experimentally simpler. Titration outside the cuvette (procedure (B)) served rather for obtaining the molar absorption coefficients as a function of the wavelength while titration in the cuvette (procedure (A)) was used for obtaining reliable and precise dissociation constant values. In strongly acid solutions, only procedure (B) could be used.

The  $A$  vs pH curves of the two indicators measured by procedure (A) directly in the cuvette are given in Figs 8 and 9. In Tables V and VI, the dissociation constants are given together with the values of function  $U$  and the standard deviations of the absorbance,  $\sigma(A)$ , calculated from this function. The values obtained from the graphical evaluation are in good agreement with those determined by non-linear regression. The fit of the computed regression  $A$ -pH curves to the experimental points is depicted in Fig. 10.

Tables VII and VIII summarize the molar absorption coefficient values for the  $H_2L^-$ ,  $HL^{2-}$  and  $L^{3-}$  forms of the two indicators, evaluated by various graphical and numerical techniques and measured by two experimental procedures. The values read directly from the graph and obtained by graphical extrapolation of the linear

TABLE III

Survey of the Results of Matrix Treatment of the Spectra from Figs 1-4 for SNAZOXS and Naphthylazoxine 6 S and for Various pH Values Using the FA 608 + EY 608 Program (ref.<sup>20</sup>)

Item	SNAZOXS		Naphthylazoxine 6S	
	$H_4L^+ \rightarrow H_3L \rightarrow H_2L^-$	$H_2L^- \rightarrow HL^{2-} \rightarrow L^{3-}$	$H_4L^+ \rightarrow H_3L \rightarrow H_2L^-$	$H_2L^- \rightarrow HL^{2-} \rightarrow L^{3-}$
The data set	10th	1st	12th	1st
No of solutions	13	24	16	26
No of wavelengths	25	25	25	25
No of degrees of freedom	275	550	308	600
No of coloured species	3	3	3	3
Residual error of the absorbance ( $s_k$ )	0.0035	0.0040	0.0025	0.0031
$pK_{a4}$	—	$6.954 \pm 0.012$	—	$7.377 \pm 0.021$
$pK_{a3}$	—	$2.912 \pm 0.007$	—	$3.162 \pm 0.011$
$p(K_{a2})_c$	$0.082 \pm 0.001$	—	$0.295 \pm 0.148$	—
$p(K_{a1})_c$	$-1.371 \pm 0.000$	—	$-0.958 \pm 0.102$	—
$U$	$4.95 \cdot 10^{-3}$	$5.83 \cdot 10^{-3}$	$5.69 \cdot 10^{-3}$	$7.44 \cdot 10^{-3}$
$\sigma(A)$	0.0035	0.0043	0.0051	0.0068

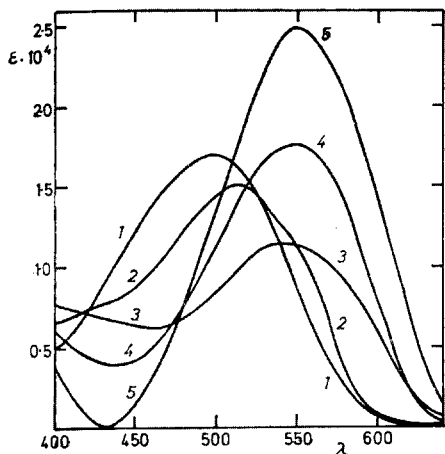


FIG. 5

Molar Absorption Coefficients of Variously Protonated Forms of SNAZOXS as a Function of the Wavelength

$c_L = 1.00M$ ;  $d 10.00$  mm. Curves of individual forms: 1  $L^{3-}$ ; 2  $HL^{2-}$ ; 3  $H_2L^{-}$ ; 4  $H_3L$ ; 5  $H_4L^+$

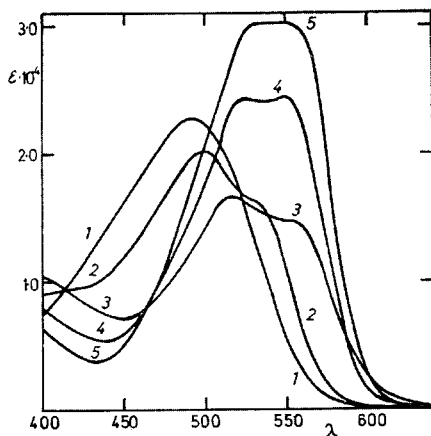


FIG. 6

Molar Absorption Coefficients of Variously Protonated Forms of Naphthylazoxine 6S as a Function of the Wavelength

$c_L = 1.00M$ ;  $d 10.00$  mm. Curves for individual forms: 1  $L^{3-}$ ; 2  $HL^{2-}$ ; 3  $H_2L^{-}$ ; 4  $H_3L$ ; 5  $H_4L^+$

FIG. 7

Fit of the Computed Regression Spectral Curves to the Experimental Points, Evaluated by the FA 608 + EY 608 Program

SNAZOXS:  $\sigma(A)$  values for individual solutions (Open circles), resultant standard deviation for all solutions  $\bar{\delta}(A)$  (dashed line (a)), residual absorbance error  $s_k$  (dashed line (b)) Naphthylazoxine 6 S:  $\sigma(A)$  values for individual solutions (full circles), resultant standard deviation  $\bar{\delta}(A)$  (solid line (a)), residual absorbance error  $s_k$  (solid line (b)). A: Concentrated  $HClO_4$  medium, spectra evaluated from Figs. 2 and 4. B: Aqueous medium, spectra evaluated from Figs 1. and 3.

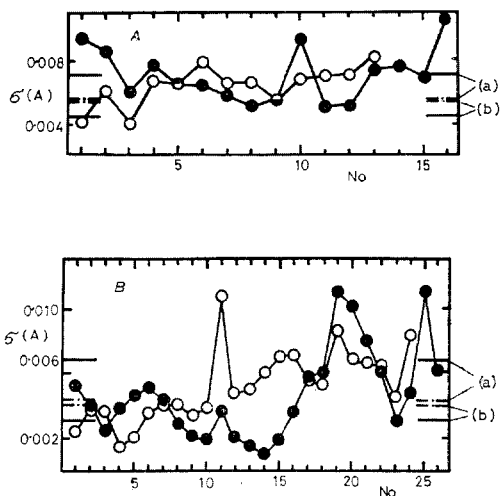


TABLE IV  
Comparison of the Results of Non-Linear Regression Using the JDC-LETAG Program for SNAZOXS, Measured Using Procedures A and B.  
 $\Delta A = 10^3(A_{\text{calc.}} - A_{\text{exp.}})$

Method A				Method B				
pH	A	$\Delta A$	pH	A	$\Delta A$	pH	A	$\Delta A$
11.223	0.651	2	6.950	0.563	1	10.580	1.205	1
10.515	0.651	2	6.885	0.556	0	9.960	1.211	7
9.515	0.651	2	6.808	0.550	0	9.500	1.208	5
8.858	0.649	2	6.751	0.545	2	9.010	1.200	-2
8.390	0.644	1	6.712	0.540	0	8.460	1.192	-3
8.045	0.638	2	6.645	0.535	1	8.010	1.173	-5
7.924	0.633	1	6.598	0.532	2	7.720	1.155	-1
7.858	0.630	0	6.497	0.527	5	7.500	1.127	-5
7.807	0.628	1	6.430	0.522	4	7.250	1.090	-4
7.698	0.620	-2	6.305	0.515	5	7.030	1.053	-1
7.608	0.613	-4	6.035	0.501	3	6.750	1.014	13
7.523	0.608	-4	5.712	0.492	3	6.500	0.958	-2
7.425	0.600	-5	5.481	0.488	2	6.040	0.908	-1
7.330	0.593	-4	4.470	0.479	2	5.650	0.891	3
7.263	0.588	-3	4.190	0.473	1	4.660	0.864	-4
7.195	0.582	-3	3.956	0.464	-2	3.940	0.844	4
7.128	0.578	-1	3.922	0.459	-6	3.600	0.804	-4
7.053	0.572	0	3.868	0.458	-4			

Method A		Method B	
3rd set: $\lambda$ 470 nm, $t$ 0.1, 25°	59	1st set: $\lambda$ 470 nm, $t$ 0.1, 25°	24
$pK_{a4}$	6.985 $\pm$ 0.013	$pK_{a4}$	6.948 $\pm$ 0.018
$pK_{a3}$	2.880 $\pm$ 0.013	$pK_{a3}$	2.934 $\pm$ 0.022
$\epsilon_{HL-}$	6.380 $\pm$ 31	$\epsilon_{HL-}$	6.290 $\pm$ 59
$\epsilon_{HL-2}$	10.770 $\pm$ 24	$\epsilon_{HL-2}$	10.890 $\pm$ 37
$\epsilon_{L-3}$	14.690 $\pm$ 23	$\epsilon_{L-3}$	15.040 $\pm$ 25
$U$	3.77 $\cdot 10^{-4}$	$U$	4.40 $\cdot 10^{-4}$
$\sigma(A)$	0.003	$\sigma(A)$	0.005

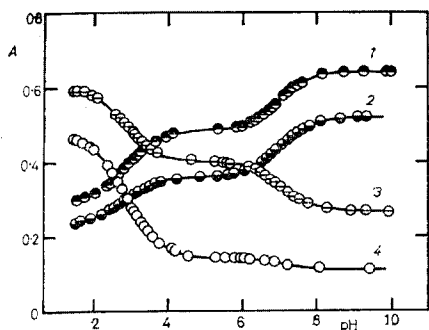


FIG. 8

Absorbance-pH Curves for SNAZOXS Solutions for Various Wavelengths, Measured by Procedure (A)

$c_L = 8.6 \cdot 10^{-6} M$ ;  $I 0.10$  ( $Na_3PO_4$ , NaCl, KIII);  $t 25.0^\circ C$ ;  $d 50.01$  mm; pH adjusted with  $HClO_4$ . Curves for wavelength in nm: 1 470; 2 450; 3 560; 4 580.

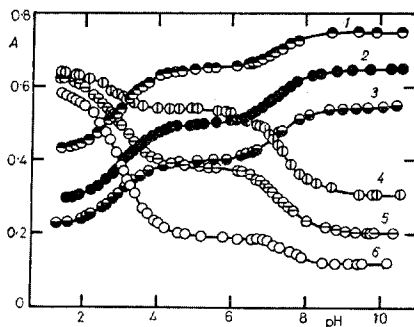


FIG. 9

Absorbance-pH Curves for Naphthylazoxine 6S Solutions for Various Wavelengths, Measured by Procedure (A)

$c_L = 6.8 \cdot 10^{-6} M$ ;  $I 0.10$  ( $Na_3PO_4$ , NaCl, KIII);  $t 25.0^\circ C$ ;  $d 50.01$  mm; pH adjusted with  $HClO_4$ . Curves for wavelength in nm: 1 490; 2 470; 3 450; 4 540; 5 550; 6 560.

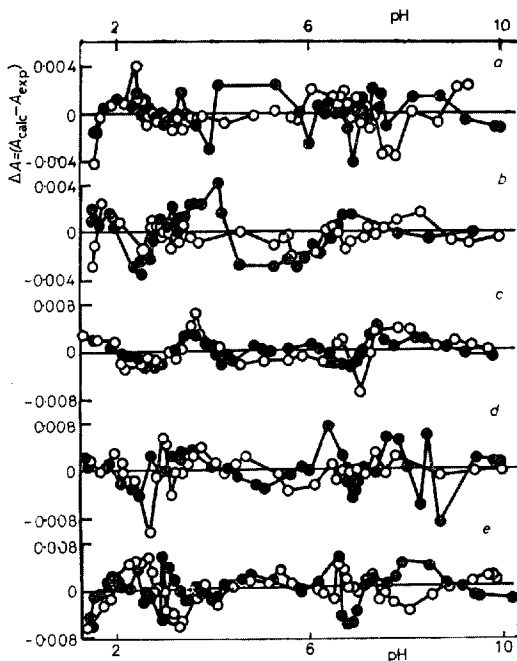


FIG. 10

Fit of the Calculated  $A$  vs pH Regression Curves to the Experimental Points for Various Wavelengths in nm

SNAZOXS: *a* ● 470; ○ 450; *b* ● 580; ○ 560; Naphthylazoxine 6S: *c* ● 470; ○ 450; *d* ● 540; ○ 490; *e* ● 560; ○ 550.

TABLE V  
 SNAZOXs Dissociation Constants Evaluated by the Graphical Method and by Non-Linear Regression of the  $A$  vs pH Curves Using the JDC-LETAG Program; Comparison of Techniques  $A$  and  $B$

Set	$\lambda$ nm	Graphical method		No of degrees of freedom	$U$	JDC-LETAG			
		$pK_{a3}$	$pK_{a4}$			$\sigma(A)$	$pK_{a3}$	$pK_{a4}$	
1	440	3.24 <sup>a</sup>	6.93	19	$2.06 \cdot 10^{-4}$	0.003	$3.200^a \pm 0.045$	$6.932 \pm 0.023$	
	450	3.06	6.93	19	$2.32 \cdot 10^{-4}$	0.005	$3.069 \pm 0.031$	$6.951 \pm 0.016$	
	470	2.95	6.97	19	$4.40 \cdot 10^{-4}$	0.005	$2.934 \pm 0.022$	$6.948 \pm 0.018$	
	500	2.84	7.02	19	$3.91 \cdot 10^{-4}$	0.004	$2.852 \pm 0.016$	$7.010 \pm 0.027$	
	550	3.64 <sup>a</sup>	7.01	19	$3.25 \cdot 10^{-4}$	0.006	$3.074 \pm 0.149$	$7.011 \pm 0.024$	
	560	3.16	7.00	19	$5.85 \cdot 10^{-4}$	0.005	$3.056 \pm 0.063$	$6.997 \pm 0.029$	
	570	3.06	6.97	19	$4.72 \cdot 10^{-4}$	0.005	$2.998 \pm 0.027$	$6.960 \pm 0.034$	
	580	2.96	6.87	19	$4.58 \cdot 10^{-4}$	0.003	$2.959 \pm 0.019$	$6.930 \pm 0.070$	
	<i>Average</i>		3.00	6.96			$2.992 \pm 0.047$	$6.967 \pm 0.030$	
2	470	2.90	6.99	40	$8.19 \cdot 10^{-5}$	0.001	$2.880 \pm 0.007$	$6.986 \pm 0.008$	
	3 470	2.89	7.06	54	$3.77 \cdot 10^{-4}$	0.003	$2.880 \pm 0.013$	$6.985 \pm 0.013$	
	4 470	3.00	7.06	36	$1.20 \cdot 10^{-4}$	0.002	$2.877 \pm 0.011$	$7.075 \pm 0.010$	
	5 470	2.89	7.08	40	$5.47 \cdot 10^{-4}$	0.004	$3.006 \pm 0.019$	$6.986 \pm 0.020$	
	6 470	2.92	7.03	49	$5.34 \cdot 10^{-4}$	0.003	$2.864 \pm 0.016$	$7.025 \pm 0.015$	
	7 450	2.89	7.03	41	$1.13 \cdot 10^{-4}$	0.002	$2.845 \pm 0.014$	$6.982 \pm 0.009$	
	8 560	2.83	7.03	38	$9.72 \cdot 10^{-5}$	0.002	$2.811 \pm 0.007$	$7.015 \pm 0.011$	
	9 580	2.88	7.17 <sup>a</sup>	32	$1.38 \cdot 10^{-4}$	0.002	$2.859 \pm 0.006$	$6.973 \pm 0.086$	
	<i>Average</i>		2.90	7.04			$2.878 \pm 0.012$	$7.003 \pm 0.022$	

<sup>a</sup> Extraneous value which was not included in the calculation of the average value.

TABLE VI  
Naphthylazoxine 6S Dissociation Constants Evaluated by the Graphical Method and by Non-Linear Regression of the  $A$  vs pH Curves Using the JCD-LETAG Program; Comparison of Techniques  $A$  and  $B$

Set	$\lambda$ nm	Graphical method		No of degrees of freedom	$U$	JDC-LETAG			
		$pK_{a,3}$	$pK_{a,4}$			$\sigma(A)$	$pK_{a,3}$	$pK_{a,4}$	
I	430	2.97	7.44	20	$7.70 \cdot 10^{-5}$	0.002	2.917 ± 0.042	7.390 ± 0.020	
	450	3.10	7.35	20	$9.34 \cdot 10^{-5}$	0.002	3.070 ± 0.017	7.346 ± 0.015	
	470	3.15	7.35	20	$3.27 \cdot 10^{-4}$	0.004	3.116 ± 0.022	7.309 ± 0.027	
	490	3.10	7.33	20	$1.75 \cdot 10^{-4}$	0.003	3.084 ± 0.014	7.249 ± 0.027	
	540	3.03	7.48	20	$1.05 \cdot 10^{-3}$	0.007	1.822 <sup>a</sup> ± 0.413	7.502 ± 0.036	
	550	3.00	7.43	20	$2.77 \cdot 10^{-4}$	0.004	2.955 ± 0.035	7.404 ± 0.022	
	560	3.09	7.33	20	$1.83 \cdot 10^{-4}$	0.003	3.105 ± 0.014	7.303 ± 0.031	
570	3.16	7.40	20	$1.92 \cdot 10^{-4}$	0.003	3.140 ± 0.013	7.185 ± 0.078		
Average		3.08	7.39			3.055 ± 0.022	7.336 ± 0.032		
2	470	3.11	7.26	44	$1.58 \cdot 10^{-4}$	0.002	3.101 ± 0.010	7.203 ± 0.011	
3	470	3.10	7.24	59	$1.76 \cdot 10^{-4}$	0.002	3.108 ± 0.007	7.228 ± 0.009	
4	470	3.04	7.26	36	$7.37 \cdot 10^{-4}$	0.005	3.012 ± 0.026	7.204 ± 0.030	
5	470	3.09	7.27	45	$2.54 \cdot 10^{-4}$	0.002	3.096 ± 0.011	7.226 ± 0.012	
6	470	3.09	7.28	37	$6.48 \cdot 10^{-4}$	0.004	3.078 ± 0.022	7.179 ± 0.024	
7	450	3.02	7.27	31	$2.73 \cdot 10^{-4}$	0.003	3.099 ± 0.020	7.199 ± 0.024	
8	490	3.02	7.39	35	$2.89 \cdot 10^{-4}$	0.003	3.006 ± 0.013	7.279 ± 0.030	
9	540	2.68 <sup>a</sup>	7.39	38	$4.14 \cdot 10^{-4}$	0.003	2.697 <sup>a</sup> ± 0.035	7.417 <sup>a</sup> ± 0.013	
10	550	2.94	7.38	49	$4.81 \cdot 10^{-4}$	0.003	2.901 ± 0.012	7.332 ± 0.015	
11	560	3.00	7.49 <sup>a</sup>	43	$4.89 \cdot 10^{-4}$	0.003	2.995 ± 0.008	7.547 <sup>a</sup> ± 0.043	
Average		3.05	7.30			3.044 ± 0.014	7.231 ± 0.020		

<sup>a</sup> Extraneous value which was not included in the calculation of the average value.

TABLE VII

Molar Absorption Coefficients for the  $H_2L^-$ ,  $HL^{2-}$  and  $L^{3-}$  Forms of SNAZOXs Evaluated by Various Graphical and Numerical Methods from the  $A$  vs pH Curves Measured by Techniques  $A$  and  $B$  (The charges on the species are omitted in the table)

Set	$\lambda$ nm	Read from the experimental curves			Graphical methods			JDC-LETAG					FA 608 + EY 608		
		$\epsilon_L$	$\epsilon_{HL}$	$\epsilon_{H_2L}$	$\epsilon_L$	$\epsilon_{HL}$	$\epsilon_{H_2L}$	$\epsilon_L \pm \sigma(\epsilon)$	$\epsilon_{HL} \pm \sigma(\epsilon)$	$\epsilon_{H_2L} \pm \sigma(\epsilon)$	$\epsilon_L$	$\epsilon_{HL}$	$\epsilon_L$	$\epsilon_{HL}$	
1	440	10 360	8 120	6 680	10 370	8 130	6 750	10 350 $\pm$ 17	8 090 $\pm$ 27	6 720 $\pm$ 32	10 390	8 040	10 390	8 040	6 560
	450	12 110	8 620	6 430	12 080	8 630	6 430	12 040 $\pm$ 18	8 660 $\pm$ 29	6 410 $\pm$ 38	12 100	8 610	12 100	8 610	6 280
	470	15 170	10 930	6 490	15 040	10 940	6 320	15 040 $\pm$ 25	10 890 $\pm$ 37	6 290 $\pm$ 59	15 060	10 900	15 060	10 900	6 270
	500	17 050	14 300	8 490	16 970	14 310	8 150	16 970 $\pm$ 24	14 310 $\pm$ 33	8 170 $\pm$ 61	16 950	14 380	16 950	14 380	8 400
	550	8 550	10 890	11 300	8 590	10 900	11 280	8 550 $\pm$ 20	10 950 $\pm$ 33	11 380 $\pm$ 60	8 610	11 050	8 610	11 050	11 260
2	560	6 810	8 930	10 800	6 160	8 940	10 710	6 150 $\pm$ 29	9 020 $\pm$ 43	10 780 $\pm$ 61	6 120	9 100	6 120	9 100	10 800
	570	4 310	6 240	9 870	4 050	6 250	9 880	4 050 $\pm$ 26	6 310 $\pm$ 38	9 970 $\pm$ 57	4 080	6 380	4 080	6 380	10 110
	580	2 370	3 560	8 550	2 440	3 560	8 800	2 440 $\pm$ 26	3 510 $\pm$ 37	8 850 $\pm$ 58	2 430	3 550	2 430	3 550	8 950
	470	14 810	11 300	6 830	14 870	11 310	6 720	14 850 $\pm$ 15	11 190 $\pm$ 17	6 690 $\pm$ 22					
	3	470	14 750	10 880	6 540	14 690	10 850	14 690 $\pm$ 23	10 770 $\pm$ 24	6 380 $\pm$ 31					
3	470	15 000	11 060	6 370	15 000	11 120	6 270	14 950 $\pm$ 41	11 010 $\pm$ 40	6 280 $\pm$ 51					
	5	470	15 000	10 690	6 940	11 310	6 430	14 990 $\pm$ 39	10 670 $\pm$ 28	6 190 $\pm$ 46					
	6	470	15 020	10 730	6 850	14 960	6 300	15 040 $\pm$ 25	11 150 $\pm$ 25	6 440 $\pm$ 25					
	7	450	12 020	8 380	5 460	12 000	8 390	11 990 $\pm$ 18	8 310 $\pm$ 17	5 430 $\pm$ 28					
	8	560	6 157	9 210	13 680	6 180	9 220	13 950	6 140 $\pm$ 13	9 270 $\pm$ 13	13 930 $\pm$ 20				
9	580	2 520	3 290	10 690	2 550	3 290	2 520 $\pm$ 25	3 340 $\pm$ 25	10 890 $\pm$ 25						



TABLE VIII

Molar Absorption Coefficients for the  $H_2L^-$ ,  $HL^{2-}$  and  $L^{3-}$  Forms of Naphthylazoxine 6S Evaluated by Various Graphical and Numerical Methods from the *A* vs pH Curves Measured by Techniques *A* and *B*. (The charges on the species are omitted in the table)

Set	$\lambda$ nm	Read from the experimental curves			Graphical methods			JDC-LETAG			FA 608 + EY 608		
		$\epsilon_L$	$\epsilon_{HL}$	$\epsilon_{H_2L}$	$\epsilon_L$	$\epsilon_{HL}$	$\epsilon_{H_2L}$	$\epsilon_L \pm \sigma(\epsilon)$	$\epsilon_{HL} \pm \sigma(\epsilon)$	$\epsilon_{H_2L} \pm \sigma(\epsilon)$	$\epsilon_L$	$\epsilon_{HL}$	$\epsilon_{H_2L}$
1	430	12 610	9 870	7 870	12 620	9 870	7 970	12 560 $\pm$ 28	9 770 $\pm$ 20	7 950 $\pm$ 50	12 560	9 800	8 060
	450	15 980	11 860	6 990	15 980	11 870	7 170	16 000 $\pm$ 30	11 750 $\pm$ 23	7 150 $\pm$ 47	16 050	11 820	7 240
	470	19 860	15 610	8 740	19 990	15 630	8 840	19 200 $\pm$ 56	15 520 $\pm$ 43	8 770 $\pm$ 86	19 990	15 600	8 840
	490	22 600	19 480	11 740	22 540	19 500	11 770	22 530 $\pm$ 40	19 330 $\pm$ 32	11 760 $\pm$ 64	19 430	22 660	11 960
	540	9 120	14 860	15 730	9 100	14 880	14 960	9 090 $\pm$ 10	14 740 $\pm$ 54	15 370 $\pm$ 27	9 310	14 860	15 060
	550	5 620	10 740	14 990	5 660	10 750	14 900	5 670 $\pm$ 54	10 860 $\pm$ 38	14 940 $\pm$ 91	5 760	10 770	14 820
2	560	3 000	5 990	13 990	3 030	6 000	14 150	3 040 $\pm$ 43	6 030 $\pm$ 33	14 120 $\pm$ 66	3 020	5 910	14 330
	570	1 500	2 500	11 740	1 490	2 500	11 470	1 510 $\pm$ 42	2 740 $\pm$ 35	11 490 $\pm$ 65	1 470	2 640	11 590
	470	19 010	14 620	8 630	18 970	14 620	8 600	18 920 $\pm$ 19	14 600 $\pm$ 15	8 500 $\pm$ 27			
	3	470	19 040	14 620	8 600	19 010	14 620	18 990 $\pm$ 14	14 650 $\pm$ 14	8 460 $\pm$ 16			
	4	470	19 120	14 560	8 510	19 070	14 560	19 000 $\pm$ 60	14 560 $\pm$ 49	8 420 $\pm$ 67			
	5	470	19 040	14 620	8 570	19 060	14 620	19 000 $\pm$ 21	14 600 $\pm$ 22	8 430 $\pm$ 26			
	6	470	19 120	14 420	8 570	19 140	14 560	19 110 $\pm$ 54	14 380 $\pm$ 42	8 430 $\pm$ 58			
	7	450	16 080	11 670	6 610	15 920	11 700	15 880 $\pm$ 30	11 630 $\pm$ 33	6 300 $\pm$ 37			
	8	490	21 930	19 010	12 570	22 030	19 010	21 920 $\pm$ 31	18 970 $\pm$ 28	12 460 $\pm$ 37			
	9	540	8 950	15 790	18 600	8 950	15 700	8 960 $\pm$ 35	15 710 $\pm$ 28	18 780 $\pm$ 51			
	10	550	5 910	11 110	17 980	5 940	11 110	5 940 $\pm$ 29	11 210 $\pm$ 29	18 220 $\pm$ 41			
11	560	3 540	5 700	16 670	3 460	5 700	3 470 $\pm$ 35	5 670 $\pm$ 30	17 040 $\pm$ 38				

part of Eqs (2) and (3) are given. In addition, numerical non-linear regression of the  $A$  vs pH curve using the JDC-LETAG program and factor analysis of absorbance matrices were employed. Various numerical and graphical techniques and two experimental procedures yield identical results. The values obtained by the JDC-LETAG program are considered to be the more reliable.

A survey of all the determined dissociation constants and comparison with the literature data are given in Table IX. We consider the dissociation constants obtained by non-linear regression with the JDC-LETAG program and denoted by letter  $f$  in Table IX to be the final values for the individual media.

TABLE IX  
Survey of Dissociation Constants of SNAZOXS and Naphthylazoxine 6S

Equilibrium	Medium	$-\log K^a$	
		SNAZOXS	Naphthylazoxine 6S
$L^{-3}/HL^{-2}$	$I$ 0.1, 25°C	6.96 <sup>c,e</sup>	7.39 <sup>c,e</sup>
	(Na <sub>3</sub> PO <sub>4</sub> , NaClO <sub>4</sub> ,	6.967 ± 0.030 <sup>c,f</sup>	7.336 ± 0.032 <sup>c,f</sup>
	K III, HClO <sub>4</sub> )	6.954 ± 0.012 <sup>d,g</sup>	7.377 ± 0.021 <sup>d,g</sup>
	$I$ 0.1, 25°C	7.04 <sup>b,e</sup>	7.30 <sup>b,e</sup>
	(Na <sub>3</sub> PO <sub>4</sub> , NaCl,	7.003 ± 0.022 <sup>b,f</sup>	7.231 ± 0.020 <sup>b,f</sup>
	K III, HClO <sub>4</sub> )		
	$I$ 0.1, 25°C	7.0 <sup>h</sup>	7.4 <sup>h</sup>
$HL^{-2}/H_2L^{-}$	$I$ 0.1, 25°C	3.00 <sup>c,e</sup>	3.08 <sup>c,e</sup>
	(Na <sub>3</sub> PO <sub>4</sub> , NaClO <sub>4</sub> ,	2.992 ± 0.047 <sup>c,f</sup>	3.055 ± 0.022 <sup>c,f</sup>
	K III, HClO <sub>4</sub> )	2.912 ± 0.007 <sup>d,g</sup>	3.162 ± 0.011 <sup>d,g</sup>
	$I$ 0.1, 25°C	2.90 <sup>b,e</sup>	3.05 <sup>b,e</sup>
	(Na <sub>3</sub> PO <sub>4</sub> , NaCl,	2.878 ± 0.12 <sup>b,f</sup>	3.044 ± 0.014 <sup>b,f</sup>
	K III, HClO <sub>4</sub> )		
	$I$ 0.1, 25°C	3.0 <sup>h</sup>	3.1 <sup>h</sup>
$H_2L^{-}/H_3L$	HClO <sub>4</sub> , 25°C	0.082 ± 0.001 <sup>d,g</sup>	0.295 ± 0.148 <sup>d,g</sup>
$H_3L/H_4L^{+}$	HClO <sub>4</sub> , 25°C	-1.371 ± 0.000 <sup>d,g</sup>	-0.958 ± 0.102 <sup>d,g</sup>

<sup>a</sup> Average taken from selected values (Tables VII, VIII); <sup>b</sup> from  $A$  vs pH curves measured by technique (A); <sup>c</sup> from  $A$  vs pH curves measured by technique (B); <sup>d</sup> from pH-absorbance spectra measured by technique (B); <sup>e</sup> evaluated graphically; <sup>f</sup> evaluated by non-linear regression using the JDC-LETAG program; <sup>g</sup> evaluated by matrix analysis of the spectra using the FA 608 + EY 608 program; <sup>h</sup> literature data, see ref.<sup>16</sup>.

*Reactions of SNAZOXS and Naphthylazoxine 6S with Various Metal Cations*

The two azoxines S are often used in analytical practice as metallochromic indicators, as they form complexes with many metals which are mostly yellow coloured. In Table X is given a survey of their colour reactions with a number of metal cations. In our laboratory, complexation equilibria of the two azoxines S with many metals

TABLE X  
Survey of Colour Reactions of SNAZOXS and Naphthylazoxine 6S with Various Metal Cations

Cation	Metal : indicator concentration ratio	SNAZOXS $c = 1.6 \cdot 10^{-4}M$ ; pH 5.3 (red solution)	Naphthylazoxine 6S $c = 1.4 \cdot 10^{-4}M$ ; pH 5.3 (red solution)
Pb <sup>2+</sup> Cu <sup>2+</sup> Ni <sup>2+</sup> Co <sup>2+</sup> Zn <sup>2+</sup> Al <sup>3+</sup> Sc <sup>3+</sup> Zr <sup>4+</sup>	10 : 1	yellow solution	
Sn <sup>2+</sup> Ce <sup>4+</sup>	2 : 1	solution remains yellow at pH < 1.3	
Ag <sup>+</sup> Th <sup>+</sup> La <sup>3+</sup> Cr <sup>3+</sup>	10 : 1	orange solution	
Hg <sub>2</sub> <sup>2+</sup> Hg <sup>2+</sup>	10 : 1 2 : 1	red-orange solution blackens due to reduced Hg orange solution; at higher metal-to-indicator ratios the solution becomes turbid	
Bi <sup>3+</sup>	2 : 1	orange solution becomes turbid at higher excesses due to hydrolysis of excess Bi	
Fe <sup>3+</sup>	10 : 1	yellow-brown solution turbid due to the presence of Fe(OH) <sub>3</sub> precipitate	
Mn <sup>2+</sup>	10 : 1	orange solution slowly becomes turbid due to the presence of a brown precipitate	
Ca <sup>2+</sup> Sr <sup>2+</sup> Ba <sup>2+</sup>	10 : 1 10 : 1 10 : 1	no colour change red precipitate slowly formed purple solution; purple precipitate formed slowly	slow formation of a red precipitate formed slowly

have been studied. The results will be published in the following communications in this series.

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#### REFERENCES

1. Fritz J. S., Lane W. J., Bystroff A. S.: *Anal. Chem.* 29, 821 (1957).
2. Busev A. I., Talipova L. L.: *Zh. Anal. Khim.* 17, 447 (1962).
3. Guerrin G., Sheldon M. V., Reilley C. N.: *Chemist Analyst* 49, 36 (1960).
4. Fritz J. S., Abbink J. E., Payne M. A.: *Anal. Chem.* 33, 1381 (1961).
5. Busev A. I., Talipova L. L.: *Vestn. Mosk. Univ., Khim.* 2, 63 (1962).
6. Busev A. I., Skrebkova L. M., Talipova L. L.: *Zh. Anal. Khim.* 17, 831 (1962).
7. Goyal S. S., Tandon J. P.: *Talanta* 14, 1449 (1967).
8. Goyal S. S., Tandon J. P.: *Talanta* 16, 106 (1969).
9. Tsuneo Shimizu: *Bunseki Kagaku* 16, 233 (1967).
10. Ingrid N., Lagerström G., Frydman M., Sillén L. G.: *Acta Chem. Scand.* 11, 1034 (1957).
11. Püschel R., Stefanec Z.: *Mikrochim. Acta* 1962, 1108.
12. Püschel R.: *Talanta* 10, 1125 (1963).
13. Vytřas K.: *Thesis*. Institute of Chemical Technology, Pardubice 1971.
14. Vytřas K., Vytřasová J., Kotrlý S.: *Talanta* 22, 529 (1975).
15. Meloun M., Čermák J.: *Talanta* 23, 15 (1976).
16. Meites L.: *Handbook of Analytical Chemistry*, p. 105, 108 McGraw-Hill, New York 1963.
17. Meloun M.: *Thesis*. Institute of Chemical Technology, Pardubice 1973.
18. Meloun M., Čermák J.: *Sb. Věd. Pr. Vys. Šk. Chemickotechnol., Pardubice* 30, 49 (1973).
19. Meloun M., Čermák J., Kotrlý S.: *Talanta*, in press.
20. Kankare J.: *Anal. Chem.* 42, 1322 (1970).
21. Tomíček I.: *Quantitative Analysis*, p. 144. Published by Státní zdravotnické nakladatelství, Prague 1954.
22. Holzbecher Z., Jeník J., Šůcha L., Vlášil F., Vrbský J.: *Analytical Chemistry*, p. 299. Published by SNTL, Prague 1968.
23. Biedermann G.: *Ark. Kemi* 9, 277 (1955).
24. Bates R. G.: *Determination of pH*. Wiley, New York 1964.
25. Říha V., Kotrlý S., Meloun M.: *Improvement Suggestion*, Institute of Chemical Technology, Pardubice, No 9/74 (1974).
26. Říha V., Meloun M.: *Chem. Listy* 68, 1289 (1974).
27. Říha V., Meloun M.: *Czech.* 149 408 (1972).
28. Říha V., Meloun M.: *Ger. (GDR)* 99008 (1972).
29. Havel J.: *Chem. Listy* 62, 1250 (1968).

30. Kubáň V., Sommer L., Havel J.: *This Journal* 40, 604 (1975).
31. Forsling W., Hietanen S., Sillén L. G.: *Acta Chem. Scand.* 6, 901 (1952).
32. Brown A. S.: *J. Amer. Chem. Soc.* 56, 56 (1934).
33. Sommer L., Kučerová J., Procházková H., Hnilíčková M.: *Publ. Fac. Sci. Univ. Brno* 1965, No 464, 249.
34. Sommer L., Kubáň V., Havel J.: *Folia Fac. Sci. Nat. Univ. Purkynianae Brunensis* 11, (Chemia 7) Part 1 (1970).
35. Wernimont G.: *Anal. Chem.* 39, 554 (1967).

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