Complexation Equilibria of Some Sulphoazoxines

Part VII.* Formation of Oligomers by Naphthylazoxine 6S, SNAZOXS and 2-, 3- and 4-CAPAZOXS Evaluated by Regression Analysis of Potentiometric Data

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The formation of oligomers by the five sulphoazoxines Naphthylazoxine 6S, SNAZOXS and 2-, 3- and 4-CAPAZOXS was studied potentiometrically. Two pH scales were used. Either proton activity was measured and the system was calibrated with standard buffers, or the hydrogen ion concentration was measured and the system was calibrated externally in terms of $h = [H^+]$. At concentrations higher than 1 mmol dm^{-3} dimers and trimers are formed, for which a reaction scheme is proposed. The concentration scale (h) gives more precise and more accurate values for the formation constants because external calibration of the glass electrode cell in terms of log h is more accurate than calibration with different NBS buffers. The main reason is that calibration with buffers is carried out in a medium very different from that used in the actual titration. The algorithm used has almost no influence on the precision of the formation constants in comparison with the reproducibility of the titration.

Keywords: *Computer-assisted regression analysis of potentiometric data; multiparametric curve fitting; sulphoazoxines; protonated oligomer formation; acidity constants*

The protonation equilibria of metallochromic indicators have been studied for some time in our laboratory. In this work oligomer formation was studied with Naphthylazoxine 6S [7-(6-sulpho-2-naphthylazo)-8-hydroxyquinoline-5-sulphonic acid] (**I**), SNAZOXS [7-(4-sulpho-1-naphthylazo)-8-hydroxy-quinoline-5-sulphonic acid] (**II**) and 2-, 3- and 4-CAPAZOXS [7-(2-, 3-, and 4-carboxyphenylazo)-8-hydroxyquinoline-5-sulphonic acid] (**III, IV** and **V**).
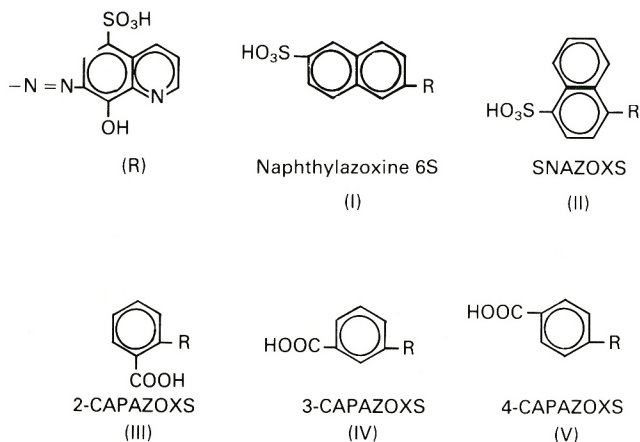
At a concentration of 10^{-6} mol dm^{-3} only monomers are formed and their protonation equilibria have been studied.[1-6] Above this concentration oligomers are formed and even in the range 2<pH< 4 some sulphoazoxines agglomerate and sometimes precipitation occurs.

The aim of this study was to determine the oligomers formed by the above five sulphoazoxines and their protonation equilibria. The reliability of the chemical model found is discussed, together with the influence of the regression algorithm used and the reproducibility of the titrations.

Experimental

Chemicals and Solutions

SNAZOXS and Naphthylazoxine 6S of analytical-reagent grade were obtained from Spolana (Neratovice, Czechoslovakia) and purified from a concentrated solution with acetone. In the first portion of the precipitate, coloured impurities were detected chromatographically. In the remainder of the precipitate the pure indicator was found. The purified indicator was washed with acetone and dried at 60 °C (333 K) and then converted into the acid form by passing it through a hydrogen-saturated cation exchanger.

The synthesis of 2-, 3- and 4-CAPAZOXS has been described elsewhere.[4] Their purity was checked by paper chromatography with Chelaton 3 using the descending technique and eluted with water - pyridine - ethanol (2 + 1 + 4). Solutions of all three derivatives are stable in alkaline and neutral media for at least 200 h. In acidic solutions (pH ≈1)

they begin to be salted-out and precipitate. Fortunately, this process is reversible.

The concentration of each sulphoazoxine was determined by potentiometric titration with NaOH using the MAGEC program[7,8] and the log h scale. This program uses the Gran method to determine the number of protons titrated. In this way the purity of the indicators was found to be Naphthylazoxine 6S 78.3, SNAZOXS 97.3, 2-CAPAZOXS 79.0, 3-CAPAZOXS 56.3 and 4-CAPAZOXS 83.2%. The impurities were mostly inorganic salts.

The sodium hydroxide solution was prepared from a 50% m/V solution stored for several days in a polyethylene bottle. The clear supernatant solution was siphoned off and diluted with carbon dioxide-free water to obtain a 1 mol dm^{-3} solution. This solution was standardised against potassium hydrogen phthalate using the MAGEC program and the data were refined by the non-linear least-squares program ACBA.[9]

Perchloric acid was prepared by dilution of 70% $HClO_4$ of analytical-reagent grade with doubly distilled, de-ionised water and standardised against HgO and KI with a reproducibility of ±0.2%.

Apparatus

All e.m.f. measurements were carried out at 298 ± 0.1 K. The e.m.f. (in millivolts) was measured on a PHM-64 digital voltmeter (Radiometer, Copenhagen, instrumental precision ±0.1 mV). The electrodes were a G202B glass electrode (Radiometer) and an OP-0830P SCE reference electrode (Radelkis, Budapest, Hungary). Titrations were performed in a 100-cm^3 water-jacketed glass vessel closed with a bung containing the electrodes, an argon inlet, a thermometer, a propeller stirrer and the polyethylene capillary tip of a microburette.

During the experiments a stream of argon was bubbled through the solution for stirring and maintaining an inert atmosphere. Before the argon entered the titration vessel it was passed through a pure ionic medium ($I = 0.25$ mol dm^{-3} for Naphthylazoxine 6S and SNAZOXS and $I = 0.12$ for 2-, 3- and 4-CAPAZOXS).

As the reliability of stability constant work is dependent on the precision and accuracy of the burette used, a set of four home-made syringe microburettes with micrometer screws was developed in our laboratory.[10] Of the microburettes PK0250 (capacity 250 μl), PK0500 (500 μl), PK1250 (1250 μl) and PK2500 (2500 μl) only two, PK1250 [$v = 1245.4$ μl, $s(v) = 0.25$ μl, $s_{rel}(v) = 0.020\%$] and PK2500 [$v = 2496.8$ μl, $s(v) = 0.31$ μl, $s_{rel}(v) = 0.012\%$] were used here. The microburettes were calibrated by weighing the delivered water on a Sartorius 1712 MP8 balance in a weighing bottle closed with a top-cap. The polyethylene capillary tip of the microburette was immersed in the water through a hole of 1 mm diameter in the top-cap. The piston cylinder was demonstrated to have uniform dimensions over its whole length by making ten successive additions of water using a 2.5 mm rise each time over the 25 mm range of the micrometer screw. The microburette was calibrated by ten replicate determinations of the total volume of the delivered water, and the arithmetic mean, standard deviation and relative standard deviation were calculated.

When sulphoazoxines were titrated, the polyethylene capillary tip of the microburette was immersed in the solution when adding the reagent, but removed after each addition in order to prevent leakage. The sodium hydroxide was titrated with standard $HClO_4$ before each sulphoazoxine titration in order to obtain the correct concentration (maximum change up to 0.5%).

Titration Procedure

The potentiometric titrations were performed with the use of two different pH scales: proton activity, a_{H^+}, calibrated with standard buffers, and hydrogen ion concentration, $h = [H^+]$, calibrated externally by titrating a known amount of acid with sodium hydroxide, and the data were evaluated with the MAGEC program.

For the pa_{H^+} scale, the pH meter was calibrated using the Irving method[11] and the commercial buffers S1500, 1510 and 1316 (Radiometer) with pH values at 298 K of 6.865, 7.410 and 4.010, respectively, the actual values of temperature and Nernstian slope being adjusted on the voltmeter. For the log h scale, the hydrogen ion concentration, h, was determined by measuring the e.m.f. for each addition of base to a solution of perchloric acid in the ionic medium used and the data were treated using the MAGEC program. Special attention was paid to the "group" parameters $E^{\circ\prime}$ and the Nernstian slope, S, in the expression

$$E = E^{\circ\prime} + \frac{RT}{F}\ln h + E_j - E_{SCE} = E^{\circ\prime} + S\log h \quad . . \quad (1)$$

Data were taken in the acidity range where variations in E_j can be neglected so that this quantity, like E_{SCE}, can be included in $E^{\circ\prime}$. The ionic strength was kept at 0.25 mol dm^{-3} (Na, H)ClO$_4$ for Naphthylazoxine 6S but at 0.12 mol dm^{-3} (Na, H)ClO$_4$ for 2-, 3- and 4-CAPAZOXS. The parameters $E^{\circ\prime}$, S and pK_w were refined iteratively by the MAGEC program. The resulting values represent the calibration adjustment of the hydrogen concentration scale and were used in the input to the program MINIQUAD[12] for final treatment of the sulphoazoxine data. In MINIQUAD, $E^{\circ\prime}$ can be further refined together with H, L and K_w (where H and L are the total proton and ligand concentrations, respectively).

The titrations of ligand were performed in the same way as the calibration titrations.

Computation

In the data treatment the programs MINIQUAD, MIQUV, MAGEC and PSEQUAD[13–15] were used. The computations were performed on an EC 1033 computer at the Computing Centre, College of Chemical Technology, Pardubice, Czechoslovakia.

Determination of chemical model

Assume that protons (H) and ligand (L) form various species according to the reaction

$$r\text{H} + q\text{L} \rightleftharpoons \text{H}_r\text{L}_q \; (\beta_{qr}) \quad . . \quad . . \quad . . \quad (2)$$
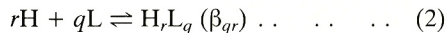
where the formation constant is given by

$$\beta_{qr} = [\text{H}_r\text{L}_q]/(h^r \, l^q) \quad . . \quad . . \quad . . \quad (3)$$

where h and l are the free concentrations of hydrogen ion and ligand, respectively. The mass balance conditions are

$$L = l + q\Sigma\beta_{qr}h^r l^q \quad . . \quad . . \quad . . \quad (4a)$$

and

$$H = h + r\Sigma\beta_{qr}h^r l^q \quad . . \quad . . \quad . . \quad (4b)$$

The activity coefficients are assumed to be kept constant by the ionic medium. For the pa_{H^+} scale, h in equation (3) is substituted for a_{H^+}, the constant β_{qr} now being a mixed constant.

For potentiometric titrations, the following relationship holds for the total hydrogen ion concentration:

$$H_{exp} = (H_0V_0 + H_T v_T)/(V_0 + v_T) \quad \cdot \cdot \quad (5)$$

where H_0 = total initial concentration of hydrogen ions in the titrand, H_T = total initial concentration of hydrogen ions in the titrant (for hydroxide $-H_T$ is given), V_0 = initial volume of solution in the titration vessel (titrand) and v_T = volume of titrant added.

The program MINIQUAD and the revised version MINIQUAD-83[13] calculates a set of formation constants and concentrations of all species present for a given model by minimising the residual-squares sum U_1 defined by

$$U_1 = \sum_i \left[w(L_{exp} - L_{calc})^2 \right]_i + \left[w(H_{exp} - H_{calc})^2 \right]_i = \text{minimum} \quad .. \quad .. \quad (6)$$

The summation is made over all experimental points, w is the statistical weight, usually taken as unity, and $L_{calc,i}$ and $H_{calc,i}$ are calculated from the mass balance conditions, equations (4a) and (4b).

A number of models can be tested and the one that gives the lowest U_1 value and also fulfils some other statistical criteria is selected as the most plausible (degree-of-fit test).

The degree-of-fit is performed by statistical analysis of the residuals in L and H as given in equation (6). The residuals should be randomly distributed about the predicted regression curve. Systematic departures indicate that the model is not

Table 1. Search for the best model for the formation of oligomers in the system H^+ - Naphthylazoxine 6S by regression analysis of one potentiometric titration curve (*cf.,* Fig. 1) using the program MINIQUAD. In parentheses are given the estimated standard deviation in units of the last digit(s) in log β_{qr}. The lowest and highest values in % of each species within titration are also given

Species L_qH_r in model	Parameter	Estimated log β_{qr}, $s(\log \beta_{qr})$, $[L_qH_r]_{min}$ and $[L_qH_r]_{max}$						
		1st model	2nd model	3rd model	4th model	5th model	6th model	7th model
LH ..	Log $\beta_{qr}(s)$	8.91(14)	7.40	7.40	7.40	7.40	7.40	7.40
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %	9.4, 99.2	0.2, 0.8	0.2, 0.8	0.2, 0.8	0.2, 0.8	0.2, 0.8	0.2, 0.8
LH_2 ..	Log $\beta_{qr}(s)$	13.01(29)	10.50	10.50	10.50	10.50	10.50	10.50
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %	0.0, 67.3	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0
L_2H_2 ..	Log $\beta_{qr}(s)$	—	20.17(9)	20.17(4)	20.28(1)	20.28(1)	20.23(1)	19.89(49)
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %		5.6, 99.1	6.0, 98.2	6.0, 97.7	5.7, 97.7	5.7, 97.2	1.0, 71.3
L_2H_4 ..	Log $\beta_{qr}(s)$	—	28.98(13)	28.21(61)	28.32(8)	28.60(2)	28.55(1)	27.95(83)
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %		0.0, 94.2	0.0, 17.3	0.0, 17.1	0.0, 31.0	0.0, 31.0	0.0, 23.4
L_2H_3 ..	Log $\beta_{qr}(s)$	—	—	25.03(8)	25.15(1)	25.06(1)	25.01(1)	24.62(49)
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %			0.0, 76.0	0.0, 76.2	0.0, 58.9	0.0, 58.9	0.0, 71.2
L_2H_5 ..	Log $\beta_{qr}(s)$	—	—	31.52(119)	31.64(14)	Negative	—	—
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %			0.0, 5.7	0.0, 5.8			
L_2H ..	Log $\beta_{qr}(s)$	—	—	—	11.09(2)	11.09(1)	10.90(2)	Negative
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %				0.0, 40.2	0.0, 40.2	0.0, 28.5	
L_3H_4 ..	Log $\beta_{qr}(s)$	—	—	—	—	35.69(7)	35.61(3)	Negative
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %					0.0, 16.0	0.0, 16.1	
L_3H_2 ..	Log $\beta_{qr}(s)$	—	—	—	—	—	21.65(3)	Negative
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %						0.0, 12.4	
L_4H_3 ..	Log $\beta_{qr}(s)$	—	—	—	—	—	—	35.24(77)
	$[L_qH_r]_{min}$, $[L_qH_r]_{max}$, %							0.0, 90.87
L_4H_4 ..	Log $\beta_{qr}(s)$	—	—	—	—	—	—	Negative

Degree-of-fit achieved by statistical analysis of residuals:

U_{min}	2.21×10^{-3}	2.54×10^{-4}	5.39×10^{-5}	7.58×10^{-7}	4.41×10^{-7}	9.90×10^{-8}	8.84×10^{-4}
$\|\bar{r}\| \times 10^5$	433	132	46.4	7.9	5.4	2.5	220
$s(r) \times 10^5$	490	166	76.5	9.1	6.9	3.3	310
R-factor, %	14.34	4.86	2.24	0.27	0.20	0.10	9.06
Model testing	Rejected	Rejected	Rejected	Rejected	Rejected	Accepted	Rejected

Table 2. Formation constants of oligomers for five sulphoazoxines by regression analysis of potentiometric titration curves using MINIQUAD. The upper row for each constant refers to the hydrogen ion scale (h) and the lower row to the activity scale (a_{H^+}). In parentheses are given the estimated standard deviation in units of the last digit(s) in log β_{qr}. ($\times$) indicates that $s(\log \beta_{qr})$ could not be estimated

| Parameter	Naphthylazoxine 6S	SNAZOXS	2-CAPAZOXS	3-CAPAZOXS	4-CAPAZOXS
Experimental conditions:					
$L \times 10^2$/mol dm^{-3} ..	4.071	5.205	0.8687	0.6052	0.9318
	5.701	6.166	1.263	0.4007	2.806
Ionic strength	0.25	0.25	0.12	0.12	0.12
Log β_{qr} [s($\log \beta_{qr}$)]:					
Log β_{22}	20.05(1)	18.80(1)	21.26(1)	18.27(2)	18.50(1)
	19.66(24)	18.22(54)	20.58(1)	16.85($\times$)	18.17(2)
Log β_{24}	28.16(1)	27.22(1)	—	27.97(25)	28.01(2)
	27.25(34)	25.06	—	27.03(80)	27.75(9)
Log β_{23}	24.75(1)	23.43(1)	26.09(2)	—	23.44(2)
	24.31(34)	21.97($\times$)	26.05(3)	—	22.89(11)
Log β_{21}	10.79(3)	10.00(2)	11.50(2)	10.24(4)	10.22(1)
	10.79(61)	10.09(55)	10.05(24)	9.33($\times$)	10.17(3)
Log β_{34}	35.17(5)	—	—	33.78(11)	—
	34.70(99)	—	—	32.29($\times$)	—
Log β_{32}	21.32(6)	—	—	—	—
	19.19($\times$)	—	—	—	—
Degree-of-fit achieved:					
R-factor, %	0.12	0.14	0.41	0.62	0.27
	5.18	19.26	0.48	11.45	0.95
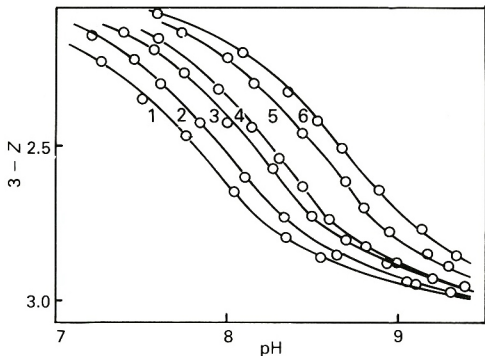
Fig. 1. Potentiometric titration of Naphthylazoxine 6S with sodium hydroxide in normalised co-ordinates (pH, Z) for six different concentrations of sulphoazoxine: (1) 2.30; (2) 4.64; (3) 9.40; (4) 17.72; (5) 34.48; and (6) 59.03 mmol dm^{-3}

adequate and/or some other parametric estimates are not satisfactory. The arithmetic mean of the residuals, $m_{r,1}$, should be close to zero and the mean residual, $|\bar{r}|$, should be close to the instrumental error of the variable from which the residuals are calculated. For a Gaussian distribution the skewness should be close to zero while the kurtosis, representing the "peakedness" of the distribution curve, should be close to 3. The Hamilton R-factor of relative fitness is conveniently expressed in per cent. and permits a comparison of the fit obtained by different titrations.

The program MIQUV[14] calculates those values of β_{qr} which minimise the residual-squares sum (U_2), again taken over all experimental points:

$$U_2 = \sum_i [w(E_{exp} - E_{calc})^2]_i = \text{minimum} \quad .. \quad (7)$$

The statistical weight w_i is defined by

$$w_i = 1/\sigma_i^2 \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where σ_i^2 is the estimated variance of the ith experimental point. In MIQUV only instrumental uncertainties in the e.m.f. readings σ_E and in the added titrant volume, σ_v, are taken into account, *i.e.*,

$$\sigma_i^2 = \sigma_E^2 + \left(\frac{\partial E_i}{\partial v_i}\right)^2 \sigma_v^2 \quad .. \quad .. \quad .. \quad (9)$$

In addition to the formation constants (common parameters), MIQUV can also refine group parameters (parameters characteristic of each titration). Such parameters are H_0, H_T, $E^{\circ\prime}$ and S in equation (1) and V_0.

The reliability of the model tested is performed in the same manner as with MINIQUAD.

The program PSEQUAD[15] solves the mass balance conditions [*cf.*, equations (4a) and (4b)] using a derivative method of minimising the residual-squares sum U_3:

$$U_3 = w_1 \sum_i (\Delta v_i)^2 + \sum_j \sum_i w_j (\Delta E_j)^2 \quad .. \quad (10)$$

where v_i = residuals in titrant volume for the ith point or residual in L or H, ΔE_j = residual in e.m.f. for the ith point as measured by the jth electrode; w_1 = weighting factor in volume or L, H; and w_j = weighting factor for the e.m.f. ($E_{cell, j}$) measured by the jth electrode.

PSEQUAD is able to evaluate simultaneously different types of measurements carried out in solutions of varying composition but also in a group of solutions, where the number of components differ from one group to another. As mentioned, there are several options, minimisation of volume or total concentrations as well as various e.m.f.s either alone or together, such as v_i and E_i together (orthogonal regression).

Errors in estimated formation constants

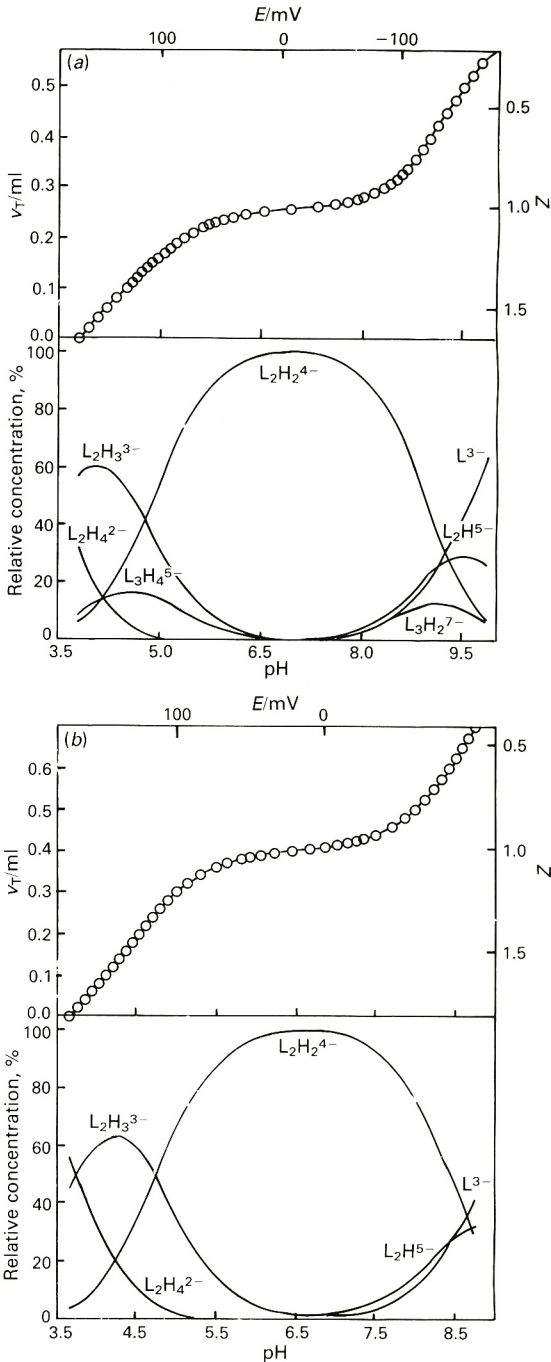The analysis of variance, ANOVA, can be applied to formation constants using k identical titrations treated by m

Fig. 2. Potentiometric titration curves of (a) Naphthylazoxine 6S and (b) SNAZOXS with 0.988 mol dm^{-3} NaOH in original (v_T, E) and normalised (pH, Z) co-ordinates and their chemical interpretation using distribution diagrams of the relative concentration population of all species in the chemical model found using MINIQUAD. $Z = (H - h)/L$, $L = 40.71$ mmol dm^{-3} Naphthylazoxine 6S or 52.05 mmol dm^{-3} SNAZOXS, $I = 0.25$ (NaClO$_4$), $T = 298$ K

different programs in order to examine the accuracy of the calculated formation constants.

The dependence of β_{qr} on various errors may be written as

$$\log\beta_{qr,exp} = \log\beta_{qr} + \varepsilon_{tit} + \varepsilon_{prog} + \varepsilon_i \quad .. \quad (11)$$

where $\log \beta_{qr}$ = "true" value of β_{qr}, ε_{tit} = systematic error in the k titrations performed, ε_{prog} = systematic error in the m programs used and ε_i = random error.

The following standard deviations are introduced: the intra-titration (or point-to-point) standard deviation, σ_i, the inter-titration (or titration to titration) standard deviation,

σ_{tit}, the inter-program (or program to program) standard deviation, σ_{prog}. The resulting standard deviation in $\log \beta_{qr}$ can be now expressed by[16]

$$\sigma_{qr} = \sqrt{\sigma_i^2 + \sigma_{\text{tit}}^2 + \sigma_{\text{prog}}^2} \quad .. \quad .. \quad (12)$$

Two null hypotheses can be tested in order to find if the effects of titrations and programs are significant:

(1) H_0: $\varepsilon_{\text{tit}} = 0$ versus H_1: $\varepsilon_{\text{tit}} \neq 0$;
(2) H_0: $\varepsilon_{\text{prog}} = 0$ versus H_1: $\varepsilon_{\text{prog}} \neq 0$.

(i) If the variance ratio $F_{\text{exp}} = \sigma_{\text{tit}}^2/\sigma_{\text{res}}^2 < F_{\text{crit}} [\alpha, (k-1), (k-1)(m-1)]$ where α is the significance level and σ_{res}^2 is the variance coming from random errors in ANOVA, then hypothesis H_0, i.e., $\varepsilon_{\text{tit}} = 0$, is accepted and all experimental

points belong to the same population and there is no significant difference between titrations.

(ii) In the same manner, if $F_{\text{exp}} = \sigma_{\text{prog}}^2/\sigma_{\text{res}}^2 < F_{\text{crit}} [\alpha, (m-1), (k-1)(m-1)]$, the hypothesis H_0, i.e., $\varepsilon_{\text{prog}} = 0$, is accepted and all formation constants evaluated in each program belong to the same population and there is no significant difference between programs.

In order to establish if all titration points belong to the same data population, each titration is first treated separately to give $(\log \beta_{qr} \pm \sigma)_i, i = 1, \ldots, k$. Then all k titrations are used to calculate $\log \beta_{qr,\text{av}}$ with the variance σ_{av}^2 and $(k-1)$ degrees of freedom.

Statistical testing is then carried out by setting $\sigma_{\text{av}}^2 = \sigma_i^2/n_j + \sigma_{\text{tit}}^2$, where n_j is the number of points in the jth titration.[16] If

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Table 3. Formation constants, $\log \beta_{qr}$, of Naphthylazoxine 6S oligomers from identical titrations (1st to 6th columns) and a set of all six titrations together (last column) by the following five regression programs given from top to bottom of each row: A, MINIQUAD(L,H); B, Miquv(E); C, PSEQUAD(v_H); D, PSEQUAD(E); and E, PSEQUAD(ortho). Group parameters: $S = 56.54$ mV/log h, p$K_w = 13.78$ and $E^{\circ\prime}$ obtained from MAGEC. For each constant the estimated standard deviation is given in parentheses in units of the last digit(s) of $\log \beta_{qr}$. ($\times$) means that $s(\log \beta_{qr})$ could not be estimated

						Titration			
			Program	1st	2nd	3rd	4th	5th	6th	All
Points ..	..	..	..	48	55	45	41	42	46	277
$E^{\circ\prime}$/mV	..	..	..	382.3	395.5	382.3	396.3	394.3	382.3	382.3
$\log \beta_{21}$ ..	..	..	A	10.770(27)	10.724(11)	10.792(27)	10.853(16)	10.766(30)	10.799(21)	10.716(70)
			B	10.758(49)	10.726(20)	10.779(74)	10.849(28)	10.759(44)	10.801(41)	10.699(46)
			C	10.763(35)	10.728(15)	10.778(33)	10.851(20)	10.756(44)	10.799(28)	10.584(170)
			D	10.616(100)	10.776(44)	10.441(180)	10.772(58)	10.701(43)	10.794(55)	10.102(430)
			E	10.617(1)	10.788(1)	10.458(1)	10.791(1)	10.703(1)	10.791(1)	10.577(1)
$\log \beta_{22}$ ..	..	..	A	20.007(7)	19.773(4)	20.105(7)	20.071(5)	19.962(9)	20.030(6)	19.977(18)
			B	20.005(12)	19.773(6)	20.102(11)	20.070(8)	19.959(12)	20.031(10)	19.972(11)
			C	20.006(9)	19.775(5)	20.102(8)	20.070(6)	19.959(13)	20.030(7)	19.746(40)
			D	19.975(19)	19.786(17)	20.048(22)	20.051(13)	19.946(10)	20.029(13)	19.671(38)
			E	19.972(1)	19.781(1)	20.043(1)	20.042(1)	19.941(1)	20.030(1)	19.739(1)
$\log \beta_{32}$ ..	..	..	A	21.302(57)	20.900(60)	21.496(46)	21.199(69)	21.405(62)	21.469(33)	21.461(107)
			B	21.325(95)	20.885(113)	21.518(74)	21.220(118)	21.427(84)	21.470(63)	21.457(62)
			C	21.320(66)	20.883(78)	21.522(49)	21.217(78)	21.429(80)	21.473(41)	21.384(170)
			D	21.537(63)	18.095($\times$)	21.798(45)	21.518(83)	21.571(46)	21.486(66)	21.392(110)
			E	21.525(1)	19.738(8)	21.795(1)	21.500(1)	21.567(1)	21.488(1)	21.390(1)
$\log \beta_{23}$ ..	..	..	A	24.667(10)	24.715(10)	24.805(11)	24.827(10)	24.837(41)	24.702(9)	24.713(36)
			B	24.667(15)	24.717(13)	24.804(16)	24.827(13)	24.347(44)	24.705(14)	24.661(18)
			C	24.670(11)	24.721(9)	24.805(11)	24.828(10)	24.363(43)	24.707(10)	24.389(60)
			D	24.611(24)	24.656(42)	24.737(28)	24.787(22)	24.431(28)	24.670(18)	24.235(66)
			E	24.613(1)	24.654(1)	24.756(1)	24.756(1)	24.706(1)	24.663(1)	24.389(1)
$\log \beta_{24}$ ..	..	..	A	28.017(12)	28.450(12)	28.249(13)	27.883(26)	28.278(21)	28.126(11)	28.145(45)
			B	28.011(17)	28.450(15)	28.244(17)	27.881(29)	28.291(20)	28.125(17)	28.233(19)
			C	28.010(12)	28.449(11)	28.239(12)	27.881(22)	28.286(26)	28.121(12)	27.662(76)
			D	28.023(25)	28.538(40)	28.206(30)	27.923(38)	28.231(19)	28.169(19)	27.712(67)
			E	28.020(1)	28.537(1)	28.203(1)	27.920(1)	28.239(1)	28.168(1)	27.660(1)
$\log \beta_{34}$ ..	..	..	A	35.072(46)	35.146(39)	35.285(48)	34.617(220)	35.523(47)	35.211(35)	35.229(142)
			B	35.043(64)	35.136(52)	35.265(66)	34.591(426)	35.557(47)	35.194(55)	35.333(53)
			C	35.043(46)	35.130(38)	35.255(46)	34.596(210)	35.546(51)	35.186(36)	34.657(280)
			D	35.206(49)	35.417(67)	35.273(70)	35.037(110)	35.411(34)	35.377(34)	34.824(120)
			E	35.197(1)	35.414(1)	35.276(1)	35.166(1)	35.435(1)	35.375(1)	34.651(1)

Degree-of-fit achieved by statistical analysis of residuals:

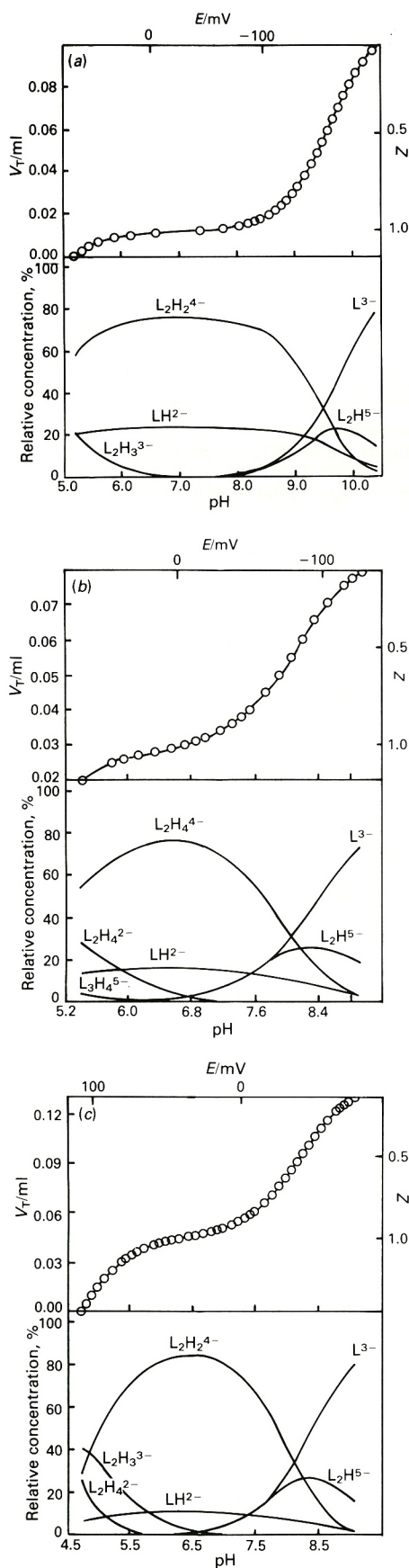|										
$\|\bar{r}\| \times 10^5$/M ..	..	..	A	3.68	3.14	3.58	3.47	6.08	2.60	27.06
$\|\bar{r}\| \times 10^5$/mV	..	..	B	0.47	0.42	0.45	0.43	0.90	0.34	3.63
$\|\bar{r}\| \times 10^3$/µl ..	..	..	C	1.12	1.16	1.04	1.06	2.17	0.90	$\times$
$\|\bar{r}\| \times 10^3$/mV	..	..	D	0.96	2.01	1.09	1.12	0.88	0.72	$\times$
$\|\bar{r}\| \times 10^3$/mV	..	..	E	0.97	2.07	1.30	1.24	5.51	0.75	$\times$
$s(r) \times 10^5$/M ..	..	..	A	4.32	4.50	4.16	4.25	7.71	3.38	39.27
$s(r) \times 10^5$/mV	..	..	B	0.60	0.65	0.55	0.58	1.31	0.48	5.23
$s(r) \times 10^3$/µl	..	..	C	1.20	1.23	1.12	1.15	2.34	0.97	17.31
$s(r) \times 10^3$/mV	..	..	D	1.03	2.13	1.17	1.21	0.95	0.78	7.58
$s(r) \times 10^3$/µl, mV ..	..	E	3.68, 1.04	4.68, 2.19	4.58, 1.39	4.69, 1.34	19.08, 5.95	2.86. 0.81	34.12, 7. 78
R-factor $\times 100$, %	..		0.130	0.136	0.124	0.130	0.229	0.100	1.177
				0.503	0.589	0.474	0.484	1.041	0.413	4.723
				$\times$	$\times$	$\times$	$\times$	$\times$	$\times$	$\times$
				$\times$	$\times$	$\times$	$\times$	$\times$	$\times$	$\times$
				$\times$	$\times$	$\times$	$\times$	$\times$	$\times$	$\times$

Fig. 3. Potentiometric titration curves of (*a*) 2-CAPAZOXS, (*b*) 3-CAPAZOXS and (*c*) 4-CAPAZOXS with 0.988 mol dm^{-3} NaOH in original (v_T, *E*) and normalised (pH, *Z*) co-ordinates and their chemical interpretation using distribution diagrams of relative concentration population of all species in the chemical model found using MINIQUAD. $Z = (H - h)/L$; $L = $ (*a*) 86.87, (*b*) 60.52 and (*c*) 93.18 mmol dm^{-3}; $I = 0.12$ (NaClO$_4$); $T = 298$ K

$\sigma_{av}^2 \approx \sigma_i^2/n_j$ then $\sigma_{tit}^2 \approx 0$. The *F*-test is then used to assess the hypothesis at a given significance level α. If H_1 is accepted then $\sigma_{tit}^2 > 0$ and the refinement of the constants must be performed separately for each titration.

A completely analogous analysis can then be performed for σ_{prog}. For each algorithm there is $(\log \beta_{qr} \pm \sigma)_i$, $i = 1, \ldots, m$. All the data are used to obtain $\log \beta_{qr,av}$ with the variance σ_{av}^2 and $(m - 1)$ degrees of freedom. Then one sets $\sigma_{av}^2 = \sigma_{tit}^2/k + \sigma_{prog}^2$. If H_0, $\sigma_{prog}^2 \approx 0$, holds there is no significant difference between the programs used and all $\log \beta_{qr}$ values belong to the same population. If hypothesis H_1 holds then there is a significant difference between the programs used. If H_1 holds for both σ_{tit}^2 and σ_{prog}^2 the differences among titrations and among programs are significant and the search for causes of error with possible covariances becomes difficult.

Results

Search for the Best Model

For each ligand six different concentrations were prepared and solutions containing ligand and perchloric acid were titrated with sodium hydroxide. The concentrations studied were as follows: Naphthylazoxine 6S 1.88, 3.74, 7.53, 14.08, 27.26, 46.79 mmol dm^{-3}; SNAZOXS 2.51, 5.26, 10.41, 20.96, 43.36. 75.53 mmol dm^{-3}; 2-CAPAZOXS 0.40, 1.12, 3.25, 5.04, 5.73, 11.54 mmol dm^{-3}; 3-CAPAZOXS 0.46, 0.87, 1.38, 2.17, 2.20, 3.90 mmol dm^{-3}; and 4-CAPAZOXS 0.27, 1.19, 3.07, 4.69, 6.26, 23.10 mmol dm^{-3}.

In Fig. 1 the data for Naphthylazoxine 6S are given in normalised variables $Z = f(pa_{H^+})$. The curves do not coincide, indicating the formation of polynuclear complexes.

For each ligand all six titrations were analysed by MINIQUAD and 20 different models were tried to fit the data. For each set of data, distribution functions can be obtained in the printout such that in a certain concentration range only the species that contribute in that range are used in the minimisation. In this search certain criteria have to be fulfilled: (i) the degree-of-fit achieved; (ii) all the species found must have meaningful concentrations; and (iii) the standard deviations in $\log \beta_{qr}$ are examined and tested. In this way the following species were found: monomers, L, LH, LH$_2$ and LH$_3$; dimers L$_2$H$_2$, L$_2$H$_3$, L$_2$H$_4$ and L$_2$H; trimers, L$_3$H$_2$ and L$_3$H$_4$ (charges have been omitted for simplicity). In these calculations pa_{H^+} was used as an independent variable.

In order to obtain best possible β_{qr} values titration data, $E = f(v)$, were collected and the hydrogen ion concentration scale used. For external calibration of the glass electrode cell the MAGEC program was utilised. Moreover, in order to be certain that the data treated belong to the same population, it was decided to treat one titration curve only, selected such that all species were present. This is shown in Table 1 for Naphthylazoxine 6S. The first model in Table 1 assumes that no aggregates are formed and only the protonation constants of H$_2$L$^-$ and HL^{2-} are to be calculated. The constants $\log \beta_{11} = 7.4$ and $\log \beta_{12} = 10.5$ obtained by spectrophotometry in very dilute solution (1 μmol dm^{-3})[6] are used as input values. On minimisation this model terminates with a poor fit to the experimental titration curve, indicating that the model is inadequate.

In the second model β_{11} and β_{12} are kept constant while the protonation constants of two dimers are calculated, L$_2$H$_2^{4-}$ and L$_2$H^{2-} estimated and so on; see Table 1, where another five models are tested. Species that contribute less than 5% in the concentration range studied have been rejected. A low value of the Hamilton *R*-factor together with the 5% limit can be regarded as giving species that have a physical meaning.

As the total monomer content was always less than 1% they have been excluded from the final model. In addition to dimers, trimers were also tested but in most instances were rejected from the final model using the criteria mentioned above.

Table 4. Grand averages and weighted grand averages of formation constants of Naphthylazoxine 6S oligomers calculated from values in Table 3. ANOVA testing of significance of influence of programs used and titration replications. $F_{crit}(0.95, 4, 20) = 5.80$, $F_{crit}(0.95, 5, 20) = 4.56$

(A) Calculation of averages of formation constants

	$\text{Log } \beta_{21}$	$\text{Log } \beta_{22}$	$\text{Log } \beta_{32}$	$\text{Log } \beta_{23}$	$\text{Log } \beta_{24}$	$\text{Log } \beta_{34}$
Grand average $\log \beta_{qr}$:	10.743(97)	19.983(103)	21.236(702)	24.677(133)	28.172(192)	35.199(250)
Weighted grand average $(\log \beta_{qr})_w$:	10.692(25)	19.987(18)	21.569(68)	24.693(11)	28.181(36)	35.310(28)

(B) Analysis of variance of formation constants

(1) ANOVA testing: H_0: $\varepsilon_{tit} \approx 0$ versus H_1: $\varepsilon_{tit} > 0$:

F_{exp}	3.53	352.18	5.42	16.38	246.82	18.53
Accepted	H_0	H_1	H_1	H_1	H_1	H_1

(2) ANOVA testing: H_0: $\varepsilon_{prog} \approx 0$ versus H_1: $\varepsilon_{prog} > 0$:

F_{exp}	2.84	4.35	0.36	3.03	0.50	3.49
Accepted	H_0	H_0	H_0	H_0	H_0	H_0

(3) Statistical testing: H_0: $\sigma^2_{tit} \approx 0$ versus H_1: $\sigma^2_{tit} > 0$:

$\sigma_i^2 \times 10^6$	529.3	42.6	3109.8	363.8	282.5	9829.2
$\sigma_{av}^2 \times 10^6$	1849.0	13924.0	49284.0	24964.0	40804.0	90000.0
F_{exp}	17.47	1634.27	79.24	343.1	722.19	46.73
Accepted	H_1	H_1	H_1	H_1	H_1	H_1

(4) Statistical testing: H_0: $\sigma_{prog}^2 \approx 0$ versus H_1: $\sigma_{prog}^2 > 0$:

$\sigma_{av}^2 \times 10^6$	6561	324	13924	961	36	6724
F_{exp}	61.98	38.03	22.39	13.21	0.64	3.49
Accepted	H_1	H_1	H_1	H_1	H_0	H_0

The final model for Naphthylazoxine 6S consists of L_2H^{5-}, $L_2H_2^{4-}$, $L_2H_3^{3-}$, $L_2H_4^{2-}$ $L_3H_2^{7-}$ and $L_3H_4^{5-}$ in addition to L^{3-}. The search for the best model with the other sulphoazoxines was performed in the same manner, giving the results in Table 2. Here both activity scales have been tested. From Table 2 it is evident that the use of pa_{H_+} and mixed constants β_{qr} leads to a much poorer fit than using the hydrogen ion concentration.

Sources of Errors in Formation Constants

Table 3 gives the results of treating the data for Naphthylazoxine 6S with the programs MINIQUAD, MIQUV, PSEQUAD(v), PSEQUAD(E) and PSEQUAD(ortho). The results obtained on treating each titration separately and for all six titrations together are given.

From the data in Table 3, a grand average and a weighted grand average of the formation constants were calculated and are given in Table 4. The analysis of variance was applied in order to investigate possible differences between the five programs used. Statistical testing by the Fisher - Snedecor test leads to the conclusion that the differences between programs are insignificant, i.e., $\varepsilon_{prog} \approx 0$.

In contrast, the differences between titrations are significant as calculated by the F-test. This conclusion is valid for all formation constants except L_2H.

Analysis of variance of the same data as those leading to Table 1, i.e., for Naphthylazoxine 6S, shows that for all species formed the difference between titrations is larger than the variability within one titration, i.e., $\varepsilon_{tit} > 0$. This is to be expected when data for different total ligand concentrations (L) are treated, because the fraction of aggregates increases with increasing L, in agreement with the law of mass action. It is therefore a confirmation of the fact that oligomers are formed.

For 4-CAPAZOXS a model has been proposed earlier[6] with the same species as in the present study but without $L_2H_4^{2-}$ and LH^{2-}. This is due to the fact that different concentrations of ligand were studied.
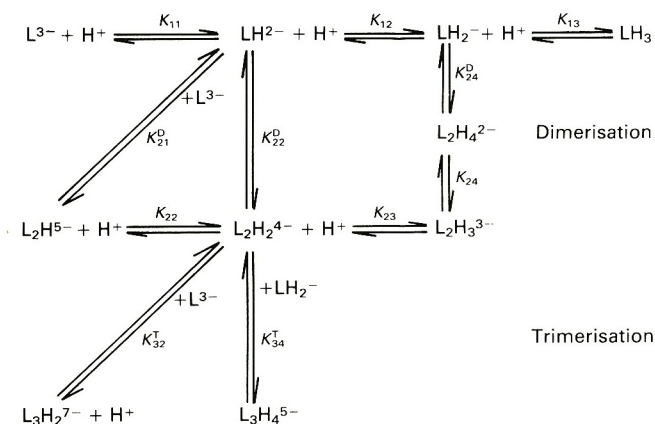
Scheme 1

The data in Table 2 lead to the following tentative reaction shown in Scheme 1.

The stepwise protonation constants were obtained from $K_{12} = \beta_{12}/\beta_{11}$, $K_{13} = \beta_{13}/\beta_{12}$, $K_{22} = \beta_{22}/\beta_{21}$ and $K_{24} = \beta_{24}/\beta_{23}$. The dimerisation constants were obtained from $K_{21}^D = \beta_{21}/\beta_{11}$, $K_{22}^D = \beta_{22}/\beta_{11}^2$, $K_{23}^D = \beta_{23}/(\beta_{12}\beta_{11})$ and $K_{24}^D = \beta_{24}/\beta_{12}^2$. The trimerisation constants were obtained from $K_{32}^T = \beta_{32}/\beta_{22}$ and $K_{34}^T = \beta_{34}/\beta_{22}$.

Conclusions

It is interesting that a much better fit with the experimental data is obtained with the log h scale compared with the pa_{H^+} scale. In this study it was assumed that activity coefficients are kept constant by the ionic medium used. This is supported by the good results obtained and permissible substitution of h for a_{H^+}. Moreover, when using the h scale the calibration is carried out in the same medium as the actual titration. The changes from buffers to medium are appreciable. The model arrived at is well supported by all the criteria used in its

evaluation. However, it might be of value to obtain independent support by some other method.

The possibility of evaluating all formation constants from one titration is demonstrated in Table 1. This is possible in favourable instances when all species found contribute more than about 5% to the total concentration in the pH range covered by the titration.

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NOTE—References 1–6 are to Parts II, III, IV, I, V and VI of this series, respectively.