Complexation Equilibria of Some Sulfoazoxines

Part IX.* Influence of Experimental Strategy on Reliability of Protonation Constants of 7-(4-Sulfo-1-naphthylazo)-8-hydroxyquinoline-5-sulfonic Acid Oligomers

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Protonation constants of variously protonated oligomers of the sulfoazoxine SNAZOXS [7-(4-sulfo-1-naphthylazo)-8-hydroxyquinoline-5-sulfonic acid] were determined potentiometrically. Instead of proton activity the hydrogen ion concentration is used. The group parameters ($E°$, Nernstian slope, concentration of titrand and titrant, *etc.*) were refined by the computer programs ESAB and MAGEC and the common parameters (log β_{qr} for species L_qH_r) by MINIQUAD. The proposed strategy of efficient experimentation was validated by determining the effect of group parameters on common parameters. The reliability of protonation constants was examined by the propagation of errors. The accuracy of the protonation constants β_{qr} depends on accuracy of the group parameters. The precision of the protonation constants β_{qr} expressed by an estimate of the standard deviation $s(\log \beta_{qr})$ is more pessimistic when a calculation of propagation of errors is applied when calculated by non-linear regression with the program MINIQUAD.

Keywords: *Dissociation constant; 7-(4-sulfo-1-naphthylazo)-8-hydroxyquinoline-5-sulfonic acid; multiparametric curve fitting; protonation constant*

Protonation and complex-forming equilibria have been studied systematically in our laboratory. The protonation constants of some sulfoazoxine oligomers were determined by regression analysis of potentiometric e.m.f. titration curves.[1,2] Whereas the program MINIQUAD[3] (or MIQUV,[4] PSEQUAD,[5] *etc.*) refines the common parameters (*i.e.*, β_{qr} for species L_qH_r) only, the programs ESAB,[6] MAGEC[7] or SUPERQUAD[8] also permit refinement of the group parameters ($E°$, Nernstian slope, concentrations of titrand and titrant, *etc.*). Protonation constants estimated by regression analysis of potentiometric titration curves are affected by (1) the instrumental technique used, (2) temperature (T), (3) ionic strength, (4) the strategy of the experimental technique used, consisting of the titration procedure, the indicator and reference electrodes, the standardization of the glass-electrode cell and the reliability of the concentration of basic components L and H, and (5) the regression algorithm.

The dissociation of the protonated sulfoazoxine SNAZOXS [7-(4-sulfo-1-naphthylazo)-8-hydroxyquinoline-5-sulfonic acid] (LH_5^{2+}) at concentrations lower than 10^{-6} mol l^{-1} when a monomer prevails in solution[9] may be expressed by the scheme

$$LH_5^{2+} \rightleftharpoons LH_4^{+} + H^{+} \rightleftharpoons LH_3 + H^{+} \rightleftharpoons LH_2^{-} + H^{+} \rightleftharpoons LH^{2-} + H^{+} \rightleftharpoons L^{3-} + H^{+}$$

where the protonated ions of SNAZOXS are LH_5^{2+} with protonation constant β_{15}, LH_4^{+} with log β_{14}, neutral molecule LH_3 with log β_{13} and anions LH_2^{-} with log β_{12}, LH^{2-} with log β_{11} and L^{3-}.

The aim of this paper is to examine the reliability of estimated protonation constants, which are affected by the experimental technique and the regression algorithm used.

Computations

All computations using the regression programs MINIQUAD, MAGEC, ESAB and CHEMSTAT (propagation of errors) were carried out on an IBM PC-AT computer.

* For Part VIII of this series, see ref. 13.

Protonation Equilibria of Oligomers

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

$$rH + qL = L_qH_r \quad (\beta qr) \tag{1}$$

When the charges are omitted for the sake of simplicity. The protonation constant is given by

$$\beta_{qr} = [L_qH_r]/(l^q h^r) \tag{2}$$

where h and l are the free concentrations of ligand [L] and hydrogen ions [H^+], respectively. The mass balance equations are

$$L = l + \Sigma q\beta_{qr} l^q h^r \tag{3}$$

$$H = h + \Sigma r\beta_{qr} l^q h^r \tag{4}$$

The activity coefficients are assumed to be kept constant by the ionic medium. For potentiometric e.m.f. titrations, the following relationship holds for the total hydrogen ion concentration:

$$L_{exp} = (L_0V_0 + L_TV_T)/V_0 + V_T) \tag{5}$$

$$H_{exp} = (H_0V_0 + H_TV_T)/(V_0 + V_T) \tag{6}$$

where H_0 and L_0 are the total initial concentrations of hydrogen ions and ligand, respectively, in the titrand (in the vessel), H_T and L_T are the total initial concentrations of hydrogen ions and ligand, respectively, in the titrant in the burette (for hydroxide $-H_T$ is used), V_0 is the initial volume of the titrand in the vessel and V_T is the volume of titrant added from the burette.

In potentiometric titration for the e.m.f. of a glass–SCE electrode cell the following relationship can be written:

$$\begin{aligned} E_{cell} &= E_H + E_j - E_{SCE} \\ &= E° + (RT/F)\ln h + (RT/F)\ln \gamma_H + j_a h \\ &\quad - j_b K_w/h - E_{SCE} \\ &= E°' + S\log h + E_j \end{aligned} \tag{7}$$

where $E°'$ is the standard potential of the glass electrode plus other terms such as the asymmetry potential, $h = [H^+]$, E_j is the liquid-junction potential ($j_a h - j_b K_w/h$), S is the slope of the

electrode response, [(*RT*/*F*)ln 10] for a Nernstian response and K_w is the operational ionic product of water.

An explicit equation for the titration volume, expressing the relationship between the volume of titrant added, $V_{T,i}$, monitored e.m.f., $E_{cell,i}$, and the common (β_{qr}) and group parameters ($\boldsymbol{k}$) is given by

$$V_{T,i} = f(E_{cell,i};\ \beta_{qr},\ \boldsymbol{k}) \tag{8}$$

in which the vector of common parameters β_{qr} contains protonation constants of all oligomers formed, the vector of group parameters $\boldsymbol{k} = (E^{\circ\prime}, S, K_w, E_j, L_0, H_0, X_0, L_T, H_T, X_T)$ containing in addition to the constants of the Nernst equation, $E^{\circ\prime}$, S, E_j, the initial concentration of ligand, L_0, and the initial concentration of hydrogen ions, H_0, in the titrand, the concentration of acid–base impurities, X_0 (*i.e.*, carbonates), and the corresponding quantities for the titrant, L_T, H_T and X_T. In most instances group parameters cannot be determined independently with sufficient accuracy.

In most regression programs for treating e.m.f. data, the task is to find a model and a set of protonation constants that give the 'best' fit to the experimental data. In ESAB the parameters β_{1r} and $\boldsymbol{k}$ are refined by minimizing the residual-square sum

$$U_V = \sum_{i=1}^{n} w_i(V_{T,exp,i} - V_{T,calc,i})^2 = \text{minimum} \tag{9}$$

where w_i is the statistical weight, equal to

$$w_i = \frac{1}{\sigma^2_{V,i}} + \left(\frac{\delta V_T}{\delta E}\right)_i \sigma^2_{E,i} \tag{10}$$

In MINIQUAD only common parameters β_{qr} are refined, by minimizing the residual-square sum U_C:

$$U_C = \sum_{i=1}^{n} w_i(C_{exp,i} - C_{calc,i})^2 = \text{minimum} \tag{11}$$

where C_i is the total concentration of ligand (L) or protons (H) at the ith point of the titration curve.

Accuracy and Precision of Protonation Constants

To classify the accuracy of estimated protonation constants, the value of each protonation constant, $\log \beta_{qr,exp}$, is considered to consist of the 'true' value, $\log \beta_{qr}$, and several sources of systematic error according to

$$\log \beta_{qr,exp} = \log \beta_{qr} + \varepsilon_{cell} + \varepsilon_{conc} + \varepsilon_{alg} + \varepsilon_i \tag{12}$$

where ε_{cell} is the systematic error due to uncertain values of the electrochemical (group) parameters $E^{\circ\prime}$ and S, ε_{conc} is the systematic error due to uncertain values of concentrations (group parameters) H_0, H_T and L_0, L_T, ε_{alg} is the systematic error due to failure or a false minimization process of the regression algorithm and ε_i is the random error.

To classify the precision of protonation constants, the standard deviations $s(\log \beta_{qr})$ is considered to be a result of errors propagated from various sources of experimental technique.

Generally, the response quantity of any experiment, y, is obviously calculated as a known functional transformation of a set of directly measured quantities $x_1, \ldots, x_m$, and the function $y = G(x_1, \ldots, x_m)$ is known. Owing to various kinds of errors (chemical, electrical, laboratory, *etc.*), the measured quantities represent random variables leading to the sample mean $\bar{x}_i$ and sample variances $s^2(x_i)$, $i = 1, \ldots, m$. Based on this input information, the mean $\bar{y}$ and its variance $s^2(y)$ may be obtained by the Taylor series expansion of the function $y = G(x_1, \ldots, x_m)$, by the two-point approximation and by Monte Carlo simulation.[11]

Using the Taylor series expansion in the neighbourhood of the mean vector $\bar{\boldsymbol{x}} = (\bar{x}_1, \ldots, x_m)^T$, we obtain

$$y \approx G(\bar{\boldsymbol{x}}) + \sum_{i=1}^{m} \frac{\delta G(\boldsymbol{x})}{\delta x_i}(x_i - \bar{x}_i) + \frac{1}{2}\sum_{i=1}^{m} \frac{\delta^2 G(\boldsymbol{x})}{\delta x_i^2}(x_i - \bar{x}_i)^2 + \sum_{i=1}^{m-1}\sum_{j=i+1}^{m} \frac{\delta^2 G(\boldsymbol{x})}{\delta x_i \delta x_j}(x_i - \bar{x}_i)(x_j - \bar{x}_j) + \ldots \tag{13}$$

where both the first and second derivatives are calculated at the vector of mean values $\bar{\boldsymbol{x}}$. Applying mean value operator $E(\,)$ to both sides of eqn. (13), the resulting relationship for the estimate of the mean $\bar{y}$ can be written as

$$\bar{y} \approx G(\bar{\boldsymbol{x}}) + \frac{1}{2}\sum_{i=1}^{m} \frac{\delta^2 G(\boldsymbol{x})}{\delta x_i^2} s^2(x_i) + \sum_{i=1}^{m-1}\sum_{j>i}^{m} \frac{\delta^2 G(\boldsymbol{x})}{\delta x_i\, \delta x_j} \operatorname{cov}(x_i, x_j) \tag{14}$$

where $\bar{y} = E(y) = E[G(\boldsymbol{x})]$ and $\operatorname{cov}(x_i, x_j)$ is the covariance, which give a measure of linear association between two variables x_i and x_j.

In determining the variance $s^2(y)$ using the approximation (13), the higher moments (*e.g.*, the skewness and kurtosis[11]) are neglected. The resulting approximate relationship for variance is often termed the rule of propagation of absolute errors and can be expressed by

$$s^2(y) \approx \sum_{i=1}^{m}\left[\frac{\delta G(\boldsymbol{x})}{\delta x_i}\right]^2 s^2(x_i) + 2\sum_{i=1}^{m-1}\sum_{j=i+1}^{m} \frac{\delta G(\boldsymbol{x})}{\delta x_i}\frac{\delta G(\boldsymbol{x})}{\delta x_j} \operatorname{cov}(x_i, x_j) + \sum_{i=1}^{m-1}\sum_{j>i}^{m} \frac{\delta^2 G(\boldsymbol{x})}{\delta x_i \delta x_j} s^2(x_i)\, s^2(x_j) \tag{15}$$

This result can be used for estimation of total error of response $s(y)$ due to the simple linear combination of errors $s(x_i)$ from m sources.

Manly's procedure[4] of two-point approximation is based on a subsitution of the probability distribution of the function $G(\boldsymbol{x})$ by the two-point distribution with the same mean and variance,

$$\bar{y} \approx \{G[\bar{x} + s(x)] + G[\bar{x} - s(x)]\}/2 \tag{16}$$

and the estimate of variance by

$$s^2(y) \approx \{G[\bar{x} + s(x)] - G[\bar{x} - s(x)]\}^2/4 \tag{17}$$

Both simple relationships give comparable results to Taylor's expansion.

For the determination of the mean $\bar{y}$ and its variance $s^2(y)$ as a function $G(\boldsymbol{x})$ of random variables x, computer-assisted Monte Carlo simulation[3] can also be applied.

Experimental

Chemicals and Solutions

SNAZOXS of analytical-reagent grade was obtained from Spolana (Neratovice, Czech Republic) and purified as described previously.[2] The actual concentration of SNAZOXS in each e.m.f. titration was determined by e.m.f. titration with NaOH and evaluated by regression analysis with the ESAB[6] and MAGEC[7] programs. The proton activity is replaced by hydrogen ion concentration, the electrodes being calibrated in terms of concentration in accordance with the Stockholm school of pH definition. Impurities in SNAZOXS were mostly inorganic salts.

Perchloric acid, 1 mol l^{-1}, was prepared by dilution of 70% $HClO_4$ of analytical-reagent grade with doubly distilled,

de-ionized water and standardized against HgO and KI using the Gran method in the MAGEC program.

Sodium hydroxide solution, 1 mol l^{-1} and carbonate-free, was prepared by reaction of sodium with doubly distilled, de-ionized and de-aerated water under an atmosphere of argon and with efficient cooling at about 275 K.

Apparatus

All e.m.f. measurements were carried out at 298.0 ± 0.1 K by means of an OP-208/1 digital voltmeter (Radelkis, Budapest, Hungary) with a G202B glass electrode (Radiometer, Bagsvaerd, Denmark) and an OP-0830P SCE reference electrode (Radelkis). A water-jacketed 100 ml glass vessel, closed with a Teflon bung carrying the electrodes, argon inlet, thermometer, stirrer and the microburette capillary tip, was used for the titrations.

During the titrations a stream of argon was bubbled through the solution both for stirring and to maintain an inert atmosphere. The argon was passed through a pure ionic medium before entering the equilibrium solution.

The burettes used were laboratory-made syringe microburettes with a micrometer screw, type PK1250, of capacity 1250 μl. The microburettes were calibrated by weighing the delivered water on a Sartorius Z200S balance in a weighing bottle closed with a top-cap. The polyethylene capillary tip of the microburette was immersed in the water though a hole of 1 mm diameter, with 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 to check the linearity of the piston. The microburette was calibrated by ten replicate determinations of the total volume of delivered water and the results were evaluated statistically, leading to a precision of ±0.015% over the volume range.

Procedure of 'Equilibrium Titration'

To determine the chemical model of protonation equilibria for SNAZOXS, the procedure of potentiometric acid–base titration consisting of the following steps was applied.

Step 1. Standardization of perchloric acid: c_{HClO_4}

The procedure for the determination of SNAZOXS oligomers is sensitive to the precise value of the concentration of $HClO_4$. Perchloric acid was standardized on HgO and KI using the relationship $c_{HClO_4} = 2m/(MV_{T,eq})$, where m is the amount of HgO in grams, M is the molecular mass of HgO (M = 216.59 g mol^{-1}) and $V_{T,eq}$ is the volume of $HClO_4$ at the equivalence point. The titration curve was evaluated by the Gran method (MAGEC) and c_{HClO_4} was also checked by the propagation of errors law (CHEMSTAT[10]).

Step 2. Calibration of glass electrode cell: $E^{\circ\prime}$, S, (pK_w), H_T *(and* X_T*)*

Before acid–base titration of a mixture of sulphoazoxine and $HClO_4$ with NaOH, the glass–SCE electrodes cell was calibrated. The hydrogen concentration $[H^+] = h$ is known from the initial concentration H_0 and measured e.m.f., E_{cell}. From $E_{cell} = E^{\circ\prime} + S \log h$ for each point $\{E_{cell}, h\}$ of the titration curve of known concentration of perchloric acid with standard sodium hydroxide, the group parameters E° and S were calculated. For evaluation, the part of titration curve for which E_j is virtually constant and can be included in $E^{\circ\prime}$ was used. Values of two group parameters, the Nernstian slope S = 59.16 mV pH^{-1} and pK_w, were taken from tables. The program MAGEC offers a choice of group parameters to be calculated; the most accurate results were obtained when only E° and S were also refined. Using ESAB, the group parameters E°, H_0, H_T and X_T were finally refined.

Step 3. Determination of concentration of sulfoazoxine and hydrogen ions: L_0, H_0

To analyse the e.m.f. titration curve for a mixture of sulphoazoxine and $HClO_4$ with NaOH by ESAB or MAGEC, the content of sulphoazoxine, L_0, and the content of protons, H_0, were determined.

Step 4. Protonation equilibria of sulphoazoxine: {q, r} *and* β_{qr}

To analyse a set of e.m.f. titration curves for a mixture of sulphoazoxine and $HClO_4$ with NaOH by MINIQUAD when previously calculated values of the group parameters E°, S, H_0 and H_T are used, the chemical model of a number of oligomers, their stoichiometry $\{q, r\}$ and protonation constants β_{qr} were determined.

Step 5. Accuracy and precision of log β_{qr} *by the propagation of errors law:* s(*log* β_{qr})

Substituting values and their standard deviations for all concentration and electrochemical quantities for calculation of the stability constants into the relationships of the three different methods of propagnation of errors in the program CHEMSTAT,[10] the final values of log β_{qr} for actual oligomers were evaluated.

Conditions of 'Equilibrium Titration'

A mixture containing 10.00 ml of 0.01 mol l^{-1} SNAZOXS and 0.265 ml of 1 mol l^{-1} $HClO_4$ [$L_0^{(0)}$ = 0.01 mol l^{-1}, $H_0^{(0)}$ = 0.05 mol l^{-1}] was titrated with 1.0 mol l^{-1} sodium hydroxide ($-H_T$ = 1.00 mol l^{-1}) and the e.m.f., E_{cell}, was read. The temperature was kept constant at 298 ± 0.2 K.

Results

To increase the reliability of the protonation equilibria of SNAZOXS, it is necessary to avoid or to minimize the systematic errors ε_{cell}, ε_{conc} and ε_{alg} in β_{qr}. The errors ε_{cell} and ε_{conc} can be found by experimental strategy and ε_{alg} by application of various computational strategies with regression algorithms. To evaluate the reliability of protonation constants the following step of 'equilibrium titration' were examined.

Step 1: The 95% interval estimate of the concentration of a stock solution of $HClO_4$ was determined, for n = 3, as $\bar{c}_{HClO_4}$ = 0.9977 ± 0.0059 mol l^{-1}. Applying the Taylor series, two-point determination and Monte Carlo methods in the propagation of errors law (CHEMSTAT) for each titration, the point estimate of a weighted mean, $\bar{c}_{w,HClO_4}$ = 0.9977 mol l^{-1} with $s(\bar{c}_{w,HClO_4})$ = 0.0023 mol l^{-1} and $s(\bar{c}_{HClO_4})$ = 0.0008 mol l^{-1} was estimated.

Step 2: Calibration of the glass–SCE electrode cell was carried out on the log h concentration scale with use of $HClO_4$–NaOH e.m.f. titration. Table 1 compares various approaches to the evaluation of the group parameters $E^{\circ\prime}$, S and H_T (Table 2).

(*a*) Graphical treatment estimates the equivalence point $V_{T,eq}$ as an inflection point on the sigmoidal titration curve using the first- or second-derivative technique. This approach to the calculation of H_T is approximate as it does not consider uncertainties in other group parameters.

(*b*) Statistical treatment of various segments of both branches of the Gran plot in the MAGEC program evaluates the weighted mean of the equilvalence point $V_{T,eq}$. The parameters $E^{\circ\prime}$ and S are estimated simultaneously. When acid–base impurities (*i.e.*, mostly carbonates) are neglected, the one fitted sigmoidal curve exhibits a strong trend in residuals. This leads to the conclusion that these impurities cannot be neglected and two sigmoidal curves should be fitted through the titration data.

(*c*) When acid–base impurities are not neglected, two sigmoidal curves are fitted through the titration data. A goodness-of-fit test proves that residuals do not exhibit any trend and are randomly distributed around zero. For very low concentrations, the acid–base impurities are ill-conditioned in model eqn. (8) and their determination is uncertain. The group parameters $E°$ and S are also called 'dangerous' parameters as their estimation is sometimes not possible or uncertain, and explanation may be found in the flat-bottomed saucer shape of the hyperparaboloid U in the $(m + 1)$-dimensional space of m unknown parameters.[12] The estimation of such ill-conditioned parameters is uncertain and in searching U_{min} the programs often fail. Therefore, it is recommended that the initial guess of group parameters should be if possible equal to the best estimates sometimes obtained by another independent technique.

In estimating the concentrations of acid–base impurities X, their dissociation constants were also refined. The values found are close to the dissociation constants of carbonic acid ($pK_1 = 10.0$, $pK_2 = 6.16$ at 298 K, $I = 0.1$) and therefore the acid–base impurities in NaOH are represented by carbonates.

From the acid branch of the Gran plot the value of H_T is evaluated and from the base-bench the sum $H_T + X_T$ is obtained.

(*d*) ESAB is based on a non-linear regression of the $HClO_4$–($NaOH + Na_2CO_3$) sigmoidal titration curve and

Table 1 Calibration of the glass electrode cell ($E°'$, S, pK_w) and standardization of sodium hydroxide (H_T) evaluated by MAGEC and ESAB to achieve sufficient reliability of estimated group parameters. $V_0 = 11.0$ ml ($HClO_4$); $H_0 = 0.0907$ mol l^{-1} $HClO_4$; $T = 298$ K; $S^{(0)} = 59.16$ mV pH^{-1}. G202B SCE cell. Standard deviations (in parentheses) refer to the corresponding last figures

	Titration				
	1	2	3	4	5
(1) Graphical treatment (first and second derivatives)—					
$V_{T,eq}$/ml	1.054	1.033	1.038	1.048	1.038
H_T/mol l^{-1}	0.9422	0.9617	0.9571	0.9679	0.9571
(2) Gran method, neglecting CO_3^{2-} (MAGEC)—					
$V_{T,eq}$/ml	1.0443	1.0304	1.0376	1.0404	1.0350
H_T/mol l^{-1}	0.9556	0.9685	0.9618	0.9568	0.9642
$E°'$/mV	376.1	355.1	370.1	371.2	368.8
S/mV pH^{-1}	58.82	52.30	58.75	58.76	59.00
(3) Gran method with a correction for CO_3^{2-} (MAGEC)—					
H_T/mol l^{-1}	0.9350	0.9409	0.9468	0.9330	0.9334
$10^4 X_T$/mol l^{-1}	7.0	4.5	9.2	5.2	9.6
$E°'$/mV	372.9	360.3	366.5	368.7	366.7
S/mV pH^{-1}	58.8	58.1	58.4	58.9	59.1
(4) Non-linear regression of all titration curve when $S^{(0)}$ is given—					
H_T/mol l^{-1}	0.9546 (19)	0.9542 (126)	0.9641 (52)	0.9629 (37)	0.9674 (48)
$10^3 X_T$/mol l^{-1}	1.39(15)	1.08(11)	1.61(26)	0.80(13)	0.99(17)
$E°'$/mV	373.8(3)	365.4(9)	369.0(7)	371.2(6)	366.4(4)
$H_T + X_T$/mol l^{-1}	0.9574 (20)	0.9563 (126)	0.9673 (52)	0.9644 (38)	0.9694 (48)

Table 2 Evaluation of SNAZOXS concentration L_0 and hydrogen ion concentration H_0 of $HClO_4$ from repeated potentiometric titration of a mixture of SNAZOXS and $HClO_4$ with NaOH using the ESAB program and the MAGEC program ($T = 298$ K). Standard deviations (in parentheses) refer to the corresponding last figures

	Titration					
	1	2	3	4	5	6
Input data for programs ESAB2M and MAGEC obtained from standardization of NaOH—						
H_T/mol l^{-1}	0.9574	0.9563	0.9673	0.9632	0.9644	0.9694
$E°$/mV	373.8	365.4	369.0	370.0	371.2	366.4
S/mV pH^{-1}	58.8	58.1	58.4	59.2	58.9	59.1
Using program ESAB2M—						
c_{HClO_4}/mol l^{-1}	0.03124 (24)	0.03167 (18)	0.03130 (22)	0.03075 (24)	0.03176 (24)	0.03176 (22)
L_0/mol l^{-1}	0.01161 (13)	0.01144 (10)	0.01188 (12)	0.01199 (13)	0.01151 (12)	0.01149 (12)
pK_1	3.860 (44)	3.809 (35)	3.830 (40)	3.781 (38)	3.802 (41)	3.776 (39)
pK_2	8.102 (43)	8.065 (32)	8.068 (36)	8.035 (32)	8.078 (34)	8.006 (38)
$E°'$/mV	370.7 (5)	370.5 (4)	370.1 (4)	371.3 (4)	370.6 (4)	368.9 (4)
Using program MAGEC—						
H_0/mol l^{-1}	0.05363	0.05380	0.05429	0.05366	0.05406	0.05393
L_0/mol l^{-1}	0.01100	0.01100	0.01115	0.01111	0.01100	0.01100
pK_1	3.885	3.852	3.854	3.854	3.803	3.827
pK_2	8.044	8.027	8.023	7.976	8.077	8.023
$E°'$/mV	367.9	368.7	367.1	368.4	368.7	366.7
S/mV pH^{-1}	58.30	58.40	58.37	58.37	58.54	58.58

MAGEC makes the Gran linear transformation. From regression analysis it is known that any transformation of original data also transforms the distribution of errors of measured variables. One of the basic assumptions based on the least-squares method is not fulfilled here, *i.e.*, the error distribution is not normal. As transformation methods in regression are approximate, the group parameters $E^{\circ\prime}$, S, H_0, H_T and X_T obtained from MAGEC were used as the initial guess for the non-linear regression algorithm ESAB and the parameters $E^{\circ\prime}$, H_T, X_T and pK_w were refined. The final refinement of the titration data by ESAB leads to experimental errors of the variables used and therefore the estimates of group parameters obtained may be considered as final.

Step 3: the titration curve of a mixture of SNAZOXS and $HClO_4$ (Fig. 1) may be analysed by ESAB or MAGEC for determination of the group parameters L_0 and H_0 (Table 2). In this step SNAZOXS is assumed to be a monomer with two protonation equilibria, $LH_2^- \rightleftharpoons LH^{2-} + H^+ \rightleftharpoons L^{3-} + H^+$ which lead to dissociation constants $pK_1 = 3.8$ and $pK_2 = 8.1$. Simultaneously with the dissociation constants, the initial concentration of SNAZOXS, L_0, and protons, H_0, may be evaluated (Fig. 2). Respecting the mass-balance equation for protons, the relationship

$$H_0 = 2L_0 + c_{HClO_4}$$

holds. Whereas ESAB determines c_{HClO_4}, the program MAGEC determines the sum of protons H_0 coming from $HClO_4$ and from SNAZOXS. The parameter S is estimated by MAGEC only and E° is refined by both programs. Comparing L_0 and H_0, both programs are in agreement as these values are within the tolerance of experimental errors.

Step 4: for the group parameters $E^{\circ\prime}$, S, pK_w, H_0, H_T and L_0 the values found in steps 2 and 3 were used as input in the program MINIQUAD and the residual-square sum function U_C [eqn. (11)] was minimized. In a search for the most probable chemical model of oligomers, their stoichiometry was established using a procedure described in previous papers.[1,2,13] The chemical model found for the protonation equilibria of SNAZOXS oligomers was fitted on six repetitive e.m.f. titrations; protonation constants are given in Table 3, together with the mean with standard deviation and the weighted mean calculated by the propagation of errors law (CHEMSTAT).

Step 5: To show the computer-assisted application of the propagation of errors law, one point on the fifth titration curve under the following experimental conditions was chosen when only two species, L_2H^{5-} and $L_2H_2^{4-}$, prevail in solution (in parentheses after each value the standard deviation corresponding to the last figures is given): $V_0 = 10.265(10)$ ml, $V_{T,i} = 10.825(16)$ ml, $H_T = 0.9644(38)$ mol l^{-1}, $H_0 = 0.05406(34)$

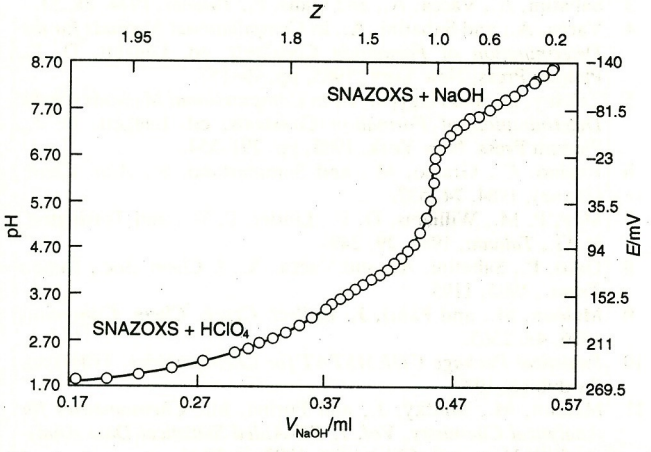

Fig. 1 Potentiometric titration curve for the titration of SNAZOXS + $HClO_4$ with 0.9644 mol l^{-1} NaOH in original (V_T, E) and normalized (pH, Z) coordinates. $Z = (H - h)/L$, $L = 11.0$ mmol l^{-1} SNAZOXS, $I = 0.03$, $T = 298$ K

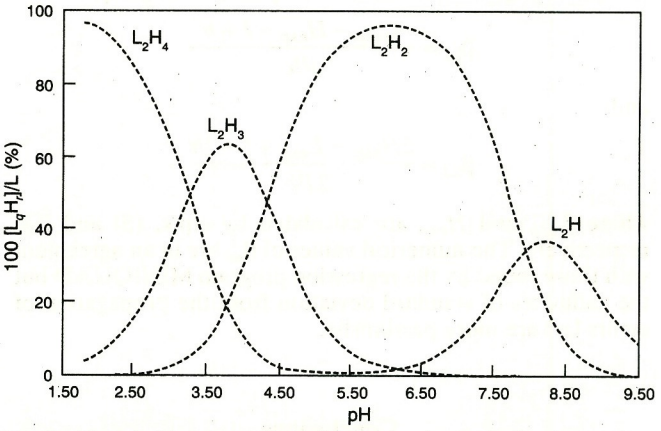

Fig. 2 Chemical interpretation of the potentiometric titration curve for SNAZOXS + $HClO_4$ shown in Fig. 1 by means of the distribution diagram of the relative concentration populations of all species in the chemical model found using MINIQUAD

Table 3 Reproducibility of protonation constants of oligomers of SNAZOXS estimated at 298 K and ionic strength $I = 0.03$ by regression analysis of e.m.f. titration curves by MINIQUAD. Group parameters $E^{\circ\prime}$, S and $-H_T^{(0)} = 1.00$ mol l^{-1} obtained from external calibration of ESAB and MAGEC. In the last column the means are given in the upper rows and the weighted means obtained by the propagation of errors law in the lower rows. Standard deviations (in parentheses) refer to the corresponding last figures

	Titration						
	1 (60 points)	2 (69 points)	3 (66 points)	4 (61 points)	5 (58 points)	6 (63 points)	Mean
Group parameters—							
L_0/mol l^{-1}	0.01100	0.01100	0.01115	0.01111	0.01100	0.01100	—
H_0/mol l^{-1}	0.05363	0.05380	0.05429	0.5366	0.05406	0.05393	—
$E^{\circ\prime}$/mV	367.9	369.0	368.2	368.4	368.7	366.7	—
S/mV pH^{-1}	58.30	58.40	58.46	58.37	58.54	58.58	—
pH range	1.8–10	1.7–9.8	1.9–10	1.8–9.7	1.8–9.8	1.8–10.1	—
Common parameters—							
Log β_{21}	10.27(3)	10.25(3)	10.31(5)	10.15(2)	10.22(3)	10.28(3)	10.247(56) 10.247(13)
Log β_{22}	18.19(2)	18.23(2)	18.28(3)	18.09(1)	18.17(2)	18.15(2)	18.185(66) 18.185(9)
Log β_{23}	22.55(3)	22.58(3)	22.73(5)	22.39(2)	22.52(3)	22.48(3)	22.542(112) 22.542(13)
Log β_{24}	25.93(3)	25.92(3)	26.15(5)	25.74(2)	25.80(3)	25.84(3)	25.897(144) 25.897(13)

Table 4 Comparison of two estimation techniques, regression analysis by MINIQUAD and straightforward calculation with propagation of errors for two protonation constants of SNAZOXS oligomers

Variable used in calculation	Regression by MINIQUAD	CHEMSTAT (propagation or errors)		
		Taylor series	Two-point determination	Monte Carlo
$h \times 10^{10}$/mol l^{-1}	9.984	9.984(15)	9.984(15)	9.949(15)
$l \times 10^{3}$/mol l^{-1}	8.175	—*	—*	—*
$H_{exp} \times 10^{3}$/mol l^{-1}	1.371	1.373(377)	1.373(377)	1.371(370)
$L_{exp} \times 10^{2}$ mol l^{-1}	1.043	1.043(11)	1.043(11)	1.044(11)
Log β_{21}	10.25(6)	10.12(19)	10.11(21)	10.12(28)
Log β_{22}	18.20(7)	18.55(68)	18.58(35)	18.58(46)

* The value was taken from the MINIQUAD output.

mol l^{-1}, $L_0 = 0.01100(12)$ mol l^{-1}, pH = 9.001, $E_{cell} = -158.2 \pm 0.1$ mV, $T = 298.0 \pm 0.2$ K, $E^{\circ\prime} = 368.7 \pm 0.1$ mV and $S = 58.54 \pm 0.11$ mV pH^{-1}. Substituting in the Nernst equation, the concentration h was calculated by the three methods of CHEMSTAT (Table 4). Here for reference, the values estimated by MINIQUAD are also given. As the free concentration of ligand, l cannot be calculated straightforwardly, the value calculated by MINIQUAD was used for the propagation of errors. Protonation constants β_{21} for L_2H^{5-} and β_{22} for $L_2H_2^{4-}$ are calculated by the relationships

$$\beta_{21} = \frac{L_{exp} - H_{exp} - l + h}{l^2 h}$$

and

$$\beta_{22} = \frac{2H_{exp} - L_{exp} + l - 2h}{2\,l^2 h^2}$$

where L_{exp} and H_{exp} are calculated by eqns. (5) and (6), respectively. The numerical values of β_{qr} are in an agreement with those found by the regression program MINIQUAD but the estimates of standard deviation from the propagation of errors law are more pessimistic.

Conclusions

The accuracy of protonation constants β_{qr} for SNAZOXS oligomers on a concentration scale determined potentiometrically depends on the accuracy of group parameters. The glass electrode cell should be calibrated by external standardization and the groups parameters $E^{\circ\prime}$, S, pK_w, L_0 and H_T should be refined to their best values. The precision of protonation constants expressed by an estimate of the standard deviation $s(\log \beta_{qr})$ is more pessimistic when a propagation of errors calculation is applied with calculation by the non-linear regression program MINIQUAD.

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NOTE—Reference 13 is to Part VIII of this series.

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