

# The thermodynamic dissociation constant of naphazoline by the regression analysis of potentiometric data

## Research Article

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**Abstract:** The mixed dissociation constant of naphazoline is determined at various ionic strengths  $I$  [mol dm<sup>-3</sup>] in the range of 0.01 to 0.26 and at temperatures of 25°C and 37°C using ESAB and HYPERQUAD regression analysis of the potentiometric titration data. A strategy of efficient experimentation is proposed in a protonation constant determination, followed by a computational strategy for the chemical model with a protonation constant determination. Two group parameters,  $L_0$  and  $H_T$  were ill-conditioned in the model and their determination is therefore uncertain. These group parameters,  $L_0$  and  $H_T$  can significantly influence a systematic error in the estimated common parameter  $pK_a$  and they always should be refined together with  $pK_a$ . The thermodynamic dissociation constant  $pK_a^T$  was estimated by nonlinear regression of  $\{pK_a, I\}$  data at 25°C and 37°C: for naphazoline  $pK_{a1}^T = 10.41(1)$  and  $10.13(2)$ . Goodness-of-fit tests for various regression diagnostics enabled the reliability of the parameter estimates to be found.

**Keywords:** Potentiometric titration • Dissociation constant • Naphazoline • ESAB • HYPERQUAD

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## 1. Introduction

Naphazoline, chemically 4,5-dihydro-2-(1-naphthalenylmethyl)-1H-imidazole, has the chemical structure shown in Fig. 1, and is denoted as NPZ. It is a relatively long-lasting action vasoconstrictor, which acts on the alpha receptors of the smooth vascular muscle [1-3].

It is a decongestant that acts on the alpha-adrenergic receptors in the arterioles of the conjunctiva to produce vasoconstriction, resulting in decreased conjunctival congestion [4]. Naphazoline belongs to the group of sympathomimetics, agonists of  $\alpha$ -Adrenergic receptors. It has vasoconstrictive effects that reduce the lumen of capillaries, and help relieve the oedema of nasal tissue. There are several analytical methods to determine NPZ; the majority are photometric [5,6] and chromatographic methods: thin layer chromatography [4], gas chromatography [7] and high-performance liquid chromatography [8,9]. Capillary electrophoresis [10-12] and atomic absorption and emission [13]

methods have also been published. NPZ presents intrinsic fluorescence and phosphorescence emission, thus different luminescence methods have been developed for its determination [14-17], all of which present too many complications for routine laboratory use. NPZ has been determined simultaneously with PHE [18] and other imidazolines with and without derivatization by spectrofluorimetric and derivative spectrophotometric methods [19,20] with quantitative determination. A number of additional studies have been reported for the determination of NPZ including micellar electrokinetic chromatography [21], phosphorimetry [16,22] and spectrophotometry [23]. In 1985, a PVC

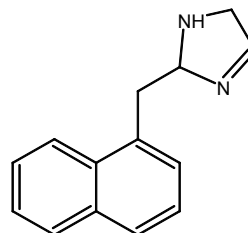


Figure 1. Basic structure of naphazoline

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membrane electrode based on the salt of naphazoline with tetraphenylborate as an ion-exchanger for the potentiometric determination of naphazoline was reported to have a usable concentration range of  $1.0 \times 10^{-5}$  to  $1.0 \times 10^{-2}$  M [24].

The protonation equilibria can play an important role because of the site of application, the nasal mucosa and/or eye (naphazoline). At the same time, the range of osmolarity of body liquids at the site of absorption (tears and nasal secretions) is better defined and much narrower than in cases of absorption in the gastrointestinal tract. (Osmolarity used here is the measure of solute concentration, defined as the number of osmoles (Osm) of solute per liter (L) of solution (osmol L<sup>-1</sup> or Osm L<sup>-1</sup>) in the same way that the molarity of a solution is expressed as „M“. Whereas molarity measures the number of moles of solute per unit volume of solution, osmolarity measures the number of *osmoles of solute particles* per unit volume of solution.) The dependence of protonation constants on ionic strength for naphazoline has been systematically investigated only once, and the thermodynamic dissociation constant was estimated as  $pK_a^T = 10.81$  ( $s=0.01$ ) and  $10.63$  ( $s=0.01$ ) at 25°C and 37°C using regression analysis of the pH-spectrophotometric titration data [25]. We have decided to complete this information and to study the protonation equilibria with the use of another instrumental technique known as potentiometric titration.

The present report investigates the dissociation constants of naphazoline at various ionic strengths and at 25°C and 37°C, to prove their reproducibility and also to estimate the thermodynamic dissociation constant  $pK_a^T$  at these two temperatures. The  $pK_a^T$  data may be used for prediction of the actual dissociation constant  $pK_a$  at a given ionic strength.

## 2. Experimental Procedure

### 2.1. Potentiometric Data Analysis

The overall protonation constant of the protonated species,  $\beta_{gr}$ , may be expressed as:

$$\beta_{gr} = \frac{[L_q H_r]}{([L]^q [H]^r)} = \frac{c}{l^q h^r}$$

where the free concentration  $[L] = l$ ,  $[H] = h$  and  $[L_q H_r] = c$ . For dissociation reactions at constant ionic strength, the so-called “mixed dissociation constants” are defined as  $K_{a,j} = [H_{j-1} L] a_{H^+} / [H_j L]$ . The mass balance equations are  $L = l + \sum_{j=1}^L \beta_{H^+} l h^j$  and  $H = h - \frac{K_w}{h} + j \sum_{j=1}^j \beta_{H^+} l h^j$ . Potentiometric readings obtained with the proton-sensitive glass and reference electrode cell

can be described by the following equation:

$$E_{cell} = E^0 + \frac{f \cdot RT \ln 10}{F} \log a_{H^+} + j_a a_{H^+} - \frac{j_b K_w}{a_{H^+}} - E_{ref} = E^0 + S \log h \quad (1)$$

where  $E^0$  is the standard potential of a glass electrode cell containing some other constants of the glass electrode such as the asymmetry potential, *etc.*, and  $a_{H^+} = [H^+] y_{H^+} = h y_{H^+}$ . The liquid-junction potential  $E_j$  is expressed by the term  $E_j = j_a a_{H^+} - j_b K_w / a_{H^+}$ , and  $S = (f \cdot RT \ln 10) / F$  is the slope of the glass electrode for a Nernstian response.  $K_w$  is the operational ion product of water at temperature  $T$  [K], and the correction factor  $f$  is taken as an adjustable parameter. At constant ionic strength, the activity coefficient does not change and the term  $E^0$  in the  $pH$  range from 3 to 11 is practically constant.

An explicit equation for the titration curve under constant ionic strength expresses a dependence between the volume of *titrant* added from the burette,  $V_i$ , and the monitored *emf*  $E_{cell,i}$  or  $pa_{H^+}$  with the vector of unknown parameters ( $\mathbf{b}$ ) separated into the vector of *common parameters* ( $\mathbf{K}_a$ ) and the vector of *group parameters* ( $\mathbf{p}$ ), i. e.  $V_i = f(E_{cell,i}; \mathbf{b}) = f(E_{cell,i}; \mathbf{K}_a, \mathbf{p})$ . Here, the vector of common parameters  $\mathbf{K}_a = (K_{a,1}, \dots, K_{a,m})$  contains  $m$  dissociation constants of the acid  $H_L$ , while a vector of group parameters

$\mathbf{p} = (E^0, S, K_w, j_a, j_b, L_0, L_T, H_0, H_T)$  contains, in addition to the two constants of the Nernstian equation,  $E^0$  and  $S$ , the total ligand concentration  $L_0$  and the hydrogen ion concentration  $H_0$  of titrand in the vessel, and the corresponding quantities of titrant  $L_T$  and  $H_T$  in the burette [26-28]. Group parameters  $\mathbf{p}$  can be refined simultaneously with the common parameters  $\mathbf{K}_a$ . Two independent regression approaches to a minimization of the sum of square residuals have been applied:

(1) The program ESAB [26,27] uses this strategy for treating the *emf* or  $pa_{H^+}$  data to find dissociation constants that give the “best” fit to experimental data. As primary data contains the total concentration  $H_T$  of protons from the burette and the measured  $pa_{H^+}$ , one could trust the  $pa_{H^+}$  value and minimize the residual sum of squares  $(V_{exp} - V_{calc})^2$ . The residual  $e$  is formulated with the volume of *added titrant*  $V$  from the burette so that  $e_i = (V_{exp,i} - V_{calc,i})$  and the resulting residual sum of squares  $U(\mathbf{b})$  is defined as:

$$U(\mathbf{b}) = \sum_{i=1}^n w_i (V_{exp,i} - V_{calc,i})^2 = \sum_{i=1}^n w_i e_i^2 \quad (2)$$

where  $w_i$  is the statistical weight usually set equal to unity, while in ESAB it may be equal to

$$\frac{1}{w_i} = s_i^2 = s_E^2 + \left[ \frac{dE_i}{dV_i} \right]^2 s_V^2 \quad (3)$$

With good equipment, we generally have  $s_E = 0.1$  mV or 0.01 pH units and  $s_V = 0.0005 - 0.0010$  cm<sup>3</sup>.

(2) In the program HYPERQUAD [29], the objective function is given in matrix notation  $U = e^T W e$ , where  $e$  is a vector of residuals representing a measurement in mV or pH, and  $W$  is a matrix of weights. To minimize the objective function, the Gauss-Newton-Marquardt method is used. The *SIGMA* criterion of a goodness-of-fit is defined as  $SIGMA = \sqrt{\frac{\sum w e_i^2}{n-m}}$ , where the weights  $w$  are calculated from estimates of the error in the *emf* or  $p a_{H^+}$  and titre, the latter only being important in regions where the titration curve slopes more steeply. Sigma squared is also a chi-squared statistic.

Let us consider the dependence of the mixed dissociation constant  $K_a = a_{H^+} [L^{z-1}]/[HL^z]$  on the ionic strength when both ions  $HL^z$  and  $L^{z-1}$  have roughly the same ion-size parameter  $\hat{a}$  in the dissociation equilibrium  $HL^z \rightleftharpoons L^{z-1} + H^+$ , with the thermodynamic dissociation constant  $K_a^T = a_{H^+} a_{L^{z-1}}/a_{HL^z}$  and that the overall salting-out coefficients is given by  $C = C_{HL^z} - C_{L^{z-1}}$ . This dependence is expressed by the extended Debye-Hückel equation:

$$pK_a = pK_a^T - \frac{A(1-2z)\sqrt{I}}{(1+B\hat{a}\sqrt{I})} + CI \quad (4)$$

where  $A = 0.5112$  mole<sup>-1/2</sup> L<sup>1/2</sup> K<sup>3/2</sup> and  $B = 0.3291 \times 10^{10}$  mole<sup>-1/2</sup> m<sup>-1</sup> L<sup>1/2</sup> K<sup>1/2</sup> for aqueous solutions at 25°C. The mixed dissociation constant  $pK_a$  represents a dependent variable, while the ionic strength  $I$  stands for the independent variable. Three unknown parameters,  $\mathbf{b} = \{pK_a^T, \hat{a}, C\}$ , are to be estimated by a minimization of the sum of squared residuals:

$$U(\mathbf{b}) = \sum_{i=1}^n w_i [pK_{a,exp,i} - pK_{a,calc,i}]^2 = \sum_{i=1}^n w_i [pK_{a,exp,i} - f(I_i; pK_a^T, \hat{a}, C)]^2 = \text{minimum} \quad (5)$$

The mixed dissociation constant  $pK_a$  represents a dependent variable, while the ionic strength  $I$  stands for the independent variable. The nonlinear estimation problem is simply a problem of optimization in the parameter space in which the  $pK_a$  and  $I$  are known and given values, while the parameters  $pK_a^T$ ,  $\hat{a}$ , and  $C$  are unknown variables to be estimated. However, for small values of the ionic strength, the  $pK_a^T$  can only be estimated [25,30,31].

## 2.2. Reliability of Estimated Dissociation Constants

The goodness-of-fit may be examined to test the adequacy of a proposed regression model with experimental data and the reliability of the parameter estimates obtained,  $b_j, j = 1, \dots, m$ , cf. page 101 in [30].

(1) *The quality of the parameter estimates obtained*,  $b_j, j = 1, \dots, m$ , is considered according to their confidence intervals or according to their variances,  $D(b_j)$ . Often an

empirical rule is used: a parameter  $b_j$  is considered to be significantly different from zero when its estimated value is greater than three standard deviations, i.e.,  $3\sqrt{D(b_j)} < |b_j|, j = 1, \dots, m$ . Higher parameter variances are also caused by termination of a minimization process before reaching a minimum [30].

(2) *The quality of experimental data* is examined by the identification of influential points with the use of regression diagnostics, cf. page 62 in [32].

(3) *The quality of achieved curve fitting*: the adequacy of a proposed model and  $m$  parameter estimates found with  $n$  values of experimental data is examined by the goodness-of-fit test based on the statistical analysis of classical residuals. If the proposed model represents the data adequately, the residuals should form a random pattern having a normal distribution  $N(0, s^2)$  with the residual mean equal to zero,  $E(\hat{e}) = 0$ , and the standard deviation of residuals  $s(\hat{e})$  near to noise, i.e., experimental error  $\epsilon$ . Systematic departures from randomness indicate that the model and parameter estimates are not satisfactory. The following statistics of residuals can be used for a numerical goodness-of-fit evaluation, cf. page 290 in [32]: (a) The *residual bias* being the arithmetic mean of residuals,  $E(\hat{e})$  should be equal to zero; all residual values lying outside the modified Hoaglin's inner bounds  $B_L^*$  and  $B_U^*$  (cf. page 47 in [33]) are considered to be outliers. (b) The *mean of absolute values of residual*,  $s E|\hat{e}|$ , and the square-root of the residuals variance,  $s^2(\hat{e}) = U(b)/(n-m)$ , known as the estimate of the *residual standard deviation*,  $s(\hat{e})$ , should both be of the same magnitude as the instrumental error of the regressed variable  $y$ ,  $s_{inst}(y)$ . Obviously, it is also valid that  $s(\hat{e}) \approx s_{inst}(y)$ . (c) The *residual skewness*,  $g_1(\hat{e})$ , for the symmetric distribution of residuals should be equal to zero; (d) The *kurtosis*,  $g_2(\hat{e})$ , for the normal distribution should be equal to 3.

## 2.3. Materials

*Naphazoline nitrate* was purchased from LOBA Feinchemie, Austria, with a purity of 99.3%. *Hydrochlorid acid*, 1 mol dm<sup>-3</sup>, was prepared by dilution of concentrated HCl (p. a., Lachema Brno) with redistilled water and standardized against HgO and KI with a reproducibility better than 0.2% according to the following reaction scheme:  $HgO + 4 KI + H_2O \rightleftharpoons 2 KOH + K_2[HgI_4]$  and  $KOH + HCl \rightleftharpoons KCl + H_2O$ . *Potassium hydroxide*, 1 mol dm<sup>-3</sup>, was prepared from the exact weight of pellets (p. a., Aldrich Chemical Company) with carbon dioxide-free redistilled water that was previously kept for 50 minutes in a sonographic bath. The solution was stored for several days in a polyethylene bottle in an argon atmosphere. This solution was standardized

against a solution of potassium hydrogen-phthalate using the derivative method with a reproducibility of 0.1%. *Mercury oxide*, *potassium iodide* and *potassium chloride*, p. a. Lachema Brno, were not additionally purified. *Twice-redistilled water* that was previously kept for 50 minutes in a sonographic bath was used in the preparation of solutions.

## 2.4. Apparatus

The free hydrogen ion concentration  $h$  was measured on a Hanna HI 3220 digital voltmeter with a precision of  $\pm 0.002$  pH with the use of a Theta HC 103-VFR combined glass electrode. Titrations were performed in a water-jacketed double-walled glass vessel of 100 mL, closed with a Teflon bung containing the electrodes, an argon inlet, a thermometer, a propeller stirrer and a capillary tip from a micro-burette. All pH measurements were carried out at  $25.0^\circ\text{C} \pm 0.1$  or  $37.0^\circ\text{C} \pm 0.1$ . During the titrations, a stream of argon gas was bubbled through the solution both for stirring and for maintaining an inert atmosphere. The argon was passed through an aqueous ionic medium by prior passage through two vessels also containing the titrand medium before entering the corresponding titrand solution. The gas is best introduced under and also above the surface of the titrand. Sometimes the flow under the surface has to be stopped while the pH is measured. If the gas flow is too fast, solution might be lost as spray on the walls.

The burettes used were syringe micro-burettes with a capacity of 1250  $\mu\text{L}$  (META, Brno) and a 25.00 cm micrometer screw [35]. The polyethylene capillary tip of the micro-burette was immersed in the solution when adding reagent, but pulled out after each addition in order to avoid leakage of reagent during the pH reading. The micro-burette was calibrated by weighing water on a Kern 770 balance with a precision of  $\pm 0.015\%$  in added volume over the whole volume range.

## 2.5. Procedure

To determine mixed dissociation constants and/or thermodynamic dissociation constants of protonation equilibria of drug acids, the following steps were applied:

Step 1. *Calibration of glass electrode cell*,  $\text{p}K_w$ ,  $H_T$ : The hydrogen activity scale,  $\text{p}a_{\text{H}^+}$ , was used after standardization using 3 WTW standard buffers of values 4.006 (4.024), 6.865 (6.841) and 9.180 (9.088) at  $25^\circ\text{C}$  and  $37^\circ\text{C}$ , respectively, in brackets.

Step 2. *Determination of the concentration of drug acid*  $L_0$ : To analyze a  $\text{pH}$ -titration curve for a mixture of a drug acid and HCl with KOH using the ESAB [26,27] or HYPERQUAD [29] programs, the content of drug acid

$L_0$  was determined. A mixture of 15.00 mL containing  $L_0^{(0)} = 0.005$  M drug,  $H_0^{(0)} = 0.004$  M hydrochloric acid and 1 mL of indifferent solutions of KCl for an adjustment of ionic strength was titrated with standard  $H_T^{(0)} = 0.896$  M KOH at  $25^\circ\text{C}$  and about 80 - 100 titration points  $\{V, \text{pH}\}$  were recorded.

Step 3. *Protonation/dissociation equilibria of drug acid*,  $K_{a,j}$ ,  $j = 1, \dots, J$ : The dissociation constant  $K_{a,j}$ ,  $j = 1, \dots, J$  was determined to analyze a set of pH titration curves corresponding to a mixture of a drug acid and HCl with KOH using the ESAB or HYPERQUAD programs when previously estimated values of group parameters  $H_T$ ,  $L_0$  are used.

Step 4. *Reliability of dissociation constant*  $K_{a,j}$ ,  $j = 1, \dots, J$ : The reliability of the dissociation constant  $K_{a,j}$ ,  $j = 1, \dots, J$  was considered on the basis of goodness-of-fit tests performed by the statistical analysis of residuals.

## 2.6. Calculation

Computation relating to the determination of dissociation constants was performed by regression analysis of titration curves using the ESAB program, version ESAB2M and the HYPERQUAD program. The thermodynamic dissociation constant  $\text{p}K_a^T$  was estimated with the nonlinear regression program MINOPT in the statistical system ADSTAT (TriloByte Statistical Software, Ltd. Pardubice) [36].

## 2.7. Supporting Information

Complete experimental and computational procedures, input data specimens and corresponding output in numerical and graphical form for the ESAB and HYPERQUAD programs are available free of charge online at <http://meloun.upce.cz> and in the DOWNLOAD and DATA blocks.

## 3. Results and Discussion

For the adjusted value of the ionic strength, the potentiometric titration of a mixture of HCl and naphazoline acid with potassium hydroxide was carried out. The initial tentative value of the dissociation constant of the drug under study, corresponding to the midpoint value in each plateau of the potentiometric titration curve (Fig. 2a), was refined by the ESAB and/or the HYPERQUAD programs.

Table 1 shows the results of the ESAB regression analysis of a part of a particular titration curve when the minimization process terminates. Besides the original data  $\{V, \text{p}a_{\text{H}^+}\}$ , residuals and the Bjerrum protonation

**Table 1a.** ESAB refinement of common and group parameters for a titration of naphazoline acid with KOH. *Common parameters refined:*  $pK_{a1} = 10.1$  ( $s = 0.01$ ). *Group parameters refined:*  $L_0 = 4.992 \times 10^{-3}$  mol dm<sup>-3</sup>,  $H_T = -0.8961$  mol dm<sup>-3</sup>. *Constants:*  $H_0 = 3.641 \times 10^{-3}$  mol dm<sup>-3</sup>,  $t = 25.0$  °C,  $pK_w = 13.9799$ ,  $V_0 = 15.05$  cm<sup>3</sup>,  $s(V) = 0.0001$  cm<sup>3</sup>,  $j_a = 0.0$  mV,  $j_b = 0.0$  mV,  $I_0 = 0.0$  [mol dm<sup>-3</sup>] (in vessel),  $I_T = 0.8961$  [mol dm<sup>-3</sup>] (in burette).

<i>I</i>	Volume [cm <sup>3</sup> ]	Residual [cm <sup>3</sup> ]	pa <sub>H+</sub>	Protonation function
1	0.0602	-0.0001	8.169	0.99
2	0.0605	0.0000	8.341	0.99
3	0.0607	0.0000	8.412	0.99
4	0.0610	0.0000	8.517	0.99
5	0.0612	0.0001	8.587	0.98
6	0.0615	0.0002	8.662	0.98
7	0.0620	0.0001	8.766	0.98
8	0.0625	0.0001	8.870	0.97
9	0.0630	0.0000	8.937	0.96
10	0.0635	-0.0001	8.998	0.96
11	0.0640	0.0000	9.051	0.95
12	0.0650	0.0001	9.154	0.94
13	0.0660	0.0000	9.238	0.93
14	0.0670	0.0000	9.296	0.92
15	0.0680	-0.0001	9.359	0.91
16	0.0700	0.0000	9.468	0.89
17	0.0725	0.0000	9.578	0.86
18	0.0750	-0.0002	9.665	0.84
19	0.0775	-0.0001	9.748	0.81
20	0.0800	0.0001	9.824	0.78
21	0.0850	-0.0001	9.943	0.73
22	0.0900	-0.0002	10.055	0.68
23	0.0950	-0.0001	10.155	0.62
24	0.1050	0.0000	10.343	0.52
25	0.1100	-0.0002	10.426	0.47
26	0.1150	-0.0001	10.502	0.43
27	0.1200	0.0000	10.578	0.39
28	0.1250	0.0001	10.658	0.34
29	0.1300	-0.0002	10.736	0.30

**Table 1b.** Reliability of parameter estimates proved by a statistical analysis of residuals

Bias, $E(\hat{e})$	-0.0000026 cm <sup>3</sup>
Lower and upper Hoaglin's limits	-0.0002 cm <sup>3</sup> and 0.0001 cm <sup>3</sup> , no outliers
Mean of absolute values of residuals, $E \hat{e} $	0.00006 cm <sup>3</sup>
Standard deviation, $s(\hat{e})$	0.000085 cm <sup>3</sup>
Skewness, $g_1(\hat{e})$	0.12 (not differing from 0)
Kurtosis, $g_2(\hat{e})$	2.03 (not differing from 3)
Jarque-Berra normality test of a residuals	Normality accepted

**Table 2.** Mixed dissociation  $pK_a$  constant of naphazoline acid at 25°C and various values of the ionic strength  $I$  [mol dm<sup>-3</sup>] estimated by nonlinear regression programs ESAB and HYPERQUAD. Standard deviation of parameter estimates in last valid digits are in brackets.

$I$ [mol dm <sup>-3</sup> ]	ESAB		HYPERQUAD	
	$pK_a$	$ \hat{e} $ [ $\mu$ L]	$pK_a$	SIGMA
0.008	10.389(3)	0.1	10.402(4)	0.734
	10.376(4)	0.1	10.389(4)	0.891
	10.393(7)	0.2	10.400(5)	1.148
0.047	10.439(3)	0.1	10.440(4)	0.612
	10.439(3)	0.1	10.435(3)	0.535
	10.437(2)	0.1	10.438(3)	0.504
0.085	10.471(4)	0.1	10.477(5)	0.879
	10.479(4)	0.1	10.478(6)	1.007
	10.476(3)	0.1	10.476(4)	0.625
0.122	10.509(4)	0.1	10.512(5)	0.835
	10.515(4)	0.1	10.514(5)	0.869
	10.508(5)	0.1	10.510(5)	0.805
0.158	10.536(4)	0.1	10.531(5)	0.912
	10.533(4)	0.1	10.530(5)	0.970
	10.531(4)	0.1	10.532(4)	0.854
0.193	10.550(4)	0.2	10.543(5)	0.868
	10.542(5)	0.1	10.547(6)	1.111
	10.544(5)	0.1	10.544(5)	0.875
0.227	10.556(4)	0.2	10.555(5)	0.834
	10.558(4)	0.1	10.559(5)	0.721
	10.558(4)	0.1	10.557(4)	0.681
0.261	10.588(5)	0.1	10.589(5)	0.804
	10.599(5)	0.1	10.598(5)	0.684
	10.591(4)	0.1	10.592(4)	0.640

function at each point are given. Both the common and the group parameters are refined and the best curve-fitting is proven by the results of a statistical analysis of the residuals. The strategy of an efficient computation in refinement of the group parameters was described in [34]. The reliability of the protonation constant may be determined according to the goodness-of-fit. With an increasing number of group parameters being refined, a better fit is achieved and therefore a more reliable estimate of protonation constants results. As further group parameters are refined, the fit is improved. A quite sensitive criterion of the reliability of the protonation constant is the mean of absolute values of residuals,  $E|\hat{e}|$ . Comparing residuals with the instrumental noise,

$s_{inst}(y)$ , represented here by either  $s(V) = 0.001$  cm<sup>3</sup> or  $s(E) = 0.2$  mV, an excellent fit is confirmed because the mean  $E|\hat{e}|$  and the residual standard deviation  $s(\hat{e})$  are nearly the same or lower than the noise  $s_{inst}(y)$ . Here,  $E|\hat{e}| = 0.0001$  cm<sup>3</sup> and  $s(\hat{e}) = 0.0002$  cm<sup>3</sup> are similar and both are lower than the burette error,  $s(V) = 0.0010$  cm<sup>3</sup>. As the bias  $E(\hat{e})$  is equal to  $-2.6 \times 10^{-6}$ , which may be taken as zero, no systematic error in curve fitting is expected. All residuals oscillate between lower  $-0.0002$  cm<sup>3</sup> and upper  $0.0001$  cm<sup>3</sup> Hoaglin's inner bounds and no outlying residuals lie outside these bounds. Residuals exhibit a normal distribution as confirmed by the Jarque-Berra normality test for combined sample skewness and kurtosis (*cf.*



**Table 3.** Mixed dissociation  $pK_a$  constant of naphazoline acid at 37°C and various values of the ionic strength  $I$  [mol dm<sup>-3</sup>] estimated by nonlinear regression programs ESAB and HYPERQUAD. Standard deviation of parameter estimates in last valid digits are in brackets.

$I$ [mol dm <sup>-3</sup> ]	ESAB		HYPERQUAD	
	$pK_a$	$ \hat{\epsilon} $ [μL]	$pK_a$	SIGMA
0.008	10.046(5)	0.1	10.048(4)	0.502
	10.058(7)	0.3	10.051(7)	1.415
	10.032(6)	0.2	10.031(6)	1.212
0.047	10.155(5)	0.1	10.152(5)	0.835
	10.149(5)	0.2	10.147(5)	0.876
	10.164(5)	0.1	10.155(6)	0.885
0.085	10.197(4)	0.1	10.195(6)	0.712
	10.206(4)	0.1	10.203(6)	0.752
	10.208(4)	0.1	10.203(6)	0.967
0.121	10.151(6)	0.1	10.142(8)	0.917
	10.173(6)	0.1	10.171(8)	0.953
	10.182(6)	0.1	10.183(7)	0.792
0.157	10.252(4)	0.1	10.258(4)	0.585
	10.256(4)	0.1	10.261(4)	0.639
	10.267(4)	0.1	10.264(8)	1.195
0.193	10.282(4)	0.1	10.280(5)	0.649
	10.291(4)	0.1	10.287(4)	0.653
	10.292(3)	0.1	10.284(4)	0.612
0.227	10.184(11)	0.3	10.176(7)	1.365
	10.295(3)	0.1	10.295(3)	0.424
	10.293(4)	0.1	10.294(4)	0.510
0.260	10.228(6)	0.1	10.228(7)	0.672
	10.231(6)	0.1	10.224(7)	0.654
	10.235(6)	0.1	10.234(6)	0.629

page 80 in [33]), and also by the skewness  $g_1(\hat{\epsilon}) = 0.12$  (which is not significantly different from zero, suggesting a symmetric distribution), and the kurtosis  $g_2(\hat{\epsilon}) = 2.03$  (which is not significantly different from 3, suggesting a normal distribution). An excellent fitness is indicated and the regression parameter estimates are considered sufficiently reliable.

Fig. 2 shows a graphical presentation of regression analysis results showing (a) the potentiometric titration curve of a mixture of HCl and naphazoline at 25°C and (b) the overall scatterplot of classical residuals, which gives an initial impression of residuals. The validity of the model and the parameter estimates are confirmed

because the residuals exhibit a normal distribution with zero mean and also form a random pattern. The lack of systematic departures from randomness indicates that the proposed model is true and that the estimates of the parameter are reliable. As shown in Fig. 2c, the distribution diagram of the relative abundance of all variously protonated species seems to be more interesting than a single numerical value of a protonation constant would suggest. The intersection of both curves gives a value of the protonation constant on the  $pH$ -axis. Fig. 2d shows the dependence of the mixed dissociation constant  $pK_a$  of naphazoline acid on the square root of the ionic strength, which leads to a parameter estimate for  $pK_a^T = 10.41(1)$ .

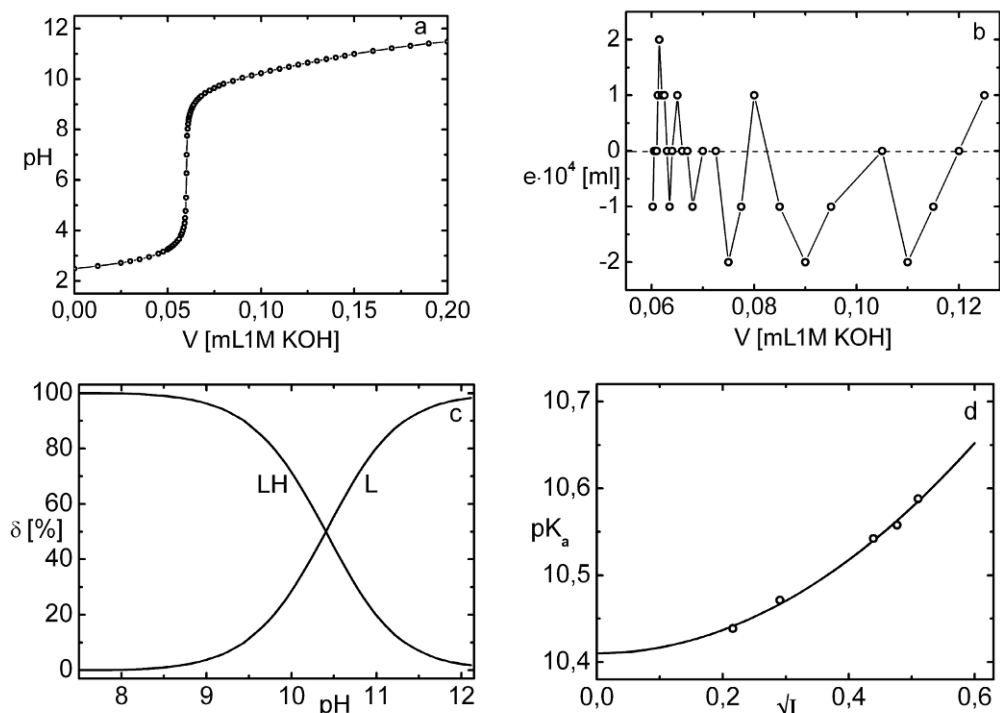
Table 2 shows results for the protonation constant of naphazoline acid determined at various values of ionic strength at 25°C as a result of regression analysis with two different mathematical approaches. The program ESAB, minimizing residuals  $e_i = (V_{exp,i} - V_{calc,i})$ , reaches 0.1 or 0.2 microliters and HYPERQUAD, minimizing  $e_i = (pa_{H^+,exp,i} - pa_{H^+,calc,i})$ , reaches a SIGMA value of about 1 or less, thereby demonstrating an excellent fit.

Table 3 shows results for the protonation constant of naphazoline determined at various values of the ionic strength at 37°C, also demonstrating an excellent fit.

Applying the extended Debye-Hückel equation (Eq. 4) to the data of Tables 2 and 3 according to a regression criterion  $U$  (Eq. 5), the  $pK_a^T$  could be estimated from three unknown parameters,  $pK_a^T$ ,  $\hat{a}$ , and  $C$ , since the range of the ionic strength contains only small values.

The thermodynamic dissociation constants were determined as follows:  $pK_a^T$  (ESAB) = 10.41(1) and  $pK_a^T$  (HYPERQUAD) = 10.42(2) at 25°C and  $pK_a^T$  (ESAB) = 10.13(2) and  $pK_a^T$  (HYPERQUAD) = 10.13(2) at 37°C.

The thermodynamic dissociation constant  $pK_a^T = 10.41$  is estimated with very small standard deviation  $s(pK_a^T) = 0.01$ , indicating a highly reliable estimation. The linear parameter  $pK_a^T$  in the regression model is well conditioned and its estimation is sensitive. It has a strong influence on the residual-square sum function  $U$ . The well-conditioned parameter  $pK_a^T$  has great influence on an elliptic hyperparaboloid shape, when the variable  $U$  is plotted against the three parameters  $pK_a^T$ ,  $\hat{a}$ ,  $C$  in  $(m + 1)$ -dimensional space (here  $m = 3$ ). For well-conditioned parameters, such a shape exhibits an obvious, sharp minimum, the pit point  $U_{min}$ . Fig. 2d provide a graphical presentation of the dependence of the mixed dissociation constant on the square root of the ionic strength.



**Figure 2.** Protonation equilibria of naphazoline acid analyzed with ESAB (a) Potentiometric titration curve of naphazoline acid with KOH;  $L_0 = 4.992 \cdot 10^{-3} \text{ mol dm}^{-3}$ ,  $H_T = 0.8961 \text{ mol dm}^{-3}$ ,  $V_0 = 15.05 \text{ cm}^3$ ,  $I = 0.0008 \text{ [mol dm}^{-3}\text{]}$ ,  $T = 25^\circ\text{C}$ ; (b) Scatterplot of residuals; (c) Distribution diagram of relative amounts of all variously protonated species in the protonation equilibrium, (d) Dependence of the mixed dissociation constant  $pK_a$  of naphazoline acid on the square root of the ionic strength, which leads to parameter estimates  $pK_{a1}^T = 10.41(1)$  at  $25^\circ\text{C}$ .

## 4. Conclusions

The reliability of the dissociation constants of naphazoline was proven even when three *group parameters*  $L_0$ ,  $H_T$  were ill conditioned in a model. Their determination is uncertain and might lead to false estimates of *common parameters*  $pK_a$  and therefore make the computational strategy important. These group parameters can have great influence on a systematic error in the estimated  $pK_a$  value, and they should be refined together with the common parameter  $pK_a$ . The external calibration of the  $p\alpha_{H^+}$  of the glass electrode cell performed during titration is sufficiently accurate. Comparing the two computational approaches, the ESAB and the HYPERQUAD

programs, it appears that ESAB led to a better fit of the potentiometric titration curve. The thermodynamic dissociation constant  $pK_a^T$  was estimated by a nonlinear regression of the dependence of the mixed dissociation constant  $pK_a$  on the ionic strength  $I \text{ [mol dm}^{-3}\text{]}$ . The goodness-of-fit proved sufficient reliability of parameter estimates for this drug at  $25^\circ\text{C}$  and  $37^\circ\text{C}$ .

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