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Thermodynamic dissociation constants of isocaine, physostigmine and pilocarpine by regression analysis of potentiometric data

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Abstract

Concentration and mixed dissociation constant(s) of three drug acids, H_jL, isocaine, physostigmine and pilocarpine, at various ionic strengths, I, in the range 0.03–0.81 and 25°C have been determined with the use of regression analysis of potentiometric titration data when common parameter, pK_a, and group parameters E'^0, L_0, and H_T are simultaneously refined. Internal calibration of the glass electrode cell in the concentration scale $[H^+]$ performed during titration was used. The estimate of ill-conditioned group parameters has a great influence on a systematic error in estimated pK_a and therefore it makes the computational strategy important. As more group parameters are refined and a better fit achieved, a more reliable estimate of dissociation constants results. The thermodynamic dissociation constant, pK_a^T, an ill-conditioned ion-size parameter, $\mathring{a}$, and the salting-out coefficient, C, were estimated by non-linear regression of $\{pK_a, I\}$ data and an extended Debye–Hückel equation. The goodness-of-fit test based on regression diagnostics is a measure of the reliability of parameters, and proves that reliable estimates for isocaine $pK_a^T = 8.96(1)$, $\mathring{a} = 8(3)$ Å and $C = 0.50(3)$ at 25°C, for physostigmine $pK_a^T = 8.07(3)$, $\mathring{a} = 19(26)$ Å and $C = 0.64(3)$ at 25°C, and for pilocarpine $pK_a^T = 7.00(1)$, $\mathring{a} = 7(1)$ Å and $C = 0.53(2)$ at 25°C were found.

Keywords: Dissociation constant; Debye–Hückel; Ion-size parameter; Salting-out coefficient; Isocaine; Physostigmine; Pilocarpine

1. Introduction

Most drugs given orally must pass through the gut wall to enter the bloodstream. This absorption process is affected by many physico-chemical factors such as the dissociation constants $pK_{a,j}$, $j = 1, \ldots, J$, of the J-protic drug, the lipid solubility of the drug, etc. The ultimate goal is to have the drug reach the site of action in a concentration that produces a pharmacological effect. The absorption of unionized molecules is favoured because they are far more lipid-soluble than those that are ionized and surrounded by a shell of water molecules [1]. Ingraham and Visscher [2]

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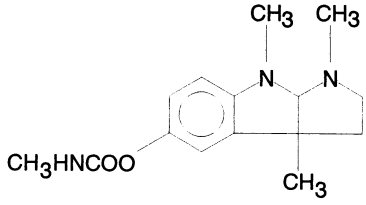
described that only the undissociated form of the compound crosses the blood–gastric juice barrier and that, therefore, the extent of secretion might depend on the dissociation constant. They pointed out that very weak acids should be secreted in measurable amounts, since at blood pH they are in partially undissociated form. This pK_a dependence may be explained by the concept that plasma and gastric juice are separated by a membrane with the characteristics of a lipoid barrier, across which only the undissociated molecule can pass. Since the pH of the two phases differs markedly, the concentration of ionized drug may differ markedly in the two phases, the difference depending on the pK_a of the drug.

The protonation of physostigmine, isocaine and pilocarpine have been studied at various temperatures and ionic strengths [3]. However, in only a few cases has the dependence of the dissociation constants on ionic strength been systematically investigated, and these methods have been reviewed [4–6]. The reliability of dissociation constants obtained by regression analysis of potentiometric data is dependent upon (i) the calibration of the glass electrode cell, (ii) the algorithm used, and (iii) the parameters selected for refinement. It was concluded [7] that an internal calibration of the glass electrode cell performed during titration is more accurate than a separate external calibration. ESAB [8,9] seems to be the most powerful program because it permits a refinement of group parameters and the application of an internal calibration.

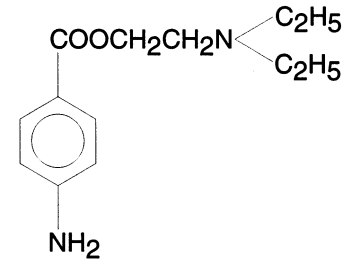
The alkaloid physostigmine, also known as eserine ($C_{15}H_{21}N_3O_2$), is the carbaminic acid ester of 2-hydroxy-(3a*S*-*cis*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]-indol. The actual drug in case is the salicylate of physostigmine (physostigminium salicylicum), having a molecular weight of 413.46 and a melting point of 185–187°C, [3].



Physostigmine

The published dissociation constant is pK_a = 8.27 at $I = 0.1$ ($NaClO_4$) and 25°C [10]. It belongs to the following therapeutic category: cholinergic (anticholinesterase), miotic.

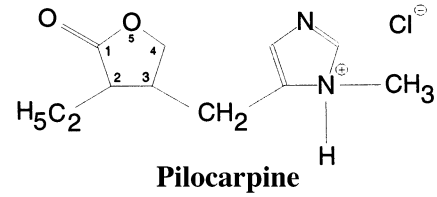
Isocaine ($C_{13}H_{20}N_2O_2$), also known as Cocainum Novum or Procaine, is of chemical structure 2-(*N*,*N*-diethylamino)ethyl-4-aminobenzoate.



Isocaine

The practical drug is procaine hydrochloride (procainum chloratum), which has the published dissociation constant pK_a = 8.98 at 25°C [10]. It belongs to the following therapeutic category: anaesthetic (local).

Pilocarpine is applied as pilocarpinium chloratum ($C_{11}H_{16}N_2O_2$.HCl), also known as 2(3*H*)-furanone, 3-ethyldihydro-4-[(1-methyl-1*H*-imidazol-5yl)methyl]-(3*S*-*cis*) hydrochloride.



Pilocarpine

The published dissociation constant of pilocarpine is pK_a = 7.21 at $I = 0.1$ ($NaClO_4$) and 25°C, or pK_a = 7.40 [3,10]. It belongs to the following therapeutic category: antiglaucoma agent, miotic, gastric secretory stimulant.

In this paper, we investigated the dissociation constants of the three drugs at various ionic strengths and at 25°C, to prove their reliability and also to estimate the thermodynamic dissociation constant, pK_a^T, and two parameters of the extended Debye–Hückel equation, an ion-size parameter, *å*, and the salting-out coefficient, *C*.

2. Theoretical

2.1. Determination of protonation/dissociation constants

The acid–base equilibrium of the drug studied is described in terms of protonation of the Brönsted base L^{z-j}

$$L^{z-j} + H^+ \rightleftarrows HL^{z-j+1}$$

characterized by the thermodynamic protonation constant

$$K_H = \frac{a_{HL^{z-j+1}}}{a^{z-j}_{L^{z-j}} a_{H^+}} = \frac{[HL^{z-j+1}]}{[L^{z-j}][H^+]} \frac{y_{HL^{z-j+1}}}{y_{L^{z-j}} y_H}$$

where the square brackets represent concentrations and y are the activity coefficients (the molar concentration scale is assumed to be used). In the case of a polyprotic species, the base L^{z-j} is protonated to yield a polyprotic acid H_JL:

$$L^{z-j} + H^+ \rightleftarrows HL^{z-j+1} \qquad K_{H1}$$

$$HL^{z-j+1} + H^+ \rightleftarrows H_2L^{z-j+2} \qquad K_{H2}$$

The subscript to K_H indicates the ordinal number of the protonation step. Direct formation of each protonated species from the base L^{z-j} can be expressed by the overall reaction

$$L^{z-j} + jH^+ \rightleftarrows H_jL^z$$

and by the overall constant $\beta_{Hj} = K_{H1}, K_{H2}, \ldots, K_{Hj} = [H_jL^z]/(lh^j)$, where j denotes the number of protons involved in the overall protonation, and l and h are the free concentrations of drug acid $[L^{z-j}]$ and hydrogen $[H^+]$, respectively. The mass balance equations are

$$L = l + \sum_{j=1}^{J} \beta_{Hj} l h^j \qquad \text{and}$$

$$H = h - \frac{K_w}{h} + \sum_{j=1}^{J} \beta_{Hj} l h^j$$

For dissociation reactions investigated at constant ionic strength, so-called 'mixed dissociation constants' are defined as $K_{a,j} = ([H_{j-1}L]a_{H^+})/[H_jL]$. These constants are found in experiments where pH values are measured with glass and reference electrodes, standardized with the practical $pH = pa_{H+}$ activity scale recommended internationally. The $pH = p(a_{H+})_c + \log \rho_s$ where index c means molar concentrations and ρ_s is the density of the solvent. For aqueous solutions and temperatures up to 35°C, this correction is less than 0.003 pH units. The value of $[H_{j-1}L]/[H_jL]$ is determined by potentiometric titration leading to 'conditional constants based upon concentrations' pK_c. If the protonation is studied at several ionic strengths or at a low value of ionic strength, the thermodynamic dissociation constant, pK^T_a, can be obtained by extrapolating to zero ionic strength ($I = 0$), the reference state for the activity coefficient being an infinitely diluted solution.

For potentiometric electromotive force (e.m.f.) titrations, the following relationship holds for the total drug acid, L, and the total hydrogen ion, H^+, concentrations:

$$L = \frac{L_0V_0 + L_TV_T}{V_0 + V_T} \qquad \text{and}$$

$$H = \frac{H_0V_0 + H_TV_T}{V_0 + V_T}$$

where H_0 (or L_0) is the total initial concentration of hydrogen ions (or the drug acid) in the titrand, H_T (or L_T) is the total initial concentration of hydrogen ions (or the drug acid) in the titrant (for hydroxide, $-H_T$ is given), V_0 is the initial volume of the titrand and V_T is the volume of titrant added from burette. Potentiometric readings obtained with the proton-sensitive glass and reference electrodes cell can be described by the equation

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

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, and $a_{H^+} = [H^+]y_{H^+} = hy_{H^+}$, y_{H^+} is the molar activity coefficient of proton, a liquid-junction potential E_j is expressed by the term $E_j = j_a a_{H^+} - j_b K_w/a_{H^+}$, and $S = (fRT \ln 10)/F$ is the slope of glass electrode for a Nernstian response. Sometimes the Nernstian slope deviates from the theoretical value, and in calibration of the electrode, the correction factor, f, is taken as an adjustable

parameter. K_w is the operational ion product of water at temperature T. The term E'^0 in equation

$$E_{cell} = E'^0 + S \log h$$

is expressed as

$$E'^0 = E^0 + S \log y_{H^+} + j_a h y_{H^+} - \frac{j_b K_w}{h y_{H^+}} - E_{ref} \quad (2)$$

For a 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 a constant ionic strength expresses a dependence between the volume of titrant added from burette, V_i, and the monitored e.m.f., $E_{cell,i}$, with the vector of unknown parameters ($\boldsymbol{b}$) being separated into the vector of common parameters ($\boldsymbol{K_a}$) and the vector of group parameters ($\boldsymbol{p}$).

$$V_i = f(E_{cell,i}; \boldsymbol{b}) = f(E_{cell,i}; \boldsymbol{K_a}, \boldsymbol{p}) \quad (3)$$

Here, the vector of common parameters $\boldsymbol{K_a} = (K_{a,1}, \ldots, K_{a,J})$ contains J protonation constants of the drug acid H_JL, while a vector of group parameters $\boldsymbol{p} = (E'^0, S, K_w, j_a, j_b, L_0, L_T, H_0, H_T)$ contains, in addition to the two constants of the Nernst 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 [8,9]. In most cases, all these group parameters cannot be determined independently with sufficient accuracy. However, when working with media of constant high ionic strength, both K_w and j_a (with j_b) may be determined by separate experiments. Group parameters $\boldsymbol{p}$ can be refined simultaneously with the common parameters $\boldsymbol{K_a}$. Since each group parameter affects a part of the residual sum of squares, $U(\boldsymbol{b})$, that comes from a single group, a certain economy can be achieved in computation. By variation of one or two, etc., of the group parameters such as E'^0, L_0 and H_T, the systematic errors in the common parameters $\boldsymbol{K_a} = (K_{a,1}, \ldots, K_{a,J})$ can be minimized or practically disappear. Of the group parameters studied, E'^0 has the greatest influence and an attempt should always be made to refine this parameter together with the protonation constants, in spite of the fact that it might be ill-conditioned and therefore make the computational strategy important [7]. The least-squares method is the best in case of an additive model of measurement and independent normally distributed errors having constant variance. The least-squares strategy is quite fast and often leads to good minima. On the other hand, if some group parameter(s) are uncertain and do not affect the residual sum of squares, $U(\boldsymbol{b})$, this uncertainty causes large standard deviations in other parameters. Two independent regression approaches to a minimization of the sum of squared residuals have been applied.

(1) The program ESAB [8] uses this strategy for treating e.m.f. 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 e.m.f. E_{cell}, one could trust E_{cell} as the independent variable 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, $U(\boldsymbol{b})$, is defined as:

$$U(\boldsymbol{b}) = \sum_{i=1}^{n} w_i (V_{exp,i} - V_{calc,i})^2 = \sum_{i=1}^{n} w_i e_i^2 \quad (4)$$

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

$$\frac{1}{w_i} = s_i^2 = s_E^2 + \left[\frac{dE_i}{dV_i}\right]^2 s_v^2 \quad (5)$$

and, with good equipment, we generally have $s_E = 0.1$–0.3 mV and $s_V = 0.001$–0.005 cm^3.

(2) The program PLUS99 [11] uses a similar strategy, but treats V_{exp} as the independent variable and minimizes the sum of residual squares $(E_{cell,exp} - E_{cell,calc})^2$. The residual e is formulated with the e.m.f. E_{cell} so that $e_i = (E_{cell,exp,i} - E_{cell,calc,i})$ and the resulting residual sum of squares, $U(\boldsymbol{b})$, is defined by

$$U(\boldsymbol{b}) = \sum_{i=1}^{n} w_i (E_{\text{cell,exp},i} - E_{\text{cell,calc},i})^2 = \sum_{i=1}^{n} w_i e_i^2 \quad (6)$$

where w_i is the statistical weight usually set equal to unity but, in PLUS99, the relation in Eq. (5) can also be used.

2.2. Determination of thermodynamic dissociation constant

Let us consider a dependence of the mixed dissociation constant $K_a = a_{H^+}[L^{z-j}]/[HL^{z-j+1}]$ on the ionic strength, I, when both ions HL^{z-j+1} and L^{z-j} have roughly the same ion-size parameter, $\mathring{a}$, in the dissociation equilibrium (for $j=1$), i.e. $HL^z \rightleftarrows L^{z-1} + H^+$ with the thermodynamic dissociation constant $K_a^T = a_{H^+}a_{L^-}/a_{HL}$ and that the overall salting-out coefficient is given by $C = C_L - C_{HL}$. This dependence is expressed by the extended Debye–Hückel equation

$$pK_a = pK_a^T - \frac{A(1-2z)\sqrt{I}}{(1+B\mathring{a}\sqrt{I})} + (C_L - C_{HL})I \quad (7)$$

where $A = 0.5112\ \text{mol}^{-1/2}\ \text{l}^{1/2}\ \text{K}^{3/2}$ and $B = 0.3291\ \text{mol}^{-1/2}\ \text{m}^{-1}\ \text{l}^{1/2}\ \text{K}^{1/2}\ 10^{10}$ for aqueous solutions and 25°C, and the ionic strength, I, is given by the usual expression $I = 0.5\Sigma_{i=1}^{n} c_i z_i^2$. The mixed dissociation constant, pK_a, represents a dependent variable while the ionic strength, I, stands for the independent variable. Three unknown parameters $\boldsymbol{b} = \{pK_a^T, \mathring{a}, C\}$ are to be estimated by a minimization of the sum of squared residuals:

$$\begin{aligned} U(\boldsymbol{b}) &= \sum_{i=1}^{n} w_i [pK_{a,\text{exp},i} - pK_{a,\text{calc},i}]^2 \\ &= \sum_{i=1}^{n} w_i [pK_{a,\text{exp},i} - f(I_i; pK_a^T, \mathring{a}, C)]^2 \\ &= \text{minimum} \end{aligned} \quad (8)$$

The non-linear 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, $\mathring{a}$, and C are unknown variables to be estimated.

2.3. Reliability of estimated dissociation constants

The reliability of determined parameter estimates, b_j, $j = 1, \ldots, m$, for m unknown parameters may be examined by the goodness-of-fit test also called the fitness test (cf. p. 101 in Ref. [6]): certain underlying assumptions have been outlined for the regression analysis such as the independence of random error, constant variance, and normal distribution for random errors. If the model represents the data adequately, the residuals should possess characteristics that agree with, or at least do not refute, the basic assumptions: the residuals should be randomly distributed about dependent variable predicted by the regression model. A source of problems may be found in components of a regression triplet (the data quality for a proposed model, the model for a given data set, and the method of estimation based on fulfilment of all least-squares assumptions).

(1) The quality of parameter estimates b_j, $j = 1, \ldots, m$, is considered according to their confidence intervals or according to their variances $D(b_j)$. Often, an empirical rule is used: parameter b_j is considered to be significantly differing from zero when its estimate is greater than three standard deviations, $3\sqrt{D(b_j)} < |b_j|$, $j = 1, \ldots, m$. Higher parameter variances are also caused by termination of a minimization process before reaching a minimum.

(2) The quality of experimental data is examined by the identification of influential points with the use of regression diagnostics (cf. p. 62 in Ref. [12]). The most suitable diagnostics are the likelihood distances LD_i and Jackknife residuals, $\hat{e}_{J,i}$. For linear regression models, all characteristics for the identification of influential points are functions of the residuals, $\hat{e}_j$, and diagonal elements, H_{ii}, of the projection matrix $\boldsymbol{H} = \boldsymbol{X}(\boldsymbol{X}^T\boldsymbol{X})^{-1}\boldsymbol{X}^T$. For non-linear regression models, the situation is rather more complicated as the parameter estimates and residuals cannot be expressed so simply as the linear combination of experimental data (cf. p. 292 in Ref. [12]). When the Taylor type linearization of original non-linear model is used, all methods of identification of influential points in

linear models can be used. The procedure starts from the one-step approximation of the parameter estimate computed without the ith point

$$b_{(i)}^{1} = b - (\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J}^{-1})J_i\frac{\hat{e}_i}{1-P_{ii}} \tag{9}$$

where P_{ii} are elements of a projection matrix, $\boldsymbol{P}=\boldsymbol{J}(\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J})^{-1}\boldsymbol{J}^{\mathrm{T}}$ [12]. The influential points may be easily identified on the basis of the one-step approximation of the Jackknife residuals, $\hat{e}_{J,i}$, calculated by the relation

$$\hat{e}_{J,i} = \frac{\hat{e}_i}{\hat{s}_{(i)}\sqrt{1-P_{ii}}} \tag{10}$$

where $\hat{s}_{(i)}^2$ is the residual variance computed by using estimates $\boldsymbol{b}_{(i)}$

$$\hat{s}_{(i)}^2 = \frac{U(\boldsymbol{b}) - [\hat{e}_i^2/(1-P_{ii})]}{n-4} \tag{11}$$

Jackknife residuals higher than 3 indicate highly influential points. A non-linear measure of the influence of the ith point on the parameter estimates is represented by the regression diagnostic called the likelihood distance

$$LD_i = 2[\ln\ L(b) - \ln\ L(b_{(i)})] \tag{12}$$

When $LD_i > \chi^2_{1-a}(2)$ is valid, the ith point is strongly influential. The significance level α is usually chosen to be equal to 0.05, then $\chi^2_{0.95}(2) = 5.992$.

(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 $s(\hat{e})$ being near to noise, i.e. experimental error ε. Systematic departures from randomness indicate that the model and parameter estimates are not satisfactory. Examination of residual plots may assist graphical analysis of residuals (cf. p. 288 in Ref. [12]). The overall diagram of residuals gives an initial impression: detection of outliers, detection of a trend in the residuals, detection of a sign change, and detection of an abrupt shift of level in the experiment. The following statistics of residuals can be used for a numerical goodness-of-fit evaluation (cf. p. 290 in Ref. [12]). (1) 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. p. 81 in Ref. [13]) are considered to be outliers. (2) The mean of absolute values of residuals, $E|\hat{e}|$, and the square-root of the residuals variance, $s^2(\hat{e}) = U(\boldsymbol{b})/(n-m)$ known as the estimate of the residual standard deviation, $s(\hat{e})$, should be both of the same magnitude as the experimental error of regressed variable $\mathrm{p}K_{\mathrm{a}}$, $s(\mathrm{p}K_{\mathrm{a}})$. Obviously, it is also valid that $s(\hat{e}) \approx s(\mathrm{p}K_{\mathrm{a}})$. (3) The residual skewness, $g_1(\hat{e})$, for symmetric distribution of residuals should be equal to zero. (4) The kurtosis, $g_2(\hat{e})$, for normal distribution should be equal to 3. (5) The determination coefficient, D, calculated from the relation

$$D = 1 - U(\boldsymbol{b})/\sum_{i=1}^{n}(\mathrm{p}K_{\mathrm{a,exp},i} - \mathrm{p}\bar{K}_{\mathrm{a,exp}})^2 \tag{13}$$

multiplied by 100% is called the regression rabat and is equal to percentage of points which correspond to proposed regression model. (6) The Hamilton R-factor of relative fitness is often used, being expressed by $R-\mathrm{factor} = \sqrt{\mathrm{U}(\boldsymbol{b})/\Sigma_{i=1}^{n}y_i^2}$. There is an empirical rule of a fitness classification with the use of the Hamilton R-factor. For a good fit, the Hamilton R-factor reaches a value lower than or equal to 1%; for excellent fitness, it is lower than 0.5%. (7) To distinguish between various models, it is suitable to apply the Akaike information criterion, AIC, being defined by the relation $AIC = -2L(b) + 2m$, or $AIC = n\ln[(U(\boldsymbol{b}))/n] + 2m$, where n is the number of data and m is the number of estimated parameters. The best regression model is considered to be a model for which this criterion reaches a minimal value.

3. Experimental

3.1. Chemicals

Isocaine hydrogenchloride, physostigmine salicylate and pilocarpine hydrogenchloride were weighted directly into a reaction vessel to reach a resulting concentration of about 0.015 mol dm^{-3}. Solutions are made by dilution of standard stock solutions, prepared from highly purified components. Hydrochloric acid (1 mol dm^{-3}) was pre-pared 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 equation $HgO + 4KI + H_2O \rightleftarrows 2KOH + K_2[HgI_4]$ and $KOH + HCl \rightleftarrows KCl + H_2O$. Potassium hydroxide (1 mol dm^{-3}) was prepared from the exact weight of pellets (p.a., Aldrich Chemical Company) with a carbon-dioxide-free redistilled water. This solution was stored for several days in a polyethylene bottle and then standardized against a solution of potassium hydrogen-phthalate using the Gran method in the MAGEC program [14] with a reproducibility of 0.1%. Mercury oxide, and potassium iodide, potassium chloride (p.a., Lachema Brno) were not further purified. Twice-redistilled water was used in for the preparation of solutions.

3.2. Potentiometric apparatus and cells

The free hydrogen-ion concentration, h, was measured via e.m.f. (Eq. (1)) on a digital voltmeter OP-208/1 (Radelkis, Budapest) with a precision of ± 0.1 mV using a glass electrode G202B (Radiometer, Copenhagen) and a commercial SCE reference electrode OP-8303P (Radelkis, Budapest). Titrations were performed in a water-jacketed glass vessel of 100 cm^3, 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 e.m.f. measurements were carried out at 25 ± 0.1°C. 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 pure ionic medium in one or two vessels before entering the corresponding equilibrium solution. The gas is best introduced under surface of the titrand. Sometimes the flow has to be stopped while the e.m.f. is measured. The burettes used were syringe micro-burettes of 1250 ml capacity (META, Brno) with a 25.00 cm micrometer screw [15]. The polyethylene capillary tip of the micro-burette was immersed into a 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 Sartorius 1712 MP8 balance with a precision of $\pm 0.015\%$ in added volume over the whole volume range. All solutions were stored at the temperature to be used for measurements.

3.3. Calibration of glass electrode cell

The potentiometric titrations of drugs with potassium hydroxide were performed using a hydrogen concentration scale where the hydrogen ion concentration $[H^+] = h$ was known from a preparation of solution and the e.m.f. E_{cell} (mV) was measured. Using a set of experimental data $\{E_{cell}, h\}$ from a calibration titration of hydrochloric acid of known concentration with standard potassium hydroxide, the unknown group parameters E'^0 and S in Eq. (1) were evaluated. The internal calibration of the glass electrode cell was used when the program ESAB estimated H_T, L_0 and E'^0 from an actual titration of a mixture of drug and hydrochlorid acids with potassium hydroxide. Some group parameters are given in the input data for ESAB such as the Nernstian slope and pK_w, both of which are accessible from the literature [16]. Group parameters can be estimated by a regression analysis of both segments of a titration curve or from the acid segment only if the basic one might be affected by some carbonate as well as silicate in the alkali. With ESAB, three group parameters, E'^0, L_0 and H_T, were refined to give the best fit. The fitness may be examined by the

goodness-of-fit criteria; for example, the Hamilton R-factor of relative fitness and the mean of absolute values of residuals. Since E'^0 might change from one titration to another because of a change of the liquid-junction potential, the internal calibration of the glass electrode cell seemed to be more accurate and has been preferred.

3.4. Procedure for 'equilibrium titration'

The glass electrode cell has to be calibrated in solutions of known composition in order to find characteristic parameters. To determine mixed dissociation constants and/or thermodynamic dissociation constants of protonation equilibria of drug acids, the following steps were applied.

Step 1: Standardization of hydrochloric acid c_{HCl}. Hydrochloric acid was standardized by $HgO + KI$ titration and evaluated by the Gran method (MAGEC [14]).

Step 2: Calibration of glass electrode cell, E'^0, S, pK_w, H_T. The hydrogen concentration $[H^+] = h$ is known from an initial concentration, H_0, and measured e.m.f., E. From the equation $E = E'^0 + S \log h$ for each point $\{E, h\}$ of the titration curve of known concentration of hydrochloric acid H_0 with standard potassium hydroxide, the group parameters E'^0, S and H_T were refined.

Step 3: Determination of the concentration of drug acid L_0. To analyze an e.m.f. titration curve concerning a mixture of a drug acid and HCl with KOH by ESAB or PLUS99 programs, the content of drug acid L_0 was determined. A mixture of 20.00 cm^3 containing $L_0^{(0)} = 0.015$ mol dm^{-3} drug, $H_0^{(0)} = 0.100$ mol dm^{-3} hydrochloric acid and 10 cm^3 different solutions KCl for an adjustment of the ionic strength was titrated with standard $H_T^{(0)} = 1.0$ mol dm^{-3} KOH at 25°C and about 30–40 titration points $\{v, E_{cell}\}$ were recorded. Here, $L_0^{(0)}$ and $H_0^{(0)}$ stand for the initial guess of parameters L_0 and H_0 in regression refinement.

Step 4: Protonation equilibria of drug acid, $K_{H,j}$, $j = 1, \ldots, J$. To analyze a set of e.m.f. titration curves concerning a mixture of a drug acid and HCl with KOH by the ESAB or the PLUS99 programs when previously estimated values of group parameters E'^0, S, H_T, L_0 are used, the dissociation constant $K_{H,j}$, $j = 1, \ldots, J$ was determined.

Step 5: Reliability of dissociation constant $K_{H,j}$, $j = 1, \ldots, J$. The reliability of the dissociation constant $K_{H,j}$, $j = 1, \ldots, J$ was considered on the basis of the goodness-of-fit tests performed by the statistical analysis of residuals.

3.5. Computer data treatment

The computation of dissociation constants were performed by regression analysis of the titration curves using the ESAB program, version ESAB2M [8,9] and the PLUS99 program [11]. The thermodynamic dissociation constant pK^T, an ion-size parameter, $\mathring{a}$, and the salting-out coefficient, C, were estimated with the non-linear regression program MINOPT in the statistical system ADSTAT (TriloByte Statistical Software, Ltd. Pardubice) [17].

4. Results and discussion

4.1. Estimation of dissociation constants

For the adjusted value of an ionic strength, the potentiometric titration of a mixture of HCl and drug acid with potassium hydroxide was carried out. The initial tentative value of the dissociation constant of the drug studied, corresponding to the midpoint value in each plateau of the potentiometric titration curve (Fig. 1a), was refined by the ESAB and/or the PLUS99 programs.

Tables 1 and 2 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 $\{v, E_{cell}\}$ and $-\log h$, the Bjerrum protonation function at each point is 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 our previous paper [7]. The reliability of the protonation constant may be determined according to the goodness-of-fit: with increasing number of group parameters refined, a better fit is achieved, and therefore a more reliable estimate of protonation constants results. E'^0 has the greatest influence on the accuracy, and hence should always be refined. As further group parameters are refined, the fit is improved. A quite sensitive criteria of the reliability of the protonation constant are the Hamilton R-factor of relative fitness and the mean of absolute values of residuals $E|\hat{e}|$. Comparing residuals with the instrumental noise, $s_{\text{inst}}(y)$, represented here by either $s(V) = 0.001$ cm^3 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 and lower than the noise $s_{\text{inst}}(y)$. Here, $E|\hat{e}| = 0.0001$ cm^3 and $s(\hat{e}) = 0.0002$ cm^3 are similar, and both are lower than the burette error $s(V) = 0.0010$ cm^3. As the bias $E(\hat{e})$ is equal to -10^{-22}, which may be taken as zero, no systematic error in curve fitting is expected. All residuals oscillate between lower and upper Hoaglin's inner bounds and no residuals lay outside these bounds. Residuals exhibit a normal distribution as confirmed by the Jarque–Berra normality test for combined sample skewness and kurtosis (cf. p. 80 in Ref. [13]), and also by the skewness $g_1(\hat{e}) = 0.12$ (which is not significantly different from zero and therefore proving a symmetric distribution), and the kurtosis $g_2(\hat{e}) = 2.03$ (which is not significantly different from 3 and therefore proving a normal distribution). The regression rabat, $100D =$ 99.99%, indicates that a high percentage of titration curve points fulfils the regression model with parameter estimates found; in fact, all points. With the use of Akaike information criterion, $AIC = -296.9$, a most suitable regression model among several plausible ones and the best esti-

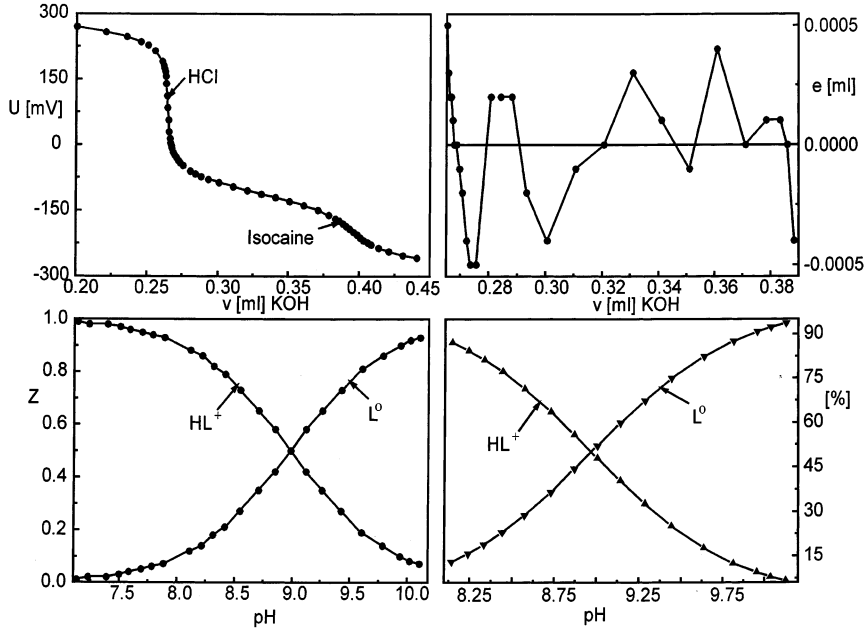


Fig. 1. Protonation equilibria of isocaine analyzed with ESAB. (a) Potentiometric titration curve of isocaine hydrogenchloride with KOH; $L_0 = 0.0152$ mol dm^{-3}, $L_T = 1.0777$ mol dm^{-3}, $V_0 = 10.25$ cm^3. $I = 0.027$, $t = 25$°C. (b) Plot of residuals. (c) Bjerrum formation function (ESAB). (d) Distribution diagram of relative presentation of all species of protonation equilibrium.

Table 1
ESAB refinement of common and group parameters for a titration of isocaine with KOH[a]

i	Volume (cm^3)	Residual (cm^3)	E_{cell} (mV)	$-\log h$	Protonation function
1	0.2617	0.0002	4.00	6.925	0.99
2	0.2622	0.0001	−5.70	7.089	0.99
3	0.2627	0.0001	−12.20	7.198	0.99
4	0.2632	0.0001	−18.20	7.300	0.98
5	0.2642	0.0001	−27.70	7.460	0.97
6	0.2652	0.0000	−34.10	7.569	0.97
7	0.2667	−0.0001	−41.40	7.692	0.96
8	0.2682	−0.0001	−47.50	7.795	0.94
9	0.2707	−0.0002	−55.50	7.930	0.93
10	0.2732	−0.0003	−61.60	8.033	0.91
11	0.2757	−0.0002	−67.40	8.131	0.89
12	0.2807	0.0001	−76.50	8.285	0.84
13	0.2857	0.0002	−83.60	8.405	0.81
14	0.2933	−0.0002	−91.90	8.546	0.75
15	0.3008	−0.0002	−99.30	8.671	0.69
16	0.3108	0.0003	−108.60	8.828	0.61
17	0.3208	−0.0001	−116.40	8.960	0.54
18	0.3309	0.0003	−124.80	9.102	0.45
19	0.3409	0.0000	−132.70	9.235	0.38
20	0.3509	−0.0001	−141.40	9.382	0.30
21	0.3609	0.0000	−151.60	9.555	0.23

[a] Common parameters refined: $\log K_{H1} = 9.022(3)$. Group parameters refined: $L_0 = 0.01387(3)$ mol dm^{-3}, $H_T = -1.1062(3)$ mol dm^{-3}, $E^0 = 413.6(3)$ mV. Constants: $H_0 = 0.02815$ mol dm^{-3}, $t = 25.0$°C, $pK_w = 13.749$, $V_0 = 10.251$ cm^3, $s(V) = 0.001$ cm^3, $s(E) = 0.2$ mV, $j_a = 0.0$ mV, $j_b = 0.0$ mV, $I_0 = 0.2935$ (in vessel), $I_T = 1.1110$ (in burette). (Standard deviation of parameter estimate in last valid digits presented in parentheses.)

mates of common and group parameters were found. The Hamilton R-factor reaches a value of 0.06%, an excellent fitness is indicated and the regression parameter estimates are considered sufficiently reliable.

Fig. 1 shows the results of a graphical presentation of regression analysis results. In Fig. 1a, the potentiometric titration curve of a mixture of HCl and isocaine shows the data at 25°C. Fig. 1b shows the overall diagram of classical residuals, giving an initial impression of residuals. The true model and the reliable parameter estimates are proven because the residuals exhibit a normal distribution with zero mean and also form a random pattern. No systematic departures from randomness indicate that the model proposed is true and the estimates of the parameter are reliable. In Fig. 1c, the Bjerrum formation function provides an overview of the dissociation of the drug acid HL and, in Fig. 1d, the distribution diagram of the relative abundance of all variously protonated species seems to be more interesting than a numerical value of the protonation constant only. The intersection of both curves gives a value of the protonation constant on the pH axis.

Table 3 provides the concentration and mixed dissociation constant of isocaine estimated by the non-linear regression programs ESAB and PLUS99 when residuals $e_i = (E_{cell,exp,i} - E_{cell,calc,i})$ are minimized. Low values of the residuals in microlitres and the Hamilton R-factor prove an excellent fit of the calculated regression curve through experimental points.

Table 4 shows results for the protonation constant of physostigmine determined at various values of ionic strength as a result of regression analysis with two various mathematical approaches. The program ESAB, minimizing residuals $e_i = (V_{exp,i} - V_{calc,i})$ reaches 0.1 or 0.2 μl, and PLUS99, minimizing $e_i = (E_{cell,exp,i} - E_{cell,calc,i})$, reaches an R-factor of about 0.2–0.3%, proving an excellent fit.

Table 2
Reliability of parameters estimates proved by a statistical analysis of residuals

Bias, $E(\hat{e})$	-6.4500×10^{-22} cm^3
Lower and upper Hoaglin's limits	−0.00082 and 0.00072 cm^3, no outliers
Mean of absolute values of residuals, $E\lvert\hat{e}\rvert$	0.0001 cm^3
Variance, $s^2(\hat{e})$	3.00×10^{-8}
Standard deviation, $s(\hat{e})$	0.0002 cm^3
Skewness, $g_1(\hat{e})$	0.12 (not differing from 0)
Kurtosis, $g_2(\hat{e})$	2.03 (not differing from 3)
Residuals sum of squares, $U(\boldsymbol{b})$	6.0×10^{-8}
Jarque–Berra normality test of a residuals	Normality accepted
Regression rabat, 100D	99.99%
Akaike information criterion, AIC	−296.9
Hamilton R-factor of relative fitness	0.06%

Table 5 shows results for the protonation constant of pilocarpine. Reliability criterion of protonation constant used for ESAB is the mean of absolute values of residuals $E\lvert\hat{e}\rvert$, here reaching 0.1 or 0.2 μl. For PLUS99, the Hamilton R-factor proves a good fit and therefore provides reliable estimates of protonation constants.

4.2. Estimation of thermodynamic dissociation constant

Applying the extended Debye–Hückel equation (Eq. (7)) to data from Tables 3–5 according to a regression criterion (Eq. (8)), the three unknown parameters pK_a^T, $\mathring{a}$, and C have been estimated.

Table 6 shows the point estimates, calculated standard deviations of each parameter, and the absolute and relative biases obtained when the minimization process terminates. Two parameters, $pK_a^T = 8.961$ and $C = 0.530$, are estimated with very small bias, −0.022 and 1.272%, and with small standard deviation $s(pK_a^T) = 0.019$ and $s(C) = 0.051$, indicating a quite reliable estimation. The ion-size parameter $\mathring{a} = 7.45$ has a larger bias, about 17.58%, and a higher value of the standard deviation $s(\mathring{a}) = 4.14$. Linear parameters, pK_a^T and C, in the regression model are well conditioned and their estimation is sensitive. They have a strong influence on the residual-square sum function U. The non-linear parameter, $\mathring{a}$, being ill-conditioned in the regression model, has a small influence on the residual-square sum function U and makes its numerical determination rather uncertain. Well-conditioned parameters, pK_a^T and C, have great influence on an elliptic hyperparaboloid shape, when the variable U is plotted against the three parameters pK_a^T, $\mathring{a}$, C in

Table 3
Concentration pK_c and mixed dissociation pK_a constants of isocaine estimated by the non-linear regression programs ESAB and PLUS99[a]

I	ESAB			PLUS99		
	pK_c	pK_a	$\lvert\hat{e}\rvert$ (ml)	pK_c	pK_a	R (%)
0.027	8.984(2)	8.928	0.2	8.969(8)	8.913	0.456
0.04	8.975(2)	8.910	0.2	8.967(4)	8.902	0.230
0.09	8.985(3)	8.907	0.1	8.992(9)	8.914	0.391
0.16	9.022(3)	8.941	0.2	9.019(6)	8.938	0.223
0.25	9.057(4)	8.980	0.2	9.007(8)	8.930*	0.209
0.36	9.085(4)	9.019	0.2	9.105(9)	9.038	0.284
0.49	9.136(3)	9.085	0.2	9.140(9)	9.089	0.302
0.64	9.199(3)	9.167	0.1	9.219(6)	9.187	0.188
0.81	9.288(3)	9.278*	0.2	9.268(6)	9.258	0.262
1.00	9.300(3)	9.317	0.1	9.293(9)	9.310*	0.367

[a] Standard deviation of parameter estimates in last valid digits is presented in parentheses. * Influential points (outliers) being excluded from a regression analysis.

Table 4
Concentration pK_c and mixed dissociation pK_a constants of physostigmine estimated by the non-linear regression programs ESAB and PLUS99[a]

I	ESAB			PLUS99		
	pK_c	pK_a	$\|\hat{e}\|$ (ml)	pK_c	pK_a	R (%)
0.027	8.105(1)	8.049	0.2	8.125(8)	8.069	0.290
0.04	8.112(2)	8.047	0.2	8.110(9)	8.045	0.171
0.09	8.156(2)	8.078	0.2	8.160(3)	8.082	0.202
0.16	8.193(2)	8.112	0.2	7.946(9)	7.865*	0.290
0.25	8.225(2)	8.148	0.2	8.236(6)	8.159	0.158
0.36	8.287(2)	8.221	0.2	8.283(5)	8.217	0.142
0.49	8.334(2)	8.283	0.1	8.331(7)	8.280	0.331
0.64	8.390(3)	8.358	0.2	8.391(7)	8.359	0.136
0.81	8.396(4)	8.386*	0.2	8.408(8)	8.398*	0.219
1.00	8.385(2)	8.402*	0.1	8.361(9)	8.378*	0.183

[a] Standard deviation of parameter estimates in last valid digits is presented in parentheses. * Influential points (outliers) being excluded from following regression analysis.

Table 5
Concentration pK_c and mixed dissociation pK_a constants of pilocarpine estimated by the non-linear regression programs ESAB and PLUS99[a]

	ESAB			PLUS99		
	pK_c	pK_a	$\|\hat{e}\|$ (ml)	pK_c	pK_a	R (%)
0.027	7.017(1)	6.961	0.1	7.068(9)	7.012	0.614
0.04	7.018(1)	6.953	0.1	7.076(8)	7.011	0.382
0.09	7.034(1)	6.956	0.2	7.078(6)	7.000	0.342
0.16	7.066(1)	6.985	0.2	7.098(4)	7.017	0.215
0.25	7.102(1)	7.025	0.2	7.132(8)	7.055	0.645
0.36	7.133(2)	7.067	0.2	7.161(4)	7.095	0.258
0.49	7.180(2)	7.129	0.2	7.185(5)	7.134	0.364
0.64	7.235(1)	7.203	0.1	7.232(7)	7.200	0.557
0.81	7.237(1)	7.227*	0.1	7.230(3)	7.221*	0.161
1.00	7.297(1)	7.311*	0.2	7.295(6)	7.312*	0.273

[a] Standard deviation of parameter estimates in last valid digits is presented in parentheses. * Influential points (outliers) being excluded from a regression analysis.

Table 6
ADSTAT refinement of the thermodynamic dissociation constant pK_a^T and parameters of extended Debye–Hückel equation $å$ and C for isocaine at 25°C[a]: point estimates of parameters

Parameter	Point estimate	Standard deviation	Absolute bias	Relative bias (%)
pK_a^T	8.961	0.019	−0.002	−0.022
$å$	7.45	4.14	1.31	17.58
C	0.530	0.051	0.007	1.272

[a] Data are in Table 4 (ESAB).

Table 7
ADSTAT refinement of the thermodynamic dissociation constant pK_a^T and parameters of extended Debye–Hückel equation $å$ and C for isocaine at 25°C[a]: statistical analysis of residuals

Point	$pK_{a,\,exp}$	$pK_{a,\,calc}$	$s(pK_{a,\,calc})$	Classical residual, e	Jackknife residual, e_J	Likelihood distance, LD_i
1	8.9280	8.9150	0.0120	−0.0003	1.0518	0.0218
2	8.9100	8.9131	0.0103	0.0000	−0.2086	0.0089
3	8.9070	8.9198	0.0075	0.0003	−0.8004	0.0160
4	8.9410	8.9420	0.0076	0.0003	−0.0620	0.0089
5	8.9790	8.9781	0.0084	0.0001	0.0545	0.0089
6	9.0200	9.0271	0.0086	−0.0001	−0.4418	0.0091
7	9.0850	9.0883	0.0079	−0.0002	−0.1993	0.0089
8	9.1680	9.1614	0.0075	−0.0002	0.3969	0.0096
9	9.2780	9.2460	0.0092	−0.0001	3.5332*	1.2536
10	9.3170	9.3420	0.0138	0.0003	−5.0442*	0.2047

[a] Data are in Table 4 (ESAB). * Outliers.

$(m+1)$-dimensional space (here, $m=3$). For well-conditioned parameters, such a shape exhibits an obvious, sharp minimum, the pit point U_{min}. The ill-conditioned parameter, $å$, leads to a 'flat-bottomed-saucer shape' of hyperparaboloid U with no obvious minimum U_{min}. A larger value of the standard deviation $s(å)$ indicates that parameter $å$ is ill-conditioned in the model and therefore its determination is rather uncertain.

Table 7 shows the fitness of the calculated regression curve and an indication of influential points, outliers. Points 9 and 10 are suspicious outliers because their Jackknife criterion, $\hat{e}_{J,9} > 3$ and $\hat{e}_{J,10} > 3$, prove it. The reliability of pK_a^T, $å$, and C estimates is proven by a goodness-of-fit test made here by a statistical analysis of classical residuals (Table 8). As $s(pK_{a,i})$ oscillates from 0.001 to 0.003 (estimated by ESAB) and $E|\hat{e}| = 0.005$ and $s(\hat{e}) = 0.017$ are of similar magnitude. The residuals exhibit symmetric, normal distribution as the skewness $g_1(\hat{e}) = 0.55$ does not significantly differ from zero indicating a symmetric distribution. The kurtosis, $g_2(\hat{e}) = 3.40$, also does not significantly differ from 3, indicating a normal distribution. The regression rabat, $100D = 99.01\%$, indicates that all points fulfil the regression model proposed with parameter estimates. With the use of the Akaike information criterion, $AIC = -78.7$, several plausible regression models were examined but the model of Eq. (7) gave the lowest value of AIC. The Hamilton R-factor proves an excellent fit and therefore provides reliable estimates of parameters. Figs. 2–4 provide a graphical presentation of the dependence of the mixed dissociation constant on the square root of the ionic strength.

5. Conclusions

The reliability of the dissociation constants of three drug acids, (isocaine, physostigmine and pilocarpine) was proven even when three group

Table 8
ADSTAT refinement of the thermodynamic dissociation constant pK_a^T and parameters of extended Debye–Hückel equation $å$ and C for isocaine at 25°C[a]: reliability of parameters estimates proved by a statistical analysis of residuals

Bias, $E(\hat{e})$	0.000100
Mean of absolute values of residuals, $E\|\hat{e}\|$	0.0050
Variance, $s^2(\hat{e})$	0.0000496
Standard deviation, $s(\hat{e})$	0.0170
Skewness, $g_1(\hat{e})$	0.55 (not differing from 0)
Kurtosis, $g_2(\hat{e})$	3.40 (not differing from 3)
Residuals sum of squares, $U(\boldsymbol{b})$	0.0020998
Jarque–Berra normality test of a residuals	Normality accepted
Regression rabat, $100D$	99.01%
Akaike information criterion, AIC	−78.7
Hamilton R-factor of relative fitness	0.16%

[a] Data are in Table 4 (ESAB).

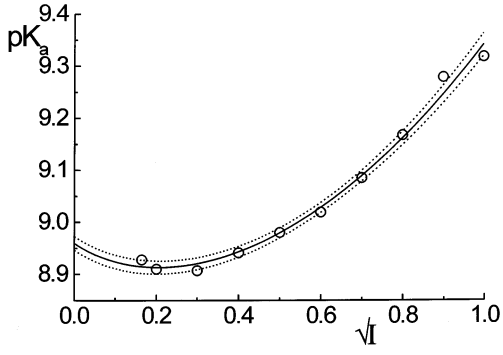


Fig. 2. Dependence of the mixed dissociation constant pK_a of isocaine on the square root of the ionic strength (solid curve) with the 95% confidence bands of prediction for the model proposed (dotted curves), which leads to parameter estimates $pK_a^T = 8.96(1)$, $\mathring{a} = 8(3)$ Å and $C = 0.50(3)$ at 25°C (ESAB).

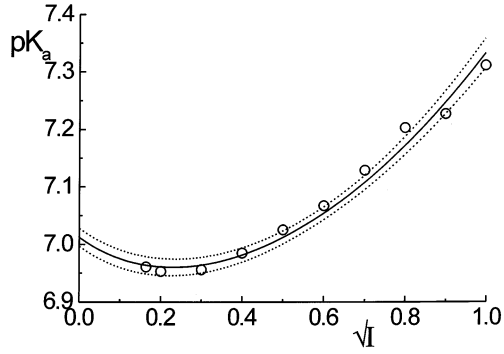


Fig. 3. Dependence of the mixed dissociation constant pK_a of physostigmine on the square root of the ionic strength (solid curve) with the 95% confidence bands of prediction for the model proposed (dotted curves), which leads to parameter estimates $pK_a^T = 8.07(3)$, $\mathring{a} = 19(26)$ Å and $C = 0.64(3)$ at 25°C (ESAB).

parameters, E'^0, L_0, H_T, were ill-conditioned in a model. Their determination is uncertain and might lead to false estimate 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 and they should be refined together with common parameters pK_a. Internal calibration of $[H^+]$ of the glass electrode cell performed during titration is more accurate than an external calibration of a_{H+}. Comparing two computational approaches, the ESAB and the PLUS99 programs, ESAB led to better a fitness of potentiometric

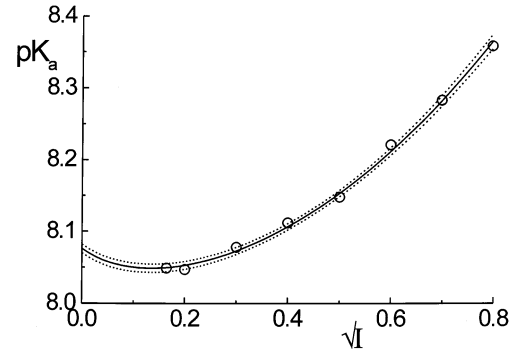


Fig. 4. Dependence of the mixed dissociation constant pK_a of pilocarpine on the square root of the ionic strength (solid curve) with the 95% confidence bands of prediction for the model proposed (dotted curves), which leads to parameter estimates $pK_a^T = 7.00(1)$, $\mathring{a} = 7(1)$ Å and $C = 0.53(2)$ at 25°C (ESAB).

Table 9
Survey of estimated thermodynamic dissociation constants[a]

Drug	pK_a^T	$\mathring{a}$	C
Isocaine	8.96(1), 8.95(2)	8(3), 8(3)	0.50(3), 0.55(4)
Physostigmine	8.07(2), 8.08(2)	19(26), 21(17)	0.64(3), 0.53(5)
Pilocarpine	7.00(1), 7.06(1)	7(1), 6(2)	0.53(2), 0.46(4)

[a] Data presented as ESAB, PLUS99.

titration curve. The thermodynamic dissociation constant pK_a^T, an ill-conditioned ion-size parameter, $\mathring{a}$, and the salting-out coefficient, C, were estimated by a non-linear regression of the dependence of the mixed dissociation constant, pK_a, on the ionic strength, I. The goodness-of-fit proved sufficiently reliable for parameter estimates for three drugs at 25°C. The standard deviation of each parameter in are in presented in parentheses (Table 9).

Acknowledgements

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References

[1] M.J. Neal, Medical Pharmacology at a Glance, Second ed., Blackwell Scientific Publications, Oxford.

[2] R.C. Ingraham, M.B. Visscher, J. Gen. Physiol. 18 (1935) 695.

[3] The Merck Index, An Encyclopedia of Chemicals. Drugs and Biologicals, Whitehouse, 1996.

[4] F.R. Hartley, C. Burgess, R.M. Alcock, Solution Equilibria, Ellis Horwood, Chichester, 1980.

[5] D.J. Leggett, Computational Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, pp. 99–157, 291–353.

[6] M. Meloun, J. Havel, E. Högfeldt, Computation of Solution Equilibria, Ellis Horwood, Chichester, 1988.

[7] M. Meloun, M. Bartoš, E. Högfeldt, Multiparametric curve fitting — XIII. Reliability of formation constants determined by analysis of potentiometric titration data, Talanta 35 (1988) 981.

[8] C. Rigano, M. Grasso, S. Sammartano, Computer analysis of equilibrium data in solution. A compact least-squares computer program for acid–base titrations ESAB, Ann. Chim. Rome 74 (1984) 537.

[9] C. De Stefano, P. Princi, C. Rigano, S. Sammartano, Computer analysis of equilibrium data in solution. ESAB2M: an improved version of the ESAB program, Ann. Chim. Rome 77 (1987) 643.

[10] D.D. Perrin, Dissociation Constants of Organic Bases in Aqueous Solution, Butterworths, London, 1995.

[11] M. Kývala, Regression program PLUS99 (latest version 1999), Proceedings of the 4th International Chemometrics Conference of the Czech Chemical Society, 3–7 July 1995, Pardubice, Czech Republic.

[12] M. Meloun, J. Militký, M. Forina, Chemometrics for Analytical Chemistry: PC-Aided Regression and Related Methods, vol. 2:, Ellis Horwood, Chichester, 1994.

[13] M. Meloun, J. Militký, M. Forina, Chemometrics for Analytical Chemistry: PC-Aided Statistical Data Analysis, vol. 1, Ellis Horwood, Chichester, 1992.

[14] P.M. May, D.R. Williams, P.W. Linder, R.G. Torrington, The use of glass electrodes for the determination of formation constants — I. A definitive method for calibration, Talanta 29 (1982) 249.

[15] M. Meloun, V. Říha, J. Žáček, Piston micro-burette for the dosage of aggressive liquids, Chem. Listy 82 (1988) 765 (in Czech).

[16] S. Capone, A. De Robertis, C. De Stefano, S. Sammartano, R. Scarcella, Ionic strength dependence of formation constants — X. Proton activity coefficients at various temperatures and ionic strengths and their use in the study of complex equilibria, Talanta 34 (1987) 593.

[17] ADSTAT 1.25, 2.0 (DOS), 3.0 (Windows 95), TriloByte Statistical Software Ltd., Pardubice, 1990 and 1999.