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The thermodynamic dissociation constants of haemanthamine, lisuride, metergoline and nicergoline by the regression analysis of spectrophotometric data

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Abstract

The mixed dissociation constants of four drugs – haemanthamine, lisuride, metergoline and nicergoline – at various ionic strengths I of range 0.01 and 0.6 and at temperatures of 25 °C and 37 °C were determined using SPECFIT32 and SQUAD(84) regression analyses of the pH-spectrophotometric titration data. A proposed strategy for efficient experimentation in a dissociation constants determination, followed by a computational strategy for the chemical model with a dissociation constants determination, is presented on the protonation equilibria of haemanthamine. Indices of precise methods predict the correct number of components, and even the presence of minor ones when the data quality is high and the instrumental error is known. The thermodynamic dissociation constant pK_a^T was estimated by non-linear regression of $\{pK_a, I\}$ data at 25 °C and 37 °C: for haemanthamine $pK_a^T = 7.22\,(1)$ and 7.05 (2), for lisuride $pK_a^T = 7.87\,(1)$ and 7.59 (1), for metergoline $pK_a^T = 7.62\,(1)$ and 7.38 (1), for nicergoline $pK_{a,1}^T = 7.94\,(1)$ and 7.69 (1). Goodness-of-fit tests for various regression diagnostics enabled the reliability of the parameter estimates to be found.
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Keywords: Spectrophotometric titration; Dissociation constant; Protonation; Haemanthamine; Lisuride; Metergoline; Nicergoline

1. Introduction

Dissociation constants/protonation constants are very important both in the analysis of drugs and in the interpretation of their mechanisms of action: whereas fat-soluble (lipophilic) drugs diffuse through the membrane according to their liposolubility, drugs of electrolyte character (acids or bases) diffuse according to the liposolubility of the *non-dissociated* parts of their molecules only. The ionised parts of molecules are lipophobic and diffuse through membranes only with difficulty. More precisely, the partition of a hydrophobic molecule across a membrane depends on its *membrane* partition coefficient, i.e. the proportion that will be present in the membrane phase compared to the aqueous phase. Most often, this coefficient is derived from measurements made between two isotropic phases, such as the octanol/water system; however, mechanisms underlying partition within a lipid bilayer are complex [1–3], especially for molecules of an amphiphilic character, and they are not adequately described by parameters such as the $\log P_{\text{octanol/water}}$. More specifically, basic lipophilic compounds often exhibit higher affinities for biological membranes than predicted by their $\log P_{\text{octanol/water}}$ value [4–6]. In particular, the protonated form of lipophilic bases has a high affinity as a result of electrostatic interactions with zwitterionic or anionic lipids [7,25]. In this study, the authors attempt to complete the information on the protonation equilibria and dissociation constants of four pharmaceutically active ingredients: lisuride, nicergoline and metergoline, which belong to family of ergoline alkaloids, and haemanthamine, which belongs among

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the Amaryllidaceae alkaloids. These compounds fall into the category of the basic lipophilic drugs mentioned above and have not previously been studied for protonation/dissociation equilibria.

Of the several physicochemical methods for studying the protonation equilibria in solution, UV–vis spectrophotometry under broad experimental conditions is, in general, highly sensitive and with subsequent computer treatment of the data can be a very powerful method [8–24]. It allows for a protonation equilibria study even in cases where other methods fail. Much more information can be extracted if multivariate spectroscopic data are analyzed by means of an appropriate multivariate data analysis [19,21]. On the other hand, protonation equilibria sometimes involve very subtle changes in the electronic vibration spectra which make it difficult to employ spectrophotometry for determination of dissociation constant.

1.1. Drug characterization

Nicergoline, metergoline and lisuride are semisynthetic derivatives of lysergic acid [29–31].

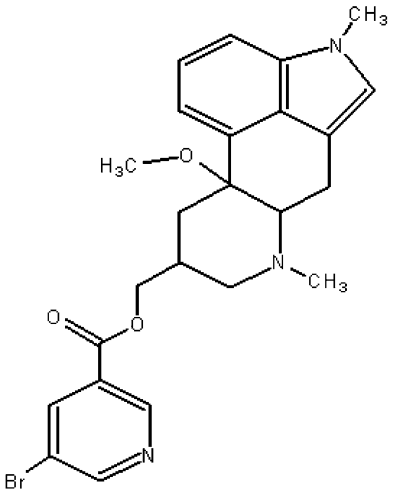
Nicergoline is a non-selective alpha-sympatolytic, antagonist on alpha-adrenergic receptors. It is used for better blood circulation in the central nervous system and legs. Due to its improving effect on the metabolism in the central nervous system, it has been used as a drug for stimulation of mental functions (nootropic) in geriatry to treat symptoms of mental deterioration associated with cerebrovascular insufficiency. It has also been used in peripheral vascular disease, and in acute myocardial infarction with diastolic hypertension. The common dosage forms of nicergoline are thus mostly tablets, intramuscular injection and slow intravenous infusion.

Lisuride and metergoline act as agonists of dopamine (D2) and serotonine (5-HT_{1A}) receptors, and for this reason are used in cases of dopamine insufficiency, such as the Parkinson's disease, or where enhanced dopamine action is needed, as is the case of hyperprolactinemia (prolactine inhibitors). Both of these drugs have also been used for migraine therapy. On other subtypes of serotonine receptors, lisuride and metergoline appear to be of neither high specificity nor selectivity and they act rather as antagonists. All three of these drugs can be prepared semisynthetically from enamel alkaloids.

Haemanthamine belongs to the class of 5,10B-ethanophenanthridines. It possesses relatively high antiretroviral properties [26], antiproliferative [27] effects and also has potent antimalarial properties (against *Plasmodium falciparum*) [28]. It is found in the bulbs of the plants Amaryllidaceae (*Clivia* species) and Liliaceae (*Hippeastrum*, *Lycoris*, *Narcissus*). To the best of the authors' knowledge, this compound has not yet been formulated into a final drug dosage form.

1.1.1. Nicergolin

The systematic chemical name of nicergoline is ergoline-8-β-methanol, 10-methoxy-l,6-dimethyl-,5-bromonicotinate (ester) and it is of the structure

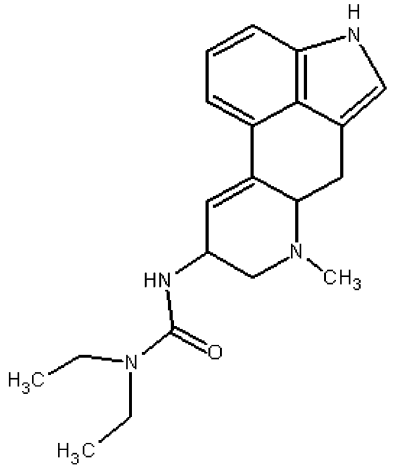


Nicergoline

Recommended INN name: nicergoline, CAS Number: 27848-84-6, EINECS Number: 248-694-6, ACX Number: XI026709-9, ATC-class: C04AE02, description: fine to granular white or yellowish powder, molecular formula: $C_{24}H_{26}BrN_3O_3$, molecular weight: 484.39, melting point: 135–138 °C, solubility: soluble in alcohol, freely soluble in dichloromethane, practically insoluble in water; pK_a is not known due to insolubility in water.

1.1.2. Lisuride

The systematic chemical name of lisuride is 3-(9,10-didehydro-6-methyl-8α-ergolinyl)-1,1-diethylurea and it is of the structure



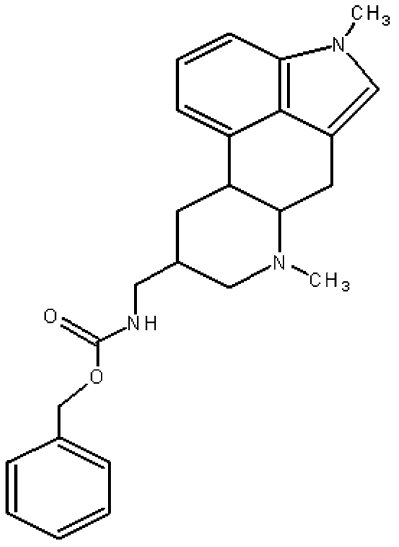
Lisuride

Recommended INN name: lisuride, CAS Number: 18016-80-3, EINECS Number: 241-925-1, ACX Number: X1063856-3, description: almost white to light yellow or brownish crystalline powder, molecular formula: $C_{20}H_{26}N_4O$, molecular weight: 338.5, melting point: 169–172 °C, solubility: slightly soluble in methanol, ethanol, dimethylformamide, dimethylsulfoxide, chloroform and dichloromethane, sparingly soluble in ether and practically

insoluble in water and hexane, pK_a is not known due to insolubility in water.

1.1.3. Metergoline

The systematic chemical name of metergoline is (+)-*N*-(carboxy)-1-methyl-9,10-dihydrolysergamin and it is of the structure

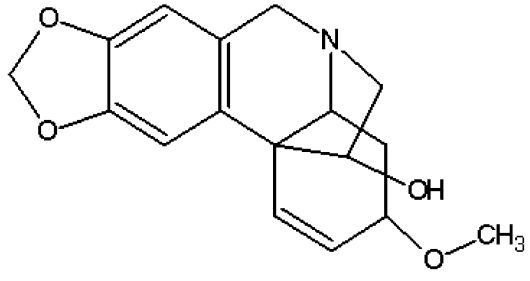


Metergoline

CAS Number: 17692-51-2, EINECS Number: 241-686-3, ACX Number: X1064044-7, appearance: white crystalline powder or granules, molecular formula: $C_{25}H_{29}N_3O_2$, molecular weight: 403.5; pK_a not known.

1.1.4. Haemanthamine

The systematic chemical name of haemanthamine is 3-methoxy-1,2-didehydrocrinan-11-ol. There are, however, several synonyms for the trivial name of the substance, including 3-epicrinamine, hemanthamine, natalensine and NSC-4, all of which belong to the same compound. Haemanthamine is of the chemical structure



Haemanthamine

CAS Number: 466-75-1, summary molecular formula: $C_{17}H_{19}NO_4$, molecular weight: 301.4, octanol/water partition coefficient as $\log P_{\text{octanol/water}}$: 1.47–1.56 (calculated); pK_a not known.

In this study, the authors attempt to complete the information on the protonation equilibria and dissociation constants of four pharmaceutically active ingredients of different origin (haemanthamine, lisuride, metergoline and nicergoline) which are formulated into a variety of different dosage forms, and whose exact pK_a knowledge is of special importance.

2. Theoretical

Computations related to the determination of protonation constants [8–11] may be performed by the regression analysis of spectra using versions of the SQUAD program family [9,12,17] and SPECFIT32 [32]. When considering a protonation of anion L^{z-1}

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

characterized by the protonation constant

$$K_H = \frac{a_{HL^z}}{a_{L^{z-1}} a_{H^+}} = \frac{[HL^z]}{[L^{z-1}][H^+]} \frac{y_{HL^z}}{y_{L^{z-1}} y_{H^+}} \tag{1b}$$

and in the case of a polyprotic species, protonated to yield a polyprotic acid H_jL:

$$L^{z-} + H^+ \rightleftarrows HL^{1-z}; \quad K_{H_1} \tag{2a}$$

$$HL^{1-z} + H^+ \rightleftarrows H_2L^{2-z}; \quad K_{H_2} \tag{2b}$$

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

$$L^{z-1} + jH^+ \rightleftarrows H_jL^z \tag{3a}$$

and by the overall protonation constant $\beta_{H_j} = K_{H_1} K_{H_2} \ldots K_{H_j}$, where j denotes the number of protons involved in the overall protonation. For dissociation reactions realized at constant ionic strength, the so called "mixed dissociation constants" are defined as

$$K_{a,j} = \frac{[H_{j-1}L] a_{H+}}{[H_jL]} \tag{3b}$$

These constants are found in experiments where pH values are measured with glass and reference electrodes, standardized with the practical $pH\,(S) = pa_{H^+}$ activity scale.

If the protonation equilibria between the anion, L (the charges are now omitted for the sake of simplicity), of a drug and a proton, H, are considered to form a set of variously protonated species L, LH, LH_2, LH_3, . . ., etc., which have the general formula L_qH_r in a particular chemical model and are represented by p the number of species, $(q, r)_i$, $i = 1,\ldots, p$, where index i labels their particular stoicheiometry, then the overall protonation constant of the protonated species, β_{qr}, may be expressed as

$$\beta_{qr} = \frac{[L_qH_r]}{([L]^q[H]^r)} = \frac{c}{l^q h^r} \tag{4}$$

where the free concentration $[L] = l$, $[H] = h$ and $[L_qH_r] = c$. For the ith solution measured at the jth wavelength, the ab-

sorbance, $A_{i,j}$, is defined as

$$A_{ij} = \sum_{n=1}^{p} \varepsilon_{j,n} c_n = \sum_{n=1}^{p} (\varepsilon_{qr,j} \beta_{qr} l^q h^r)_n \quad (5)$$

where $\varepsilon_{qr,j}$ is the molar absorptivity of the L_qH_r species with the stoichiometric coefficients q, r measured at the jth wavelength. The absorbance $A_{i,j}$ is the element of the absorbance matrix $\boldsymbol{A}$ of size $(n \times m)$ being measured for n solutions with known total concentrations of two basic components, c_L and c_H, at m wavelengths. Throughout this paper, it is assumed that the $n \times m$ absorbance data matrix $\boldsymbol{A} = \varepsilon\mathbf{C}$ containing the n recorded spectra as rows can be written as the product of the $m \times p$ matrix of molar absorptivities ε and the $p \times n$ concentration matrix $\boldsymbol{C}$. Here, p is the number of components that are absorb in the chosen spectral range. The rank of the matrix $\boldsymbol{A}$ is obtained from the equation $\text{rank}(\boldsymbol{A}) = \min[\text{rank}(\varepsilon), \text{rank}(\boldsymbol{C})] \leq \min(m, p, n)$. Since the rank of $\boldsymbol{A}$ is equal to the rank of ε or $\boldsymbol{C}$, whichever is the smaller, and since $\text{rank}(\varepsilon) \leq p$ and $\text{rank}(\boldsymbol{C}) \leq p$, then provided that m and n are equal to or greater than p, it is only necessary to determine the rank of matrix $\boldsymbol{A}$, which is equivalent to the number of dominant light-absorbing components [9,18–19]. All spectra evaluation may be performed with the INDICES algorithm [19] in the S-Plus programming environment. Most index methods are functions of the number of principal components PC(k)'s into which the spectral data are usually plotted against an integer index k, PC(k) = $f(k)$, and when the PC(k) reaches the value of the instrumental error of the spectrophotometer used, $s_{inst}(A)$, the corresponding index k^* represents the number of light-absorbing components in a mixture, $p = k^*$. In a scree plot, the value of PC(k) decreases steeply with increasing PCs as long as the PCs are significant. When k is exhausted the indices fall off, some even displaying a minimum. At this point, $p = k^*$ for all indices. The index values at this point can be predicted from the properties of the noise, which may be used as a criterion to determine p [19].

The multi-component spectra analysing program SQUAD(84) [14] may adjust β_{qr} and ε_{qr} for absorption spectra by minimising the residual-square sum function, U,

$$U = \sum_{i=1}^{n}\sum_{j=1}^{m}(A_{\text{exp},i,j} - A_{\text{calc},i,j})^2 = \sum_{i=1}^{n}\sum_{j=1}^{m}(A_{\text{exp},i,j} - \sum_{k=1}^{p}\varepsilon_{j,k}c_k)^2 = \text{minimum} \quad (6)$$

where $A_{i,j}$ represents the element of the experimental absorbance response-surface of size $n \times m$ and the independent variables c_k are the total concentrations of the basic components c_L and c_H being adjusted in n solutions. The calculated standard deviation of absorbance $s(A)$ is used as the most important criterion for a fitness test. If, after termination of the minimization process, the condition $s(A) \approx s_{inst}(A)$ is met and the R-factor is also less than 1%, the hypothesis of the chemical model is taken as being the most probable, and is accepted. SPECFIT32 is the latest version of a global analysis program for equilibrium and kinetic systems with singular value decomposition and non-linear regression modeling using the Levenberg–Marquardt method [32].

3. Experimental

3.1. Chemicals and solutions

3.1.1. Drugs

The nicergoline, lisuride, metergoline and haemanthamine were the kind gifts of IVAX Pharmaceuticals, s.r.o., Czech Republic. The nicergoline, Batch Number 10039314, was of purity 99.5% (w/w), determined by HPLC assay and recalculated on an anhydrous basis. The metergoline, Batch Number 50209MG505, was of assay (HPLC) 99.9% recalculated on an anhydrous basis. The lisuride, Batch Number SC041200/4, was of assay 99.9% (HPLC) calculated as the area ratio using the internal standard method. The haemanthamine 100.0% (HPLC) was calculated as the area ratio with the use of the internal standard method.

Perchloric acid, 1 M, was prepared from cone. $HC1O_4$ (p.a., Lachema Brno) using redistilled water and standardized against HgO and Nal with a reproducibility of less than 0.20%. Sodium hydroxide, 1 M, was prepared from pellets (p.a., Aldrich Chemical Company) with carbon dioxide-free redistilled water and standardized against a solution of potassium hydrogen-phthalate using the Gran method in the MAGEC program [9] with a reproducibility of 0.1%. Mercuric oxide, sodium iodide, and sodium perchlorate (p.a. Lachema Brno) were not further purified. The preparation of other solutions from analytical reagent-grade chemicals has been described previously [10,11]. Twice-redistilled water was used in the preparation of solutions.

3.2. Apparatus and pH-spectrophotometric titration procedure

The apparatus used and the pH-spectrophotometric titration procedures have been described previously [20].

3.3. Procedure for the determination of the chemical model and protonation constants

The experimental and computational schemes for the determination of the protonation constants of the multicomponent system are taken from Meloun et al. [9] and are described in a previous paper [20]. When a minimization process terminates, some diagnostics are examined to determine whether the results should be accepted: the physical meaning of the parametric estimates, the physical meaning of the species concentrations, the goodness-of-fit test and the deconvolution of the spectra.

3.4. Determination of the thermodynamic protonation/dissociation constants

The non-linear estimation of the thermodynamic dissociation constant $pK_a^T = a_{H^+}a_{L^-}/a_{HL}$, is simply a problem of optimization in the parameter space in which pK_a and I are known and given values, while the parameters pK_a, a, and C are the unknown variables to be estimated [9,20].

3.5. Reliability of the estimated dissociation constants

The adequacy of a proposed regression chemical model with experimental data and the reliability of parameter estimates $pK_{a,i}$ found, being denoted for the sake of simplicity as b_j, and ε_{ij}, $j = 1, \ldots, m$, may be examined by the goodness-of-fit test, cf. p. 101 in ref. [9] or may be found in a previous paper [20] and also are explained in ref. [34].

3.6. Software used

Computations were performed by regression analysis of UV–vis spectra using SPECFIT32 [32]. The thermodynamic dissociation constant pK_a^T was estimated with the MINOPT non-linear regression program in the ADSTAT statistical system (TriloByte Statistical Software Ltd., Pardubice) [33,34].

4. Results and discussion

4.1. Estimation of the dissociation constants of four drugs

4.1.1. Haemanthamine

The deprotonation haemanthamine LH form exhibits two sharp isosbestic points in spectra, and these two points indicate one simple equilibrium. pH-spectrophotometric titration enables absorbance-response data (left 3D graph in the first row of Fig. 1) to be obtained for analysis by non-linear regression, and the reliability of parameter estimates (pK's and ε's) can be evaluated on the basis of the goodness-of-fit test of residuals (middle 3D graph in the first row of Fig. 1). The A–pH curves at 224 nm and 250 nm (right graph in the first row of Fig. 1) show that a dissociation constant can be indicated. However, as the changes in spectra are quite small within deprotonation, both of the variously protonated species L and LH exhibit quite similar absorption bands. The shift of a band maximum to lower wavelengthes in the spectra set may also be indicated. The adjustment of pH value from 6.3 to 8.4 causes the absorbance to change by 0.022 of the A–pH curve only, so that a monitoring both components L and LH of protonation equilibrium is rather unsure. As the changes in spectra are very small, a very precise measurement of absorbance is necessary for the reliable detection of the deprotonation equilibrium studied.

In the first step of the regression spectra analysis, the number of light-absorbing species was estimated by the INDICES algorithm (Fig. 1). The position of the break point on the $s_k(A) = f(k)$ curve in the factor analysis scree plot is calculated and gives $k^* = 2$ with corresponding co-ordinate $s_k^*(A) = 0.27$ mAU, which also represents the actual instrumental error $s_{inst}(A)$ of the spectrophotometer used. Due to the large variations in the indicator values, these latter are plotted on a logarithmic scale. All six selected methods of the modified factor analysis estimate the two light-absorbing components L and LH of protonation equilibrium. The number of light-absorbing species p can be predicted from the index function values by finding the point $p = k$ where the slope of index function $PC(k) = f(k)$ changes, or by comparing $PC(k)$ values to the instrumental error $s_{inst}(A)$. This is the common criterion for determining p. Very low values of $s_{inst}(A)$ prove that reliable spectrophotometer and experimental techniques were used. The dissociation constant and two molar absorptivities of haemanthamine calculated for 48 wavelengths of 21 spectra constitute 1008 unknown parameters which are refined by SQUAD(84) or SPECFIT32 in the first run. The reliability of the parameter estimates may be tested with the use of following diagnostics.

The first diagnostic value indicates whether all of the parametric estimates β_{qr} and ε_{qr} have physical meaning and reach realistic values. As the standard deviations $s(\log \beta_{qr})$ of parameters $\log \beta_{qr}$ and $s(\varepsilon_{qr})$ of parameters ε_{qr} are significantly smaller than their corresponding parameter estimates (Table 1), all the variously protonated species are statistically significant at a significance level $\alpha = 0.05$. The physical meaning of the dissociation constant, molar absorptivities and stoichiometric indices is examined. The absolute values of $s(\beta_j)$, $s(\varepsilon_j)$ gives information about the last U-contour of the hyperparaboloid in the neighbourhood of the pit, U_{min}. For well-conditioned parameters, the last U-contour is a regular ellipsoid, and the standard deviations are reasonably low. High s values are found with ill-conditioned parameters and a "saucer"-shaped pit. The relation $s(\beta_j) \times F_\sigma < \beta_j$ should be met, where F_σ is equal to 3. The set of standard deviations of ε_{pqr} for various wavelengths, $s(\varepsilon_{qr}) = f(\lambda)$, should have a Gaussian distribution; otherwise, erroneous estimates of ε_{qr} are obtained. Fig. 1 shows the estimated molar absorptivities of all of the variously protonated species ε_L, ε_{LH}, of haemanthamine in dependence on wavelength.

The second diagnostic tests whether all of the calculated free concentrations of variously protonated species on the distribution diagram of the relative concentration expressed as a percentage have physical meaning, which proved to be the case (Fig. 1). The calculated free concentration of the basic components and variously protonated species of the chemical model should show molarities down to about 10^{-8} M. Expressed in percentage terms, a species present at about 1% relative concentration or less in an equilibrium behaves as numerical noise in a regression analysis. A distribution diagram makes it easier to judge the contributions of individual species to the total concentration quickly. Since the molar absorptivities will generally be in the range 10^3–10^5 l mol^{-1} cm^{-1}, species present at less than ca. 0.1% relative concentration

will affect the absorbance significantly only if their ε is extremely high. The diagram shows the protonation equilibria of LH and L.

The next diagnostic concerns the goodness-of-fit (Fig. 1). The goodness-of-fit achieved is easily seen by examination of the differences between the experimental and calculated values of absorbance, $e_i = A_{\mathrm{exp},i,j} - A_{\mathrm{calc},i,j}$. Examination of the spectra and of the graph of the predicted absorbance response-surface through all the experimental points should reveal whether the results calculated are consistent and whether any gross experimental errors have been made in the measurement of the spectra. One of the most important statistics

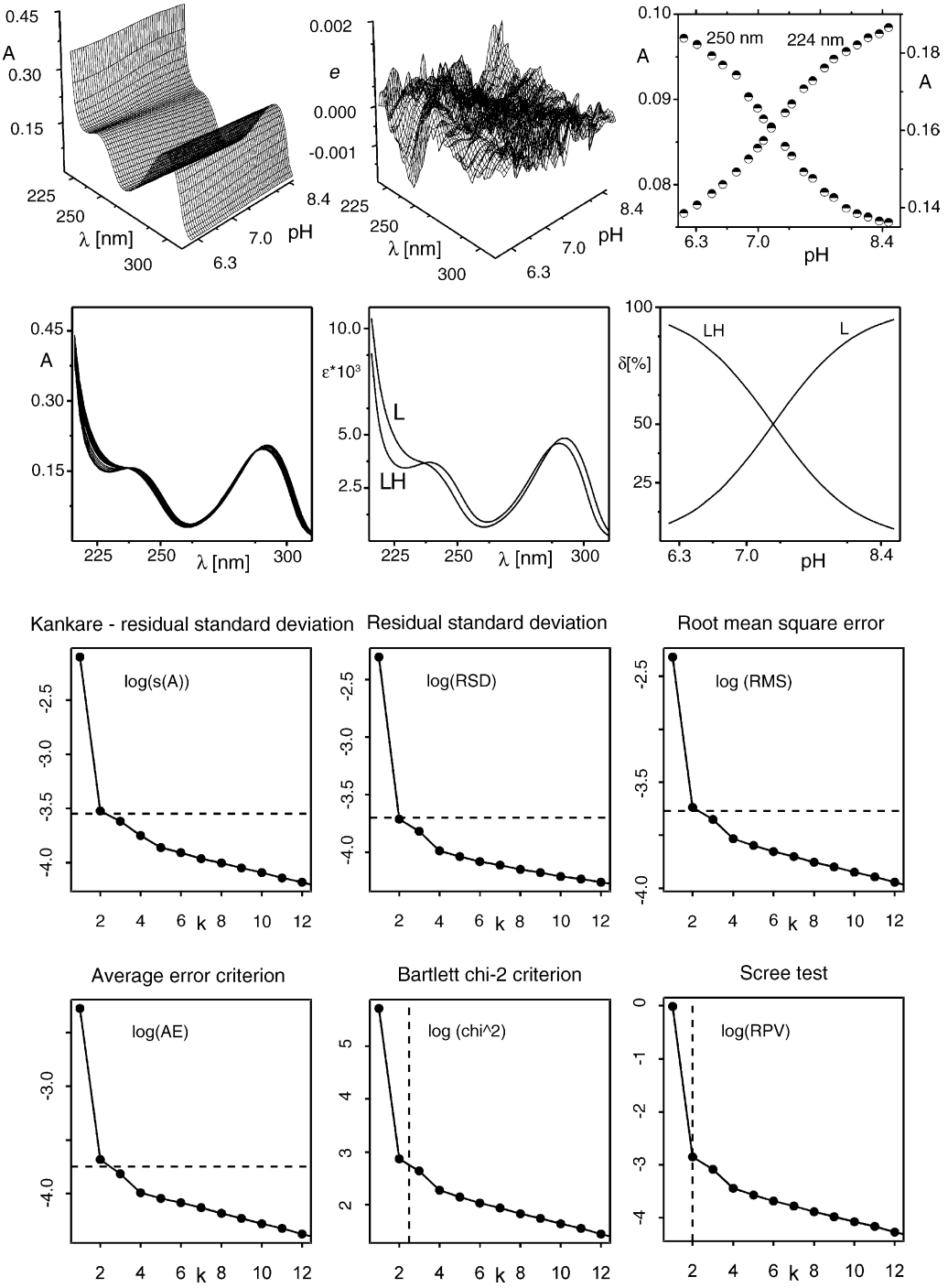


Fig. 1. Absorption spectra of the protonation equilibria of haemanthamine in dependence on pH at 25 °C. First row: The 3D-absorbance-response-surface represents input for the SQUAD(84) and SPECFIT32 programs; the 3D-overall diagram of residuals indicates the quality of a goodness-of-fit; the *A*–pH curves at two selected wavelengths. Second row: absorption spectra measured; spectra of molar absorptivities vs. wavelengths for all of the variously protonated species; distribution diagram of the relative concentrations of all of the variously protonated species. Third row: Kankare's residual standard deviation $s_k(A)$; residual standard deviation R.S.D., root mean square error RMS. Fourth row: average error criterion AE; Bartlett χ^2 criterion; scree test RPV.

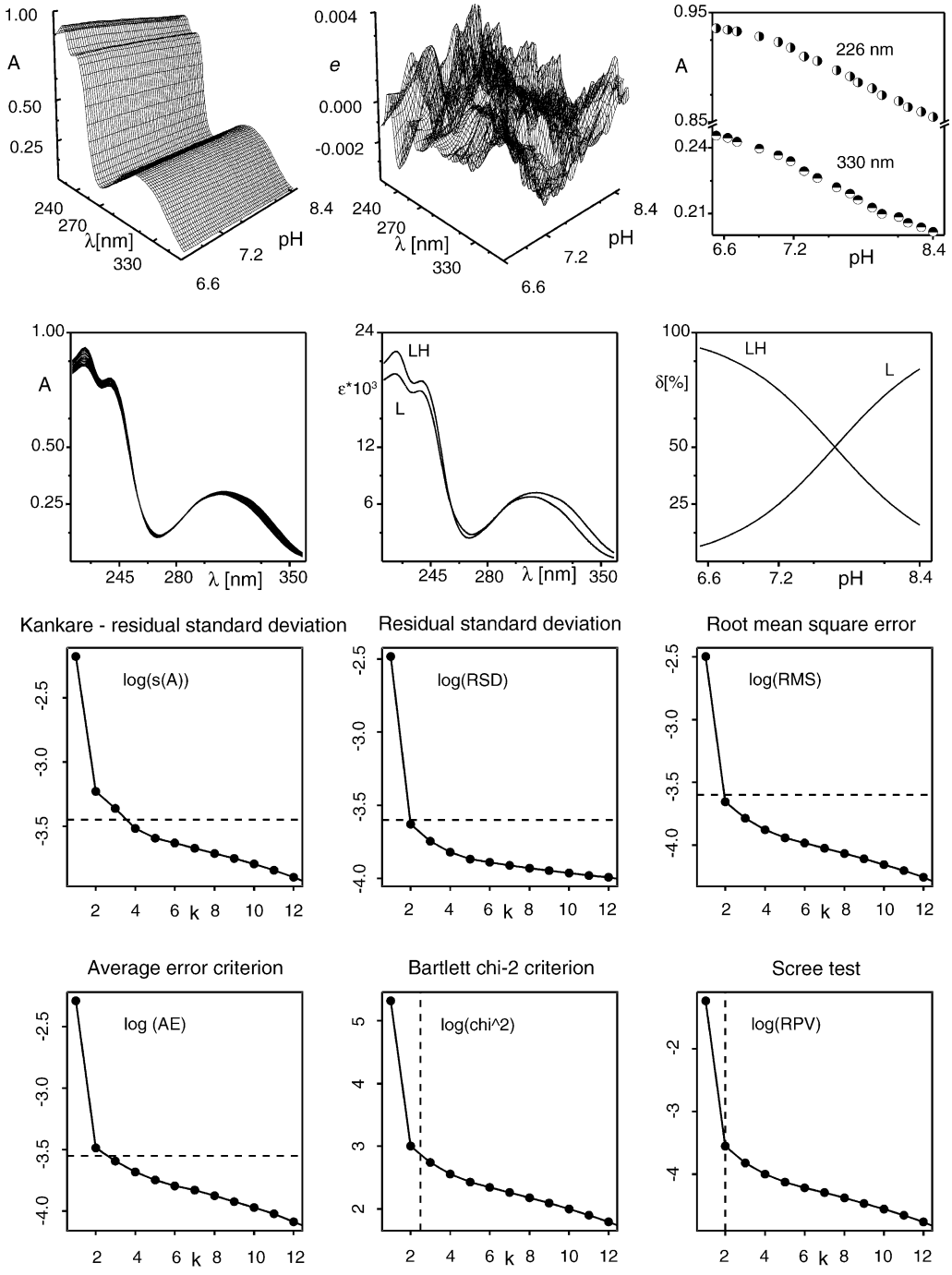


Fig. 2. Absorption spectra of the protonation equilibria of lisuride in dependence on pH at 25 °C as described in Fig. 1.

calculated is the standard deviation of absorbance, $s(A)$, calculated from a set of refined parameters at the termination of the minimization process. It is usually compared with the standard deviation of absorbance calculated by the INDICES program [19], $s_k(A)$, and if $s(A) \leq s_k(A)$ or $s(A) \leq s_{inst}(A)$, the instrumental error of the spectrophotometer used, the fit is considered to be statistically acceptable (Table 1). This proves that the $s_2(A)$ value is equal to 0.27 mAU and is quite close to the standard deviation of absorbance when the minimization process terminates, $s(A) = 0.35$ mAU. Although this statistical analysis of residuals [20,34] gives the most rigorous test of the degree-of-fit, realistic empirical limits must be used. The statistical measures of all residuals e prove that the minimum of the eliptic hyperparaboloid U is reached (Table 1): the residual standard deviation $s(e)$ always has sufficiently low values, lower than 1 mAU. The criteria of resolution used for

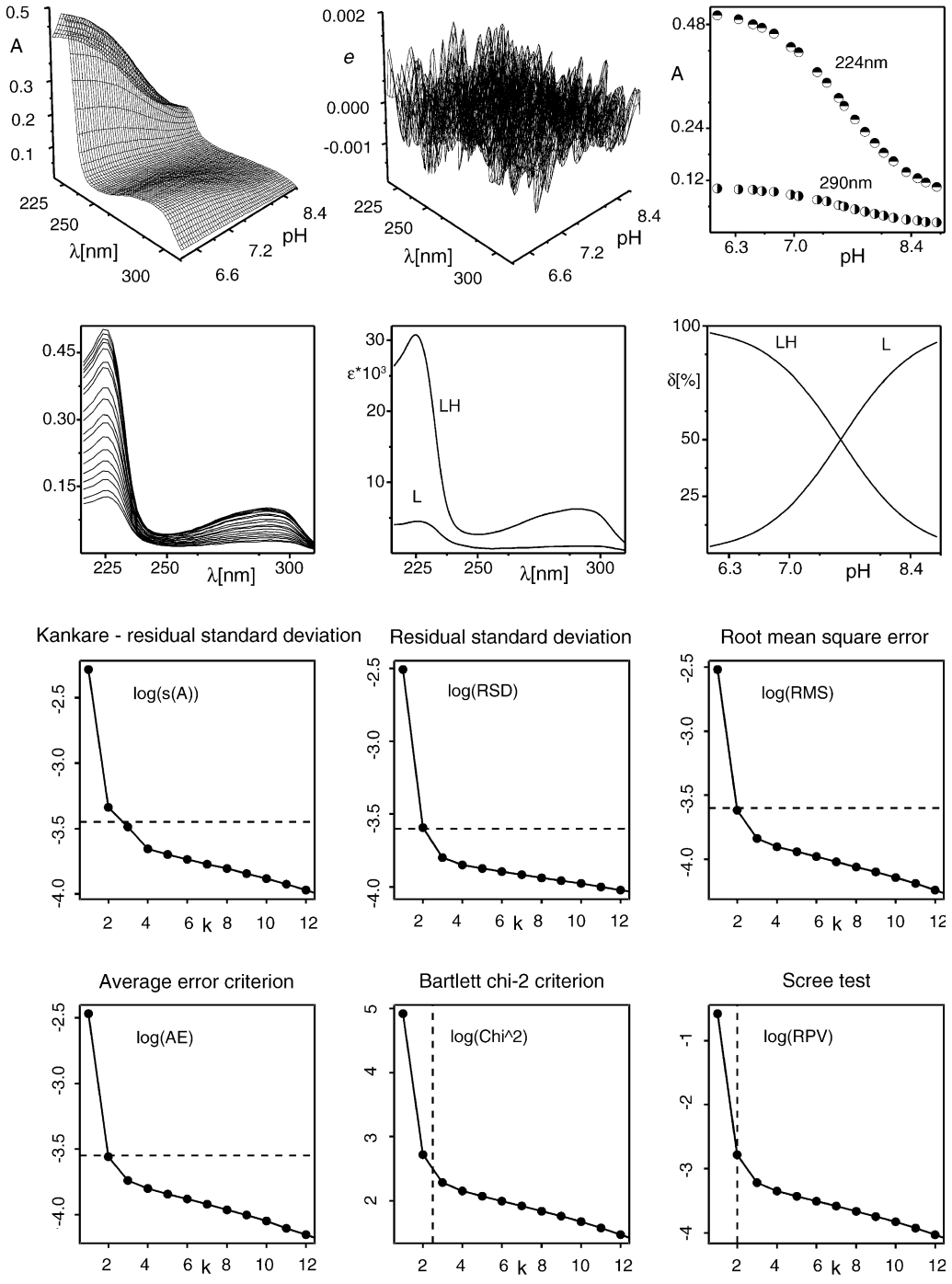


Fig. 3. Absorption spectra of the protonation equilibria of metergoline in dependence on pH at 25 °C as described in Fig. 1.

the hypotheses were: (1) a failure of the minimisation process in a divergency or a cyclisation; (2) an examination of the physical meaning of the estimated parameters if they were both realistic and positive; (3) the residuals should be randomly distributed about the predicted regression spectrum, and systematic departures from randomness were taken to indicate that either the chemical model or the parameter estimates were unsatisfactory.

Using the experimental and evaluation strategy, the protonation equilibria of lisuride (Table 2 and Fig. 2), metergoline (Table 3 and Fig. 3) and nicergoline (Table 4 and Fig. 4) were also examined. To test the reliability of the protonation/dissociation constants at different ionic strengths the goodness-of-fit test with the use of statistical analysis of the residuals was applied, and the results are given in Tables 2–4. For all four drugs studied the most efficient tool, such as the

Table 1
The dependence of the mixed dissociation constants of haemanthamine on ionic strength using regression analysis of pH-spectrophotometric data with SPECFIT32, with the standard deviations of the parameter estimates in the last valid digits in brackets

The chemical model attained contains L, LH (25 °C)												
Ionic strength	0.014	0.027	0.041	0.068	0.137	0.164	0.233	0.26	0.356	0.451	0.547	
p$K_{a,1}$	7.194 (8)	7.182 (5)	7.162 (5)	7.162 (6)	7.176 (6)	7.193 (4)	7.214 (4)	7.212 (10)	7.234 (6)	7.261 (5)	7.278 (7)	
Goodness-of-fit test												
RSS × 10^3	0.46	0.18	0.21	0.21	0.24	0.13	0.12	0.5	0.23	0.16	0.3	
$s(A)$ (mAU)	0.71	0.43	0.47	0.5	0.52	0.35	0.36	0.76	0.52	0.44	0.58	
The chemical model attained contains L, LH 37 °C												
Ionic strength	0.009	0.024	0.127	0.188	0.317	0.015	0.052	0.146	0.219	0.328	0.421	0.537
p$K_{a,1}$	7.052 (7)	7.032 (4)	7.062 (4)	7.080 (5)	7.123 (5)	7.035 (4)	7.052 (5)	7.076 (3)	7.080 (9)	7.131 (6)	7.150 (5)	7.165 (7)
Goodness-of-fit test												
RSS × 10^3	0.23	0.53	0.39	0.48	0.43	0.24	0.52	0.49	0.17	0.59	0.2	0.28
$s(A)$ (mAU)	0.69	0.54	0.71	0.61	0.72	0.41	0.65	0.86	0.58	0.63	0.37	0.38

The reliability of the parameter estimates found is proven with goodness-of-fit statistics such as the residual square sum function RSS and the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU) at 25 °C (above) and 37 °C (below).

standard deviation of residuals, was applied. The standard deviation of absorbance $s(A)$ after termination of the minimization process is always better than 1 mAU, and the proposal of a good chemical model and reliable parameter estimates is thus proven.

4.1.2. Lisuride

Lisuride also exhibits very small changes in spectra within the protonation of anion L. The adjustment of pH from 6.6 to 8.4 causes an absorbance change of 0.080 at 226 nm only, what makes a monitoring of L and LH com-

Table 2
The dependence of the mixed dissociation constants of lisuride on ionic strength using regression analysis of pH-spectrophotometric data with SPECFIT32, with the standard deviations of the parameter estimates in the last valid digits in brackets

The chemical model attained contains L, LH (25 °C)									
Ionic strength	0.017	0.072	0.099	0.167	0.195	0.29	0.386	0.414	0.55
p$K_{a,1}$	7.858 (12)	7.778 (14)	7.849 (23)	7.797 (22)	7.873 (14)	7.905 (16)	8.002 (19)	7.978 (18)	8.030 (18)
Goodness-of-fit test									
RSS × 10^3	0.82	0.53	1.25	0.74	1.12	0.86	1.08	1.19	0.78
$s(A)$ (mAU)	0.75	0.66	1.01	0.83	0.9	0.84	0.97	0.98	0.85
The chemical model attained contains L, LH (37 °C)									
Ionic strength	0.017	0.044	0.099	0.195	0.263	0.387	0.596		
p$K_{a,1}$	7.557 (6)	7.489 (13)	7.558 (19)	7.653 (10)	7.629 (9)	7.755 (12)	7.863 (8)		
Goodness-of-fit test									
RSS × 10^3	1.05	2.07	0.4	1.37	0.97	0.7	0.35		
$s(A)$ (mAU)	0.85	1.3	0.68	1.03	0.89	0.84	0.73		

The reliability of the parameter estimates found is proven with goodness-of-fit statistics as in Table 1.

Table 3
The dependence of the mixed dissociation constants of metergoline on ionic strength using regression analysis of pH-spectrophotometric data with SPECFIT32, with the standard deviations of the parameter estimates in the last valid digits in brackets

The chemical model attained contains L, LH (25 °C)								
Ionic strength	0.006	0.071	0.123	0.209	0.346	0.415	0.552	
p$K_{a,1}$	7.592 (2)	7.589 (4)	7.577 (3)	7.607 (2)	7.638 (2)	7.635 (3)	7.667 (2)	
Goodness-of-fit test								
RSS × 10^3	0.12	0.32	0.7	0.2	0.18	0.31	0.38	
$s(A)$ (mAU)	0.37	0.59	0.9	0.43	0.42	0.6	0.63	
The chemical model attained contains L, LH (37 °C)								
Ionic strength	0.013	0.044	0.071	0.14	0.168	0.359	0.455	0.55
p$K_{a,1}$	7.327 (3)	7.317 (3)	7.289 (3)	7.297 (2)	7.282 (4)	7.324 (2)	7.337 (4)	7.367 (2)
Goodness-of-fit test								
RSS × 10^3	0.31	0.17	0.34	0.11	0.43	0.19	0.29	0.18
$s(A)$ (mAU)	0.58	0.44	0.61	0.36	0.71	0.45	0.59	0.46

The reliability of the parameter estimates found is proven with goodness-of-fit statistics as in Table 1.

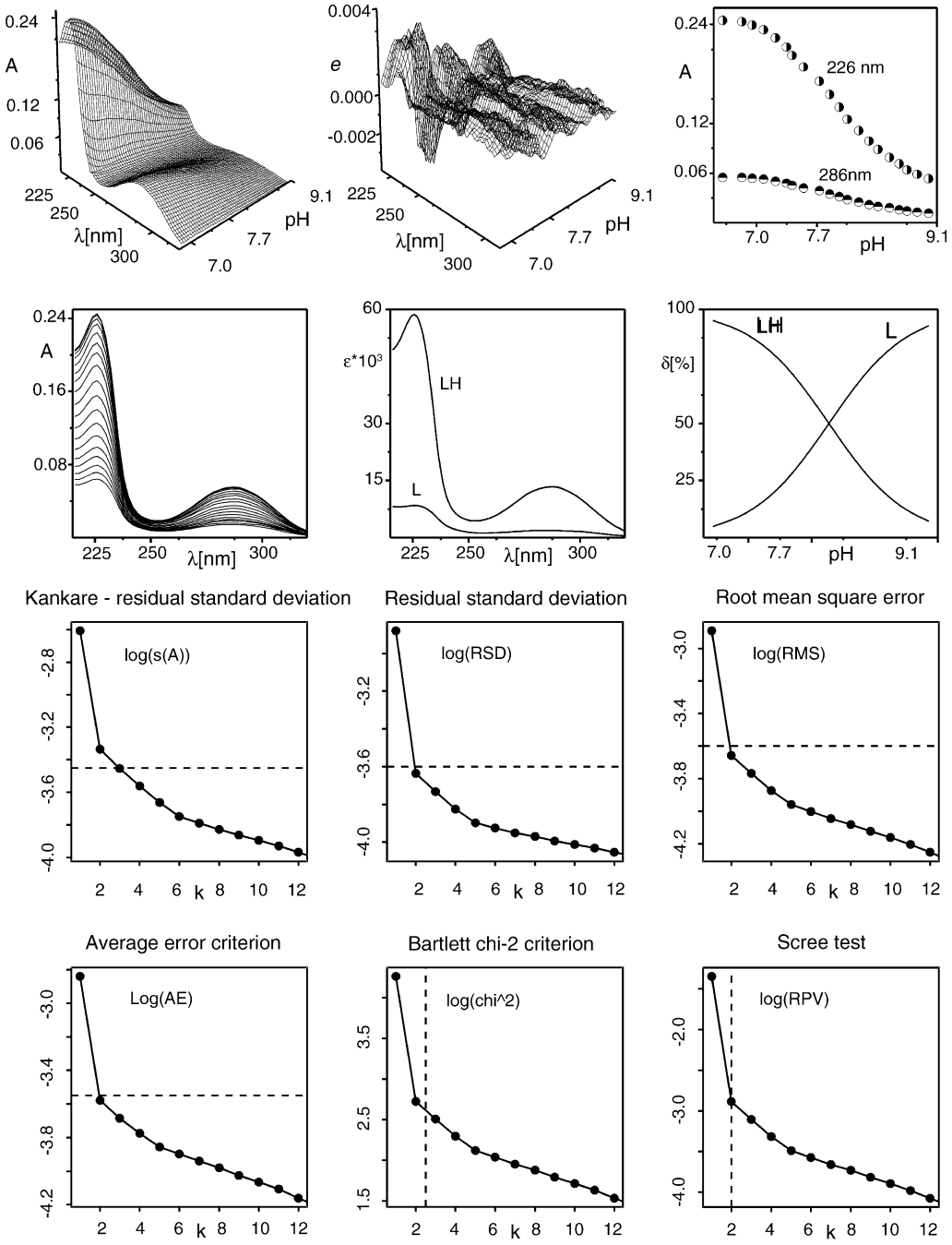


Fig. 4. Absorption spectra of the protonation equilibria of nicergoline in dependence on pH at 25 °C as described in Fig. 1.

ponent rather difficult to determine (Fig. 2). The best region of the spectrum seems to be 215–358 nm. The curves of the molar absorption coefficients for the forms L and LH cross at wavelengths 250 and 280 nm, forming two isosbestic points. Most of the selected methods of factor analysis by the INDICES algorithm lead to two light-absorbing components in the equilibrium mixture. Even small changes in the spectra of the proposed chemical model of lisuride protonation led to small values of standard deviation of absorbance $s(A)$, these being mostly under 1 mAU (Table 2). This goodness-of-fit proves a sufficiently reliable estimates of the dissociation constant and molar absorption coefficient.

4.1.3. *Metergoline*

The structure of the metergoline molecule is similar to lisuride but the spectrum is quite different. This dissimilarity is caused by various substituents on the basic

Table 4
The dependence of the mixed dissociation constants of nicergoline on ionic strength using regression analysis of pH-spectrophotometric data with SPECFIT32, with the standard deviations of the parameter estimates in the last valid digits in brackets

The chemical model attained contains L, LH (25 °C)								
Ionic strength	0.016	0.038	0.071	0.112	0.167	0.208	0.263	0.304
$pK_{a,1}$	7.893 (3)	7.87 (3)	7.879 (3)	7.868 (3)	7.865 (3)	7.874 (4)	7.882 (4)	7.883 (4)
Goodness-of-fit test								
RSS × 10^3	0.8	0.56	0.89	0.7	1.01	1.35	1.43	0.92
$s(A)$ (mAU)	0.87	0.76	0.87	0.79	1.03	1.13	1.14	0.93
The chemical model attained contains L, LH (37 °C)								
Ionic strength	0.001	0.009	0.037	0.09	0.122	0.175	0.255	0.363
$pK_{a,1}$	7.685 (4)	7.644 (3)	7.631 (4)	7.624 (3)	7.629 (3)	7.675 (3)	7.708 (4)	7.732 (3)
Goodness-of-fit test								
RSS × 10^3	0.64	0.27	0.56	0.44	0.39	0.41	0.5	0.35
$s(A)$ (mAU)	0.76	0.52	0.71	0.63	0.61	0.61	0.69	0.57

The reliability of the parameter estimates found is proven with goodness-of-fit statistics as in Table 1.

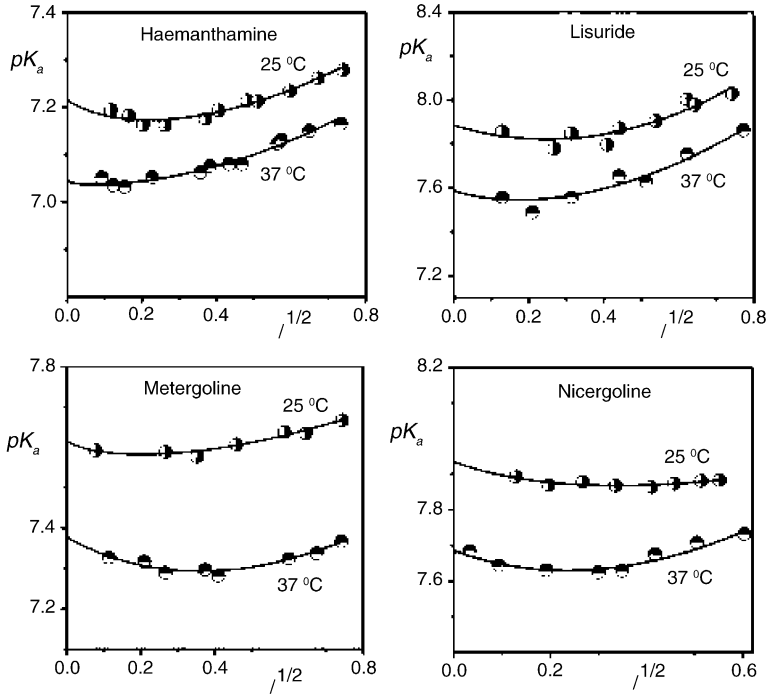


Fig. 5. Dependence of the mixed dissociation constant pK_a of four drugs on the square root of ionic strength, which lead to the parameter estimates of pK_a^T at 25 °C and 37 °C.

molecule. Fig. 3 shows that the protonation of metergoline is followed by large changes in spectra particularly in the range 215–310 nm. For large changes in spectra, the regression analysis was quite reliable and a goodness-of-fit test proved a very close curve fitting, the calculated standard deviation of absorbance $s(A)$ mostly being under l mAU (Table 3).

4.1.4. Nicergoline

Nicergoline exhibits one protonation equilibrium. The protonated form LH exhibits high values of molar absorption coefficients in comparison to the deprotonated form L (Fig. 4). Protonation causes large changes in the spectra, and therefore, the determination of the dissociation constant is easy and reliable (Table 4).

4.2. Thermodynamic dissociation constants

The thermodynamic dissociation constants of the unknown parameter pK_a^T were estimated by applying a

Table 5
Thermodynamic dissociation constants for haemanthamine, lisuride, metergoline and nicergoline at two selected temperatures with the standard deviations in the last valid digits in brackets

	pK_a^T	
	25 °C	37 °C
Haemanthamine	7.22 (1)	7.05 (2)
Lisuride	7.87 (1)	7.59 (1)
Metergoline	7.62 (1)	7.38 (1)
Nicergoline	7.94 (1)	7.69 (1)

Debye–Hückel equation to the data in Tables 1–4 and Fig. 5 according to the regression criterion [33]; Table 5 shows point estimates of the thermodynamic dissociation constants of the four drugs at two temperatures. Because of the narrow range of ionic strengths, the ion-size parameter *a* and the salting-out coefficient *C* could not be estimated.

5. Conclusions

When drugs are very poorly soluble then pH-spectrophotometric titration may be used with the non-linear regression of the absorbance-response-surface data instead of a potentiometric determination of dissociation constants. The reliability of the dissociation constants of the four drugs (i.e. haemanthamine, lisuride, metergoline and nicergoline) may be proven with goodness-of-fit tests of the absorption spectra measured at various pH. Goodness-of-fit tests for various regression diagnostics enabled the reliability of the parameter estimates to be determined.

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