Thermodynamic Dissociation Constants of Rasagiline by the Nonlinear Regression and Factor Analysis of Multiwavelength Spectrophotometric pH Titration Data

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The mixed dissociation constants of rasagiline-free base [*N*-propargyl-1-(*R*)aminoindan] and rasagiline mesylate {[*N*-propargyl-1-(*R*)aminoindan]methansulfonate} at different ionic strengths *I* in the range of (0.01 and 0.4) mol·L^{-1} and at temperatures of (25 and 37) °C were determined with the use of two different multiwavelength and multivariate treatments of spectral data, SPECFIT32 and SQUAD(84), nonlinear regression analyses, and INDICES factor analysis. Concurrently, the experimental determination of the thermodynamic dissociation constants was combined with their computational prediction of the PALLAS program based on knowledge of the chemical structures of the drug in good agreement with its experimental value. The factor analysis in the INDICES program predicts the correct number of light-absorbing components, when the data quality is high and the instrumental error is known. Most indices always predict the correct number of components when the signal-to-error ratio (SER) is higher than 10. The thermodynamic dissociation constant pK_a^T was estimated by nonlinear regression of pK_a and *I* data: for rasagiline base, pK_a^T = 7.12(1) at 25 °C and 7.01(1) at 37 °C; for rasagiline mesylate, pK_a^T = 7.07(1) at 25 °C and 7.16(1) at 37 °C, where the value in parentheses is the standard deviation in the last significant digit. The reliability of the dissociation constants of both drugs was proven with goodness-of-fit tests of the multiwavelength spectrophotometric pH titration data.

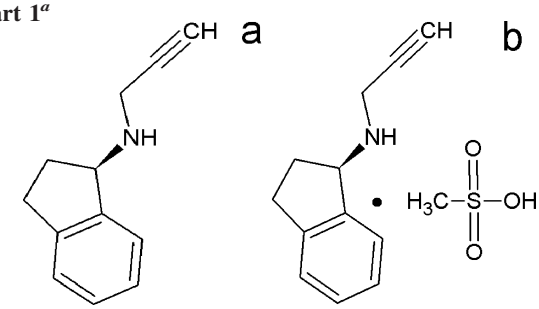
Introduction

Rasagiline [*N*-propargyl-1-(*R*)aminoindan, Chart 1[1]] is a novel, selective, and potent irreversible monoamine oxidase type B (MAO-B) inhibitor for the treatment of Parkinson's disease.[2] Symptoms of the disease include stiffness, tremors, slowness of movement, impaired balance, decreased facial expression, fatigue, apathy, and sometimes pain.

Rasagiline and its analogues are also under investigation for Alzheimer's disease.[1−9] They apparently enhance memory and learning. Rasagiline may also improve mood, motivation, and age-related memory decline in the aging but nominally well adult population.

Drug pharmaceuticals and the determination of useful dosage forms depend upon an understanding of drug dissociation and the extent of dissociation that will occur in the system of the body. The most important physicochemical characteristics of drugs are their acidity or basicity expressed by their pK_a values, their hydrophobicity, and their dependence upon pH. Dissociation constants are very important in both the analysis of drugs and the interpretation of their mechanisms of action because they are key parameters for predicting the extent of ionization of a molecule in solution at different pH. In previous work,[10−18,34] the authors have shown that the spectrophotometric method in combination with suitable chemometric tools can be used for the determination of protonation constants β_{qr} or acid dissociation constants pK_a even for barely soluble drugs. Spectrophotometry is a convenient method for pK_a determination in very dilute aqueous solutions (about 10^{-5} to 10^{-6} M), provided that the compound possesses pH-dependent light absorption because of the presence of a chromophore in proximity to the ionization center.[19−33] The most relevant algorithms are SQUAD[22−27] and SPECFIT.[30−32,35]

Chart 1[a]



[a] (a) Rasagiline base structure formula, *N*-propargyl-1-(*R*)aminoindan; (b) rasagiline mesylate stucture formula, *N*-propargyl-1-(*R*)aminoindan methansulfonate.

In this study, we have tried to complete the information on the protonation/dissociation constants for rasagiline base and rasagiline mesylate. Concurrently, the experimental determination of protonation constants was combined with their computational prediction based on a knowledge of chemical structures.[42] Dissociation of rasagiline mesylate concerns the following three equations:

$$\text{rasagiline mesylate} \leftrightarrow \text{rasagiline}^+ + \text{mesylate}^- \quad (1)$$

$$\text{rasagiline}^+ + \text{OH}^- \leftrightarrow \text{non-dissociated base of rasagiline} \quad (2)$$

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$$\text{mesylate}^- + \text{H}^+ \leftrightarrow \text{non-dissociated methansulfonic acid} \quad (3)$$

Determination of the Protonation/Dissociation Constants. An acid−base equilibrium of the drug studied is described in terms of the thermodynamic protonation of the Brönstedt base L^{z-1} according to the equation $L^{z-1} + H^+ \rightleftarrows HL^z$ characterized by the protonation constant

$$K_H = \frac{a_{HL^z}}{a_{L^{z-1}} a_{H^+}} = \frac{[HL^z]}{[L^{z-1}][H^+]} \frac{y_{HL^z}}{y_{L^{z-1}} y_{H^+}} \quad (4)$$

The protonation equilibria between the anion L (the charges are omitted for the sake of simplicity) of a drug and a proton H are considered to form a set of variously protonated species L, HL, H_2L, H_3L, etc., which have the general formula H_rL in a particular chemical model and which are represented by n_c the number of species, r_i, $i = 1, ..., n_c$, where index i labels their particular stoichiometry; the overall protonation (stability) constant of the protonated species, β_r, may then be expressed as

$$\beta_r = [H_rL]/([L][H]^r) = c/(lh^r) \quad (5)$$

where the free concentration $[L] = l$, $[H] = h$, and $[H_rL] = c$. For dissociation reactions realized at constant ionic strength, the so-called mixed dissociation constants are defined as

$$K_{a,j} = \frac{[H_{j-1}L] a_{H^+}}{[H_jL]} \quad (6)$$

Because each aqueous species is characterized by its own spectrum, for UV/vis experiments and the ith solution measured at the jth wavelength, the Beer−Lambert law relates the absorbance, $A_{i,j}$, being defined as

$$A_{i,j} = \sum_{n=1}^{n_c} \varepsilon_{j,n} c_n = \sum_{n=1}^{p} (\varepsilon_{r,j} \beta_r l h^r)_n \quad (7)$$

where $\varepsilon_{r,j}$ is the molar absorptivity and β_r is the estimate of the protonation constant of the nth H_rL species with the stoichiometric coefficient r measured at the jth wavelength. The absorbance $A_{i,j}$ is an element of the absorbance matrix $\boldsymbol{A}$ of size $n_s \cdot n_w$ being measured for n_s solutions with known total concentrations of $n_z = 2$ basic components, c_L and c_H, at n_w wavelengths. Calculations related to the determination of protonation constants may be performed by the regression analysis of spectra using versions of the SQUAD(84) program family[22−27] and SPECFIT/32[30−32,35] and have been described previously.[34,46,47]

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

$$pK_a = pK_a^T - \frac{A(1 - 2z)\sqrt{I}}{1 + Ba\sqrt{I}} + CI \quad (8)$$

where $A = 0.5112\ \text{mol}^{-1/2} \cdot \text{L}^{1/2} \cdot \text{K}^{3/2}$ and $B = 0.3291\ \text{mol}^{-1/2} \cdot \text{m}^{-1} \cdot \text{L}^{1/2} \cdot \text{K}^{1/2} \cdot 10^{10}$ 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 $\boldsymbol{b} = (pK_a, a, C)$ are to be estimated by a minimization of the sum of the squared residuals[36]

$$U(\boldsymbol{b}) = \sum_{i=1}^{n} w_i [pK_{a,exp,i} - pK_{a,calc,i}]^2 = \sum_{i=1}^{n} w_i [pK_{a,exp,i} - f(I; pK_a^T, a, C]^2 = \text{minimum} \quad (9)$$

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, a, and C are unknown variables to be estimated.[11,23,34,36,43−47]

The adequacy of a proposed regression model with experimental data and the reliability of parameter estimates $pK_{a,i}$ found, being denoted for the sake of simplicity as unknown parameters b_j, and $\varepsilon_{i,j}$, where $j = 1, ..., p$, may be examined by the goodness-of-fit test, cf. a previous tutorial.[34,47]

Determination of the Number of Light-Absorbing Species. A qualitative interpretation of the spectra aims to evaluate the quality of the data set, remove spurious data, and estimate the minimum number of factors, that is contributing aqueous species, which are necessary to describe the experimental data. The INDICES[40] program determines the number of dominant light-absorbing species present in the equilibrium mixture.

Signal-to-Noise Ratio (SNR). The level of "experimental noise" should be used in the experiment as a critical factor. Therefore, it is necessary to have a consistent definition of the SNR, so that the impact of this parameter can be critically assessed. Traditional approaches to SNR are typically based on the ratio of the maximum signal to maximum noise value. As an alternative, the concept of instrumental error was again employed and the signal-to-error ratio (SER) is defined, where for an error the instrumental standard deviation of absorbance, $s_{inst}(A)$ is used. The plot of small absorbance changes in the spectrum of the drug studied means that the value of the absorbance difference for the jth wavelength of the ith spectrum $\Delta_{i,j} = A_{i,j} - A_{i,acid}$ is divided by the instrumental standard deviation $s_{inst}(A)$, and the resulting ratios $SER = \Delta / s_{inst}(A)$ are plotted with dependence upon wavelength λ for all absorbance matrix elements, where $A_{i,acid}$ is the initial spectrum of the acid form of the drug being measured for the starting pH value of the pH range studied. This SER ratio is then compared to the limiting SER value to test if the absorbance changes are significantly larger than the instrumental noise.

The plot of the ratio $e/s_{inst}(A)$, that is the ratio of the residuals divided by the instrumental standard deviation $s_{inst}(A)$ with dependence upon wavelength λ for all of the residual matrix elements for tests if the residuals, are of the same or similar magnitude as the instrumental noise to prove the best curve fitting achieved.

Experimental Section

Chemicals. Hydrochloric acid (1 M) was prepared from concentrated HCl (p.a., Lachema Brno) using redistilled water and standardized against HgO and KI with reproducibility of less than 0.20 %. Potassium hydroxide (1 M) was prepared from pellets (p.a., Aldrich Chemical Co.) with carbon-dioxide-free redistilled water and standardized against standardized HCl with a reproducibility of 0.1 %. The preparation of other solutions from analytical-reagent-grade chemicals has been described previously.[11−17,44,46] Rasagiline base and rasagiline mesylate ($5 \cdot 10^{-5}$ M) was prepared from solid samples (IVAX a.s., Opava) using redistilled water. A high purity of the substances (over 98 %) was guaranteed by the supplier.

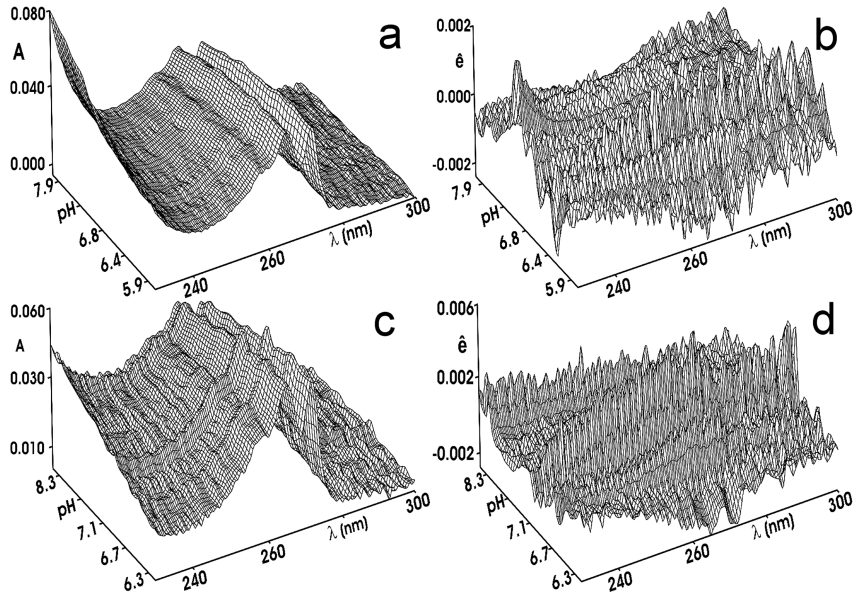

Figure 1. Three-dimensional (3D) absorbance response surface representing the measured multiwavelength absorption spectra with dependence upon pH at 25 °C for (a) rasagiline base and (c) rasagiline mesylate and the 3D residual map after nonlinear regression performed with SQUAD for (b) rasagiline base and (d) rasagiline mesylate (S-Plus).

pH Spectrophotometric Titration Procedure. The apparatus used and the pH spectrophotometric titration procedure have been described previously.[34] The experimental and computation scheme for the determination of the protonation constants of the multicomponent system is taken from Meloun et al., cf. page 226 in ref 19, and the five steps are described in a previous contribution:[34] (1) instrumental error of absorbance measurements, $s_{inst}(A)$, (2) experimental design, (3) number of light-absorbing species, (4) choice of computational strategy, and (5) diagnostics indicating a correct chemical model. When a minimization process terminates, some diagnostics are examined to determine whether the results should be accepted: the physical meaning of parametric estimates, the physical meaning of the species concentrations, the goodness-of-fit test, and the deconvolution of spectra.

Software. Computation relating to the determination of dissociation constants was performed by regression analysis of the UV/vis spectra using the SQUAD(84)[24] and SPECFIT/32[35] programs. Most of the graphs were plotted using ORIGIN 7.5[37] and S-Plus.[38] The thermodynamic dissociation constant pK_a^T was estimated with the MINOPT nonlinear regression program in the ADSTAT statistical system.[39] A qualitative interpretation of the spectra with the use of the INDICES program[40] aims to evaluate the quality of the data set, remove spurious data, and estimate the minimum number of factors, that is contributing aqueous species, which are necessary to describe the experimental data and determine the number of dominant species present in the equilibrium mixture. PALLAS[41,42] is a program for making predictions based on the structural formulas of the drug compounds.

Results and Discussion

Recently, rasagiline base and rasagiline mesylate were studied in our laboratory, and these two drugs are examples of drug acids that exhibit small changes in spectra. Other instrumental methods for determination of dissociation constants do not seem to be suitable because of the limited solubility in water.

Rasagiline Base. The deprotonation of rasagiline base LH form indicates one simple equilibrium. A pH spectrophotometric titration enables absorbance response data (Figure 1a) to be obtained for analysis by nonlinear regression, and the reliability of parameter estimates (pK and ε values) can be evaluated on the basis of a goodness-of-fit test of the residuals (Figure 1b).

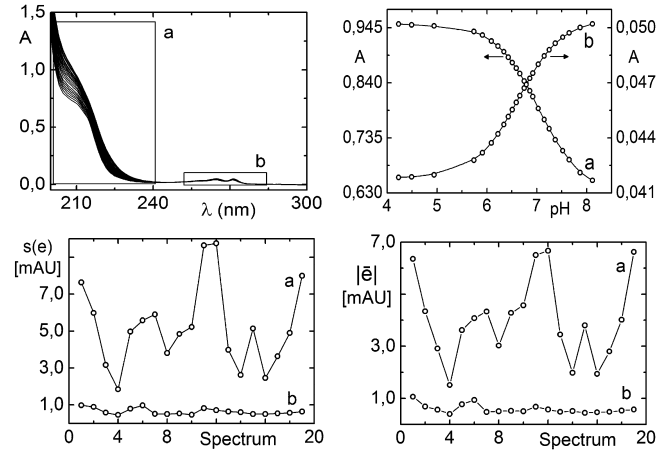

Figure 2. Two parts of a 2D absorption spectrum of rasagiline base with dependence upon pH at 25 °C were analyzed to determine pK_a. While part a led to worse curve fitting with false $pK_a = 6.30(3)$, part b led to much better results of a goodness-of-fit test with $pK_a = 7.00(8)$. The absorbance versus pH curves for (230 and 260) nm with dependence upon pH at 25 °C exhibit that the inflection point or pK_a value slightly differs for both curves. The plot of the standard deviation of absorbance fitted $s(e)$ and the mean residual $|\bar{e}|$ for every spectrum is significantly lower for part b, and therefore, this part of the spectrum was preferred for analysis of pK_a.

Two parts of the absorption spectrum of rasagiline base with a dependence on pH were analyzed to determine the pK_a (Figure 2). While part a from (205 to 240) nm in Figure 2 does not lead to reliable determination of the dissociation constant because a worse curve fitting was achieved, part b from (230 to 300) nm on Figure 2 leads to better results in a goodness-of-fit test because residuals are much lower than for part a (lower part of Figure 2).

The changes in spectra are quite small during deprotonation; however, both the deprotonated and protonated species L^- and

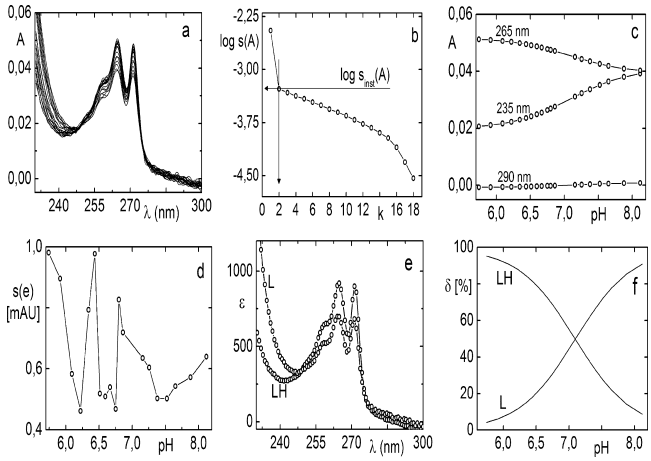

Figure 3. Nonlinear regression analysis of the protonation equilibria model and factor analysis of rasagiline base. (a) Absorption spectra with dependence upon pH at 25 °C. (b) Cattel's scree plot of the Wernimont−Kankare procedure for the determination of the number of light-absorbing species in the mixture $k^* = 2$ leads to $n_c = 2$ and the actual instrumental error of the spectrophotometer used $s_{inst}(A) = 0.55$ mAU (INDICES in S-Plus). (c) Absorbance versus pH curves for (265, 235, and 290) nm with dependence upon pH at 25 °C. (d) Detecting influential outlying spectra with the use of the goodness-of-fit test and the plot of the residual standard deviation $s(e)$ versus pH for 19 spectra with dependence upon pH at 25 °C. (e) Pure spectra profiles of molar absorptivities versus wavelengths for the variously protonated species L and HL. (f) Distribution diagram of the relative concentrations of both variously protonated species L and HL of rasagiline base with dependence upon pH at 25 °C. The charges of species are omitted for the sake of simplicity (SPECFIT and ORIGIN).

LH exhibit similar absorption bands. The small shift of a band maximum to lower wavelengths in the spectra set is shown in Figure 3a. The adjustment of the pH value from 5.5 to 8.5 causes the absorbance to change by 15 mAU only, so that monitoring of both components L^- and LH of the protonation equilibrium is rather uncertain. Because the changes in spectra are small, a very precise measurement of absorbance is required for the reliable estimation of the deprotonation equilibrium studied. In the first step of the regression spectra analysis, the number of light-absorbing species n_c is estimated by the INDICES algorithm (Figure 3b). The position of the break point on the $s_k(A) = f(k)$ curve in the factor analysis scree plot is calculated and gives $k^* = 2$, and therefore, $n_c = 2$ with corresponding coordinate $\log s_{k^*}(A) = -3.27$; that is $s_{k^*}(A) = 0.53$ mAU, which also represents the actual instrumental error $s_{inst}(A)$ of the spectrophotometer used. Because of the large variations in the indicator values, these latter values are plotted on a logarithmic scale. All other selected methods of the modified factor analysis in the INDICES algorithm estimate two light-absorbing components L^- and LH of the protonation equilibrium. The number of light-absorbing species n_c can be predicted from the index function values by finding the point $n_c = k$, where the slope of the index function $PC(k) = f(k)$ changes, or by comparing $PC(k)$ values to the instrumental error $s_{inst}(A) \approx 0.53$ mAU. This is the common criterion for determining n_c. Very low values of $s_{inst}(A)$ prove that reliable spectrophotometer and experimental techniques were used. The A−pH curves at (235, 265, and 290) nm (Figure 3c) show that a dissociation constant may be indicated. The dissociation constant and the two molar absorptivities of rasagiline base ε_L and ε_{HL} calculated for 30 wavelengths of 19 spectra (Figure 1) constitute $(2 \cdot 30) + 1 = 61$ unknown regression parameters, which are estimated and refined by SQUAD(84) or SPECFIT32 in the first run. The reliability of the parameter estimates may be tested with the use of the following diagnostics.

The first diagnostic value indicates whether all of the parametric estimates pK_a and ε_L and ε_{HL} have physical meaning and reach realistic values: for rasagiline base, $pK_a^T = 7.21$ ($s = 0.01$) at 25 °C; PALLAS, $pK_a^T = 7.13$. Because the standard deviations $s(pK_a)$ of parameters pK_a and $s(\varepsilon)$ of parameters ε_L and ε_{HL} are significantly smaller than their corresponding parameter estimates, all the variously protonated species are statistically significant at a significance level $\alpha = 0.05$. The physical meaning of the dissociation constant pK_a and molar absorptivities ε_L and ε_{HL} are examined. The absolute values of $s(pK_a)$ and $s(\varepsilon)$ give information about the last U contour of the hyperparaboloid in the neighborhood 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 relationship $s(\beta_j)F_\sigma < \beta_j$ should be met where F_σ is equal to 3 for a 99.9 % statistical probability level and β_j stands for pK_a and ε_L and ε_{HL}. The set of standard deviations of ε_L and ε_{HL} for various wavelengths, $s(\varepsilon) = f(\lambda)$, should have a Gaussian distribution; otherwise, erroneous estimates of ε are obtained. Figure 3e shows the estimated molar absorptivities of all of the variously protonated species ε_L and ε_{HL} of the rasagiline base as a function of the 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 (Figure 3f).

The next diagnostic concerns the goodness-of-fit (panels b and d of Figure 1 and Figure 3d). The goodness-of-fit achieved is easily seen by examination of the differences between the experimental and calculated values of absorbance, $e_i = A_{exp,i,j} - A_{calc,i,j}$. Examination of the spectra and the graph of the predicted absorbance response surface through all of 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 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 to the standard deviation of the absorbance calculated by the INDICES program,[36] $s_k(A)$, 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. This proves that the $s_2(A)$ value is equal to 0.53 mAU and is quite close to the standard deviation of absorbance when the minimization process terminates, $s(A) = 0.55$ mAU. Although this statistical analysis of the residuals[34,36] gives the most rigorous test of the degree-of-fit, realistic empirical limits must be used. The statistical measure of all residuals e proves that the minimum of the eliptic hyperparaboloid U is reached; the residual standard deviation $s(e)$ always has sufficiently low values. The criteria of resolution used for the hypotheses were (1) a failure of the minimization process in a divergency or a cyclization, (2) an examination of the physical meaning of the estimated parameters to ensure that they were both realistic and positive, and (3) a random distribution of the residuals about the predicted regression spectrum, with systematic departures from randomness being taken to indicate that either the chemical model or the parameter estimates were unsatisfactory.

To express small changes of absorbance in the spectral set, the absorbance differences for the jth wavelength of the ith

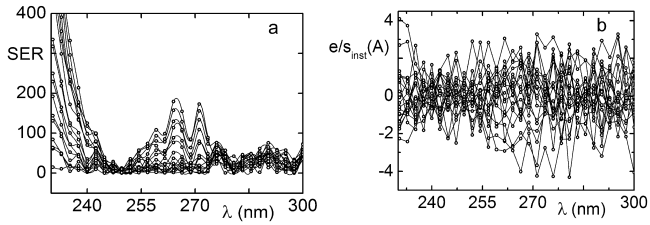

Figure 4. (a) Plot of small absorbance changes in the spectrum means that the value of the absorbance difference for the *j*th wavelength of the *i*th spectrum $\Delta_{i,j} = A_{i,j} - A_{i,\text{acid}}$ is divided by the instrumental standard deviation $s_{\text{inst}}(A)$, and the resulting ratios SER $= \Delta/s_{\text{inst}}(A)$ are plotted with dependence upon wavelength λ for all absorbance matrix elements, where $A_{i,\text{acid}}$ is the limiting spectrum of the acid form of the rasagiline base measured. This ratio is compared to the limiting SER value for the rasagiline base to test if the absorbance changes are significantly larger than the instrumental noise. (b) Plot of the ratio $e/s_{\text{inst}}(A)$, that is the ratio of the residuals divided by the instrumental standard deviation $s_{\text{inst}}(A)$, with dependence upon wavelength λ for all of the residual matrix elements for rasagiline base proved that the residuals are of the same magnitude as the instrumental noise.

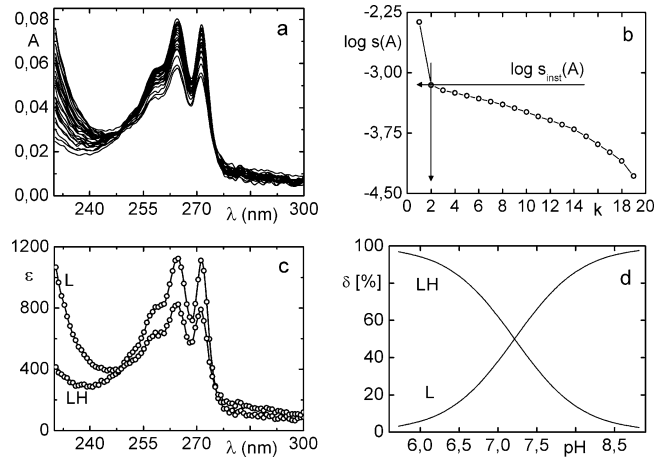

Figure 5. Nonlinear regression analysis of the protonation equilibria model and factor analysis of rasagiline mesylate. (a) Absorption spectra with dependence upon pH at 25 °C. (b) Cattel's scree plot of the Wernimont−Kankare procedure for the determination of the number of light-absorbing species in the mixture $k^* = 2$ leads to $n_c = 2$ and the actual instrumental error of the spectrophotometer used $s_{\text{inst}}(A) = 0.55$ mAU (INDICES in S-Plus). (c) Pure spectra profiles of molar absorptivities versus wavelengths for the variously protonated species L and HL. (d) Distribution diagram of the relative concentrations of both variously protonated species L and HL of the rasagiline base with dependence upon pH at 25 °C. The charges of species are omitted for the sake of simplicity (SPECFIT and ORIGIN).

spectrum $\Delta_i = A_{i,j} - A_{i,\text{acid}}$ were calculated, so that, from the absorbance value of the spectrum measured at the actual pH, the absorbance value of the acidic form was subtracted. The absorbance difference Δ_i was then divided by the actual instrumental standard deviation $s_{\text{inst}}(A)$ of the spectrophotometer used, and the resulting value represents the SER. Figure 4a is a graph of the SER as a function of the wavelength in the measured range for rasagiline base. When the SER is larger than 10, a factor analysis is sufficiently able to predict the correct number of light-absorbing species in the equilibrium mixture. To prove that nonlinear regression can analyze such data, the residuals set was compared to the instrumental noise $s_{\text{inst}}(A)$. If the ratio $e/s_{\text{inst}}(A)$ is of similar magnitude, that is nearly close to 1, it means that sufficient curve fitting was achieved by the nonlinear regression of the spectra set and that the minimization process found the global minimum of the residual-square-sum function $U_{\min}$. Figure 4b shows a comparison of the ratio $e/s_{\text{inst}}(A)$ as a function of the wavelength for rasagiline base measured. From the figure, it is obvious that most of the residuals are of the same magnitude as the instrumental noise and, therefore, proves a sufficient reliability of regression process performed.

Rasagiline Mesylate. The deprotonation of rasagiline mesylate HL indicates one simple equilibrium. A pH spectrophotometric titration enables absorbance response data (Figure 5a) to be obtained for analysis by nonlinear regression, and the reliability of parameter estimates (pK and ε values) can be evaluated on the basis of a goodness-of-fit test of the residuals (Figure 1d). Interpretation of deprotonation of rasagiline mesylate is similar to the case of rasagiline base in Figure 3.

Applying a Debye−Hückel equation to the data in Tables 1 and 2 according to the regression criterion (9), the unknown parameter pK_a^T has been estimated. Table 3 gives estimates of the thermodynamic dissociation constants of two drugs studied at two temperatures. Because of the narrow range of ionic strength, the ion size parameter a and the salting-out coefficient C could not be estimated here.

Conclusions

When drugs are poorly soluble, pH spectrophotometric titration may be preferred with the nonlinear regression of the absorbance response surface data instead of performing a potentiometric determination of the dissociation constants. The reliability of the dissociation constants of both drugs studied (rasagiline base and rasagiline mesylate) may be proven with goodness-of-fit tests of the absorption spectra measured at various pH values. The thermodynamic dissociation constant pK_a^T was estimated by nonlinear regression of pK_a and I data at 25 °C: for rasagiline base, $pK_a^T = 7.12(1)$; for rasagiline mesylate, $pK_a^T = 7.06(1)$, where the value in parentheses is the standard deviation in the last significant digit (Figure 6). Goodness-of-fit tests for the various regression diagnostics enabled the reliability of the parameter estimates to be determined. Most indices always predict the correct number of components and even the presence of a minor one when the SER is higher than 10. The Wernimont−Kankare procedure in INDICES performs a reliable determination of the instrumental standard deviation of the spectrophotometer used $s_{\text{inst}}(A)$, correctly predicts the number of light-absorbing components present n_c, and can also solve

Table 1. Dependence of the Estimated Mixed Dissociation Constant pK_a of Rasagiline Base on Ionic Strength Using Regression Analysis of pH Spectrophotometric Data with SPECFIT and SQUAD at (25 and 37) °C[a]

estimated pK_a at 25 °C							
ionic strength		0.0017	0.0224	0.0470	0.0841	0.1252	0.1527
SPECFIT	pK_a	7.064(7)	7.031(7)	7.029(7)	7.098(6)	7.163(4)	7.213(8)
	$s(A)$ (mAU)	0.68	0.76	0.72	0.55	0.44	0.90
SQUAD	pK_a	7.061(5)	7.027(5)	7.026(6)	7.098(3)	7.147(2)	7.209(5)
	$s(A)$ (mAU)	0.86	0.93	0.62	0.69	0.68	0.91

estimated pK_a at 37 °C							
ionic strength		0.0017	0.0224	0.0532	0.0738	0.1252	0.1664
SPECFIT	pK_a	7.034(6)	6.872(4)	6.814(10)	6.843(6)	6.927(7)	7.045(8)
	$s(A)$ [mAU]	0.64	0.44	1.10	0.62	0.76	0.81
SQUAD	pK_a	7.027(4)	6.868(2)	6.810(8)	6.837(3)	6.916(4)	7.041(5)
	$s(A)$ [mAU]	0.88	0.72	0.78	0.87	0.94	0.93

[a] The standard deviations of the pK_a in the last valid digits are in parentheses.

Table 2. Dependence of the Estimated Mixed Dissociation Constants pK_a of Rasagiline Mesylate on Ionic Strength Using Regression Analysis of pH Spectrophotometric Data with SPECFIT and SQUAD at (25 and 37) °C[a]

estimated pK_a at 25 °C							
ionic strength			0.0018	0.0224	0.0419	0.0635	0.1036	0.1250
SPECFIT	pK_a		7.022(5)	6.983(5)	7.001(7)	7.025(8)	7.141(7)	7.153(5)
	$s(A)$	[mAU]	0.49	0.85	0.49	0.49	0.77	0.53
SQUAD	pK_a		7.026(3)	6.986(3)	6.991(4)	7.006(5)	7.133(4)	7.153(3)
	$s(A)$	[mAU]	0.64	0.87	0.72	0.67	0.93	0.76

estimated pK_a at 37 °C							
ionic strength			0.0017	0.0103	0.0223	0.0428	0.0840	0.1252
SPECFIT	pK_a		7.003(6)	6.931(5)	6.911(7)	6.903(9)	6.927(7)	6.991(7)
	$s(A)$	[mAU]	0.76	0.76	0.79	0.61	0.87	0.79
SQUAD	pK_a		7.009(3)	6.930(3)	6.912(5)	6.900(5)	6.921(4)	6.996(3)
	$s(A)$	[mAU]	1.26	0.96	0.76	0.79	1.07	0.94

[a] The standard deviations of the pK_a in the last valid digits are in parentheses.

Table 3. Thermodynamic Dissociation Constants pK_a for Rasagiline Base and Rasagiline Mesylate at (25 and 37) °C[a]

		estimated with SPECFIT		
		value at 25 °C	value at 37 °C	predicted with PALLAS
rasagiline base	pK_a^T	7.12(1)	7.01(1)	7.13
rasagiline mesylate	pK_a^T	7.07(1)	7.05(1)	7.13

[a] The standard deviations in the last valid digits are in parentheses.

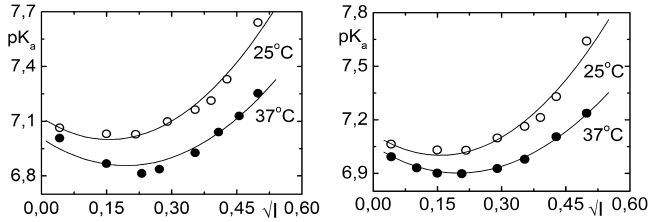

Figure 6. Dependence of the mixed dissociation constant pK_a of two drugs, (left panel) rasagiline base and (right panel) rasagiline mesylate, on the square root of ionic strength, which leads rasagiline base to the parameter estimates of pK_a^T = 7.121(9) at 25 °C and pK_a^T = 7.010(6) at 37 °C and rasagiline mesylate to the parameter estimates of pK_a^T = 7.065(10) at 25 °C and pK_a^T = 7.158(8) at 37 °C.

an ill-defined problem with severe collinearity in the spectra or very small changes in spectra.

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Complete experimental and computational procedures, input data specimens, and corresponding output in numerical and graphical form for the programs INDICES, SQUAD(84), and SPECFIT/32 are available free of charge online at http://meloun.upce.cz in the block DOWNLOAD and DATA.

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