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The thermodynamic dissociation constants of ambroxol, antazoline, naphazoline, oxymetazoline and ranitidine by the regression analysis of spectrophotometric data

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Abstract

The mixed dissociation constants of five drug acids—ambroxol, antazoline, naphazoline, oxymetazoline and ranitidine—at various ionic strengths I of range 0.01 and 1.0 and at temperatures of 25 and 37 °C were determined using SQUAD(84) regression analysis of the pH-spectrophotometric titration data. A proposed strategy of efficient experimentation in a protonation constants determination, followed by a computational strategy for the chemical model with a protonation constants determination, is presented on the protonation equilibria of ambroxol. The thermodynamic dissociation constant pK_a^T was estimated by non-linear regression of $\{pK_a, I\}$ data at 25 and 37 °C: for ambroxol $pK_{a,1}^T = 8.05\,(6)$ and 8.25 (4), $\log\beta_{21}^T = 11.67\,(6)$ and 11.83 (8), for antazoline $pK_{a,1}^T = 7.79\,(2)$ and 7.83 (6), $pK_{a,2}^T = 9.74\,(3)$ and 9.55 (2), for naphazoline $pK_{a,1}^T = 10.81\,(1)$ and 10.63 (1), for oxymethazoline $pK_{a,1}^T = 10.62\,(2)$ and 10.77 (7), $pK_{a,2}^T = 12.03(3)$ and 11.82 (4) and for ranitidine $pK_{a,1}^T = 1.89\,(1)$ and 1.77 (1). Goodness-of-fit tests for various regression diagnostics enabled the reliability of the parameter estimates to be found.

Keywords: Spectrophotometric titration; Dissociation constant; Protonation; Ambroxol; Antazoline; Naphazoline; Oxymetazoline; Ranitidine

1. Introduction

In the 1990s, the pharmaceutical industry and regulatory health care authorities adopted a new system for the classification of drugs, the Biopharmaceutics Classification System (BCS) [1–3]. BCS classifies every pharmaceutical active ingredient into one of four groups based on two basic characteristics: solubility and permeability. The system reflects contemporary experience in the evaluation of the most important features of drugs which affect the formulation of medicine preparation and the regulatory consequences. When a poorly soluble drug is to be formulated, attention is paid mainly to an improvement of its solubility, and thus mostly to the selection of appropriate pharmaceutical excipient(s). In more soluble drugs, there is generally more information on their protonation behaviour in water systems. However, the dependence of protonation constants on ionic strength has been systematically investigated only in a few cases in the literature. The authors decided to complete such information and to study the protonation equilibria of five readily soluble drugs. In three, the protonation/dissociation equilibria can play an important role because of their site of application, the nasal mucosa and/or eye (naphazoline, antazoline, and oxymetazoline). At the same time, the range of osmolality of body liquids at the site of absorption (tears and nasal secretions) is better defined and much narrower than in cases of absorption in the gastrointestinal tract. In the other two drugs, the protonation/dissociation behaviour on site of absorption is generally known, and the influence of ionic strength should not play a role, i.e. ambroxol as a representative of basic, and ranitidin as a representative of acidic drugs.

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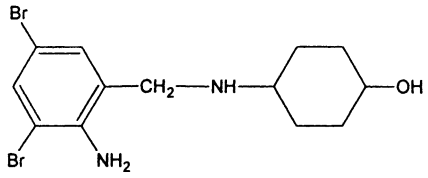
Naphazoline and oxymetazoline belong to the group of α-sympatomimetics, agonists of α-adrenergic receptors. Antazoline is a histamine H_1-receptor antagonist. They all have vasoconstrictive effects, due to which they reduce the lumen of capillaries, and help relieve the oedema of nasal tissue. Antazoline, which possess an antihistaminic effect, is often used in combination with other vasoconstrictors to treat rhinitis of allergic origin. As allergic rhinitis is accompanied by eye irritation, the final dosage forms—drops—containing these compounds are often designed both as nasal and eye drops [4]. However, the pH of nasal mucosa is slightly acidic, pH = 5.5–5.6, while the pH of tears, the conjunctival liquid, is slightly basic, pH = 7.4.

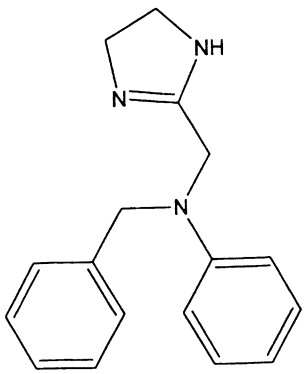
Ideally, both active compounds should not be much dissociated/protonated in this pH range to achieve good absorption. On the other hand, the pH of the nasal or eye drop formulation itself plays an important role in perception (well-being) after application on the one hand and in the stability of the formulation on the other which may lead to contradictory conditions.

Ranitidine is a competitive antagonist of histamine H_2-receptors. Due to its acidic character, it is less dissociated under low pH and is thus considered a representative of drugs well absorbed in the stomach. Nevertheless, the dissociation constant of ranitidine is not listed in the general literature, e.g. [5,6]. Ambroxol is a well-known secretolytic and mucolytic drug. It is almost completely absorbed, and due to its basic character, the sites of its absorption are tissues with basic pH. Therefore, ambroxol is a representative of drugs which are well absorbed in either the small intestine or rectum.

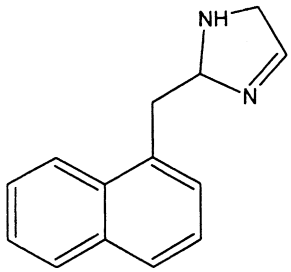
Ambroxol, chemically (2-amino-3,5-dibromo-*N*-[*trans*-4-hydroxy-cyclohexyl] benzylamin,



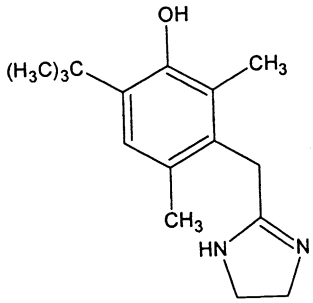
Antazoline, chemically 4,5-Dihydro-*N*-phenyl-*N*-(phenylmethyl)-1*H*-imidazole-2-methanamine, 2-[(*N*-benzylanilino) methyl]-2-imidazole,



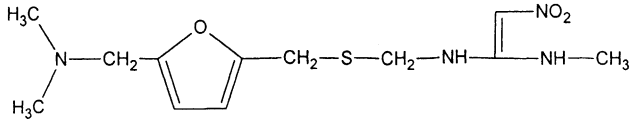
Naphazolin, chemically 4,5-Dihydro-2-(1-naphthalenylmethyl)-1*H*-imidazole,



Oxymethazoline, chemically 2-(3-hydroxy-2,6-dimethyl-4-*tert*-butylbenzyl)-2-imidazolin,



Ranitidin, chemically *N*,*N*-dimethyl-*N*-[5-[2-(1-methylamino-2-nitrovinylamino)-ethyl-thiomethyl]furfuryl]-amine,



This paper investigates the dissociation constants of the five drugs: ambroxol, antazoline, naphazoline, oxymetazoline and ranitidine at various ionic strengths and at 25 and 37 °C, to prove their reliability and also to estimate the thermodynamic dissociation constant pK_a^T at these two temperatures. The pK_a^T may be used for prediction of the actual dissociation constant pK_a at the given value of an ionic strength.

2. Theoretical

Computations related to the determination of protonation constants [7–10] may be performed by the regression analysis of spectra using versions of the SQUAD program family [8,11–16]. 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 a 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

$$\beta_{qr} = \frac{[L_qH_r]}{[L]^q[H]^r} = \frac{c}{l^q h^r} \quad (1)$$

where the free concentration $[L] = l$, $[H] = h$ and $[L_qH_r] = c$. For the ith solution measured at the jth wavelength, the absorbance, $A_{i,j}$, is defined as

$$A_{i,j} = \sum_{n=1}^{p} \varepsilon_{j,n} c_n = \sum_{n=1}^{p} (\varepsilon_{qr,j} \beta_{qr} l^q h^r)_n \quad (2)$$

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 $\boldsymbol{A} = \boldsymbol{\varepsilon C}$ containing the n recorded spectra as rows can be written as the product of the $m \times p$ matrix of molar absorptivities $\boldsymbol{\varepsilon}$ and the $p \times n$ concentration matrix $\boldsymbol{C}$. Here, p is the number of components that absorb in the chosen spectral range. The rank of the matrix $\boldsymbol{A}$ is obtained from the equation $\text{rank}(A) = \min[\text{rank}(\boldsymbol{\varepsilon}), \text{rank}(\boldsymbol{C})] \leq \min(m, p, n)$. Since the rank of $\boldsymbol{A}$ is equal to the rank of $\boldsymbol{\epsilon}$ or $\boldsymbol{C}$, whichever is the smaller, and since $\text{rank}(\boldsymbol{\varepsilon}) \leq p$ 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 [8,17,18]. All spectra evaluation may be performed with the INDICES algorithm [18] in the S-Plus programming environment. Most indices 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, $\text{PC}(k) = f(k)$, and when the PC(k) reaches the value of the instrumental error of the spectrophotometer used, $s_{\text{inst}}(A)$, 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 an increasing number 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 indices values at this point can be predicted from the properties of the noise, which may be used as a criteria to determine p [18].

The multi-component spectra analysing program SQUAD(84) [13] may adjust $\boldsymbol{\beta}_{qr}$ and $\boldsymbol{\varepsilon}_{qr}$ for absorption spectra by minimising the residual-square sum function, U,

$$U = \sum_{i=1}^{n}\sum_{j=1}^{m} (A_{\text{exp},i,j} - A_{\text{calc},i,j})^2 = \sum_{i=1}^{n}\sum_{j=1}^{m} (A_{\text{exp},i,j} - \sum_{k=1}^{p} \varepsilon_{j,k} c_k)^2 = \text{minimun} \quad (3)$$

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 $s(A)$ and the Hamilton R-factor are used as the most important criteria for a fitness test. If, after termination of the minimization process the condition $s(A) \approx s_{\text{inst}}(A)$ is met and the R-factor is less than 1%, the hypothesis of the chemical model is taken as the most probable one and is accepted.

3. Experimental

3.1. Chemicals and solutions

All the drugs were used in the form of hydrochloride, nitrate or mesylate. Ranitidine hydrochloride was purchased from SMS Pharmaceuticals, India, with a purity of 98.3%. Antazoline mesylate was purchased from SIMS S.p.A., Italy, with a purity of 99.8%. Naphazoline nitrate was purchased from LOBA Feinchemie, Austria, with purity 99.3%. Ambroxol hydrochloride was purchased from Boehringer Ingelheim, Germany, with a purity of 99.9%. Oxymetazoline hydrochloride was purchased from Sigma–Aldrich with a purity of 99.6%. Perchloric acid, 1 M, was prepared from conc. $HClO_4$ (p.a., Lachema Brno) using redistilled water and standardized against HgO and NaI with a reproducibility less than 0.20%. Sodium hydroxide, 1 M, was prepared from pellets (p.a., Aldrich) with carbon dioxide-free redistilled water and standardized against a solution of potassium hydrogen-phthalate using the Gran method in the MAGEC program [11] with a reproducibility of 0.1%. Mercuric oxide, sodium iodide, and sodium perchlorate (p.a., Lachema Brno) were not further purified. The preparation other solutions from analytical reagent-grade chemicals has been described previously [9,10]. Twice-redistilled water was used in the preparation of solutions.

3.2. Apparatus and pH-spectrophotometric titration procedure

The used apparatus and the pH-spectrophotometric titration procedure has been described previously [19].

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

The experimental and computational schemes for the determination of the protonation constants of the multicomponent system is taken from Meloun et al. [8] and are described in a previous contribution [19]. 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.

3.4. Determination of the thermodynamic protonation/dissociation constants

The non-linear estimation problem of the thermodynamic dissociation constant $K_a^T = a_{H+}a_{L-}/a_{HL}$, is simply a

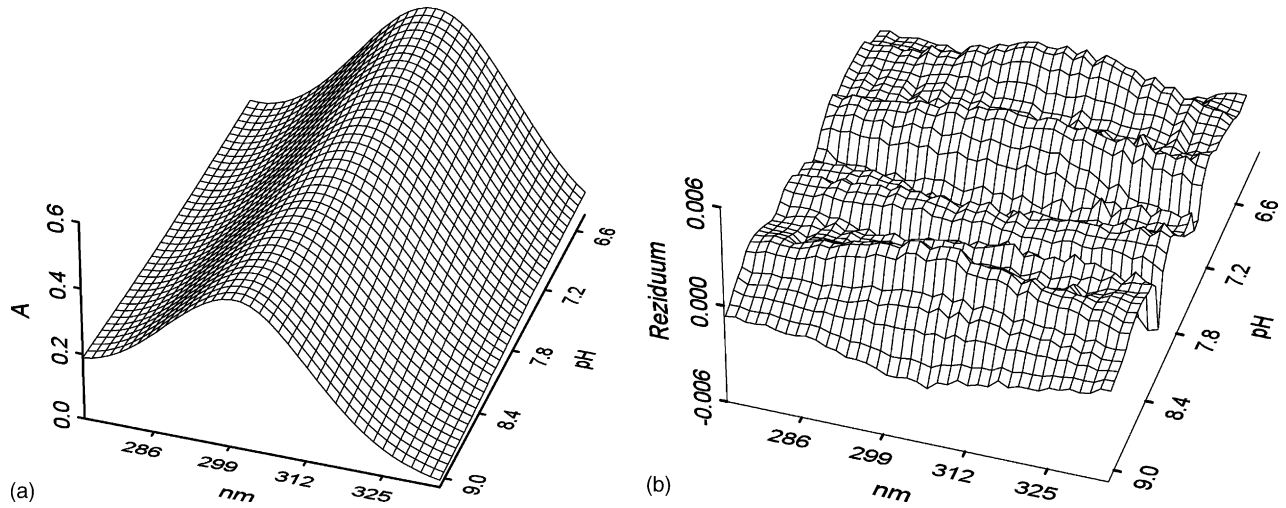


Fig. 1. Absorption spectra of the protonation equilibria of ambroxol in dependence on pH at 25 °C: (a) 3D-absorbance response-surface representing SQUAD(84) input data, (b) the 3D-overall diagram of residuals represents a response-surface indicating the quality of a goodness-of-fit.

problem of optimization in the parameter space in which the pK_a and I are known and given values while the parameters pK_a, $å$, and C are unknown variables to be estimated [8,19].

3.5. Reliability of estimated protonation/ 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. page 101 in Ref. [8] or may be found in a previous paper [19].

3.6. Software used

Computations were performed by regression analysis of UV/Vis spectra using the SQUAD(84) program [13]. The thermodynamic dissociation constant pK^T was estimated with the non-linear regression program MINOPT in the ADSTAT statistical system (TriloByte Statistical Software Ltd., Pardubice) [20].

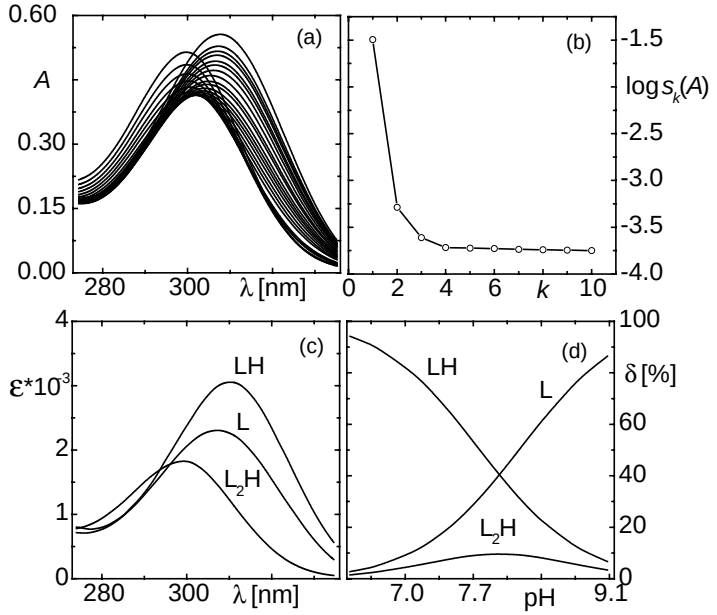


Fig. 2. Estimation of the protonation constants and molar absorptivities of ambroxol at 25 °C and $I = 0.006$: (a) scree plot for determination of the number of light-absorbing species in mixture $k^* = 3$ and the instrumental error of the spectrophotometer used $s_3^*(A) = 0.25$ mAU, (b) goodness-of-fit scatter plot: $s(e)$ and $|\bar{e}|$ bar line for each spectrum, (c) the spectra of molar absorptivities vs. wavelengths for all of the variously protonated species, (d) distribution diagram of the relative concentrations of all of the variously protonated species.

4. Results and discussion

4.1. Estimation of protonation/dissociation constants of five drugs

A proposed strategy for efficient experimentation in protonation constants determination followed by spectral data treatment is presented on the protonation equilibria of ambroxol. pH-spectrophotometric titration enables absorbance response-surface data (Fig. 1a) to be obtained for analysis with non-linear regression. The reliability of parameter estimates (pK's and ε's) may be evaluated on the basis of the goodnes-of-fit test of residuals (Fig. 1b). The SQUAD(84) program [13] analysis process starts with data smoothing followed by a factor analysis using the INDICES procedure [18]. The position of a break-point on the $s_k(A) = f(k)$ curve in the scree plot is calculated and gives $k^* = 3$ with the corresponding co-ordinate $s_3^*(A) = 0.25$ mAU which also represents the instrumental error $s_{\text{inst}}(A)$ of the spectrophotometer used (Fig. 2a). Two protonation constants and three molar absorptivities of ambroxol calculated for 39 wavelengths constitute 236 unknown parameters which are refined by the MR algorithm in the first run of the SQUAD program. In the second run, the NNLS algorithm makes the final refinement of all of the previously found parameter estimates with all molar absorptivities kept non-negative. The reliability of the parameter estimates may be tested with the use of SQUAD(84) 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 variously protonated species are statistically significant at significance level $\alpha = 0.05$.

The physical meaning of the protonation constants, molar absorptivities, and stoichiometric indices is examined: β_{qr} and ε_{qr} should be neither too high nor too low, and ε_{qr} should not be negative. The absolute values of $s(\beta_j)$, $s(\varepsilon_j)$ gives information about the last U-contour of the hyperparaboloid in 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. 2c shows the estimated molar absorptivities of all of the variously protonated species ε_L, ε_{LH}, ε_{L_2H} ambroxol in dependence on wavelength. Some spectra quite overlap and such cases may cause some resolution difficulties.

The second diagnostic tests whether all of the calculated free concentrations of variously protonated species on the distribution diagram of the relative concentration expressed in percents have physical meaning, which proved to be the case (Fig. 2d). 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 percents, a species present at about 1% relative concentration or less in an equilibrium behaves as a numerical noise in 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 that overlapping protonation equilibria of H with LH and L exist.

Table 1
Determination of protonation constants and molar absorptivities of the variously protonated species of ambroxol by regression analysis of the UV/Vis absorption spectra with SQUAD(84) for $n = 20$ spectra measured at $m = 39$ wavelengths for two basic components L and H forming $p = 3$ variously protonated species

Protonation constants			Partial correlation coefficients	
L_qH_r	$\log \beta_{qr}$	$s(\log \beta_{qr})$	L_1H_1	L_1H_2
L_1H_1	7.968	0.009	1	–
L_2H_1	11.34	0.017	0.6814	1
Determination of the number of light-absorbing species by factor analysis				
Number of light-absorbing species, p			3	
Residual standard deviation $s_3(A)$ (mAU)			0.25	
Goodnes-of-fit test by the statistical analysis of residuals				
Hamilton R-factor (%)			0.33	
Residual mean $\bar{e}$			-6.00×10^{-12}	
Mean residual $\lvert\bar{e}\rvert$ (mAU)			0.86	
Standard deviation of residuals $s(e)$ (mAU)			1.21	
Residual skewness $g_1(e)$			−0.12	
Residual kurtosis $g_2(e)$			2.64	

The charges of the ions are omitted for the sake of simplicity.

The third diagnostic concerning the matrix of correlation coefficients in Table 1 proves that there is an absence of interdependence in the pair of protonation constants LH and L_2H of ambroxol.

The fourth diagnostic concerns the goodness-of-fit (Fig. 1b). To identify the "best" or true chemical model when several are possible or proposed, and to establish whether or not the chemical model represents the data adequately, the residuals e should be analysed. The goodness-of-fit achieved is easily seen by examination of the differences between the experimental and calculated values of absorbance, $e_i = A_{\text{exp},i,j} - A_{\text{calc},i,j}$. 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 calculated is the standard deviation of absorbance, $s(A)$, calculated from a set of refined parameters at the termination of the minimization

Table 2
The search for a chemical equilibrium model of ambroxol using regression analysis of pH-spectrophotometric data with SQUAD(84), with the standard deviations of the parameter estimates in the last valid digits in brackets

q, r	Log β_{qr}	Log β_{qr}	Log β_{qr}	Log β_{qr}	Log β_{qr}
1, 0	–	–	–	–	–
1, 1	7.927 (10)	–	8.044 (9)	7.968 (9)	7.952 (14)
1, 2	–	15.773 (19)	9.000 (1927.778)	–	–
2, 1	–	–	–	11.340 (17)	–
2, 2	–	–	–	–	12.000 (***)
Degree-of-fit test by the statistical analysis of residuals					
R-factor (%)	1.84	3.18	1.13	0.33	0.70
$s(A)$ (mAU)	6.53	11.27	4.13	1.21	2.54
$s_k(A)$ (mAU), p	0.4, 2	0.52, 2	0.25, 3	0.25, 3	0.25, 3
$\bar{e}$	0.0048	0.0086	0.0028	0.0009	0.0018
$s(e)$	0.0065	0.0113	0.0041	0.0012	0.0025
$g_1(e)$	−0.87	−0.17	−0.66	−0.12	−0.34
$g_2(e)$	3.66	2.67	3.87	2.64	3.16
Model is	Rejected	Rejected	Rejected	Accepted	Rejected
1, 0	–	–	–	–	–
1, 1	7.598 (7)	8.179 (9)	7.900 (10)	7.936 (44)	7.061 (31)
2, 1	–	–	–	11.902 (42)	11.736 (46)
3, 1	14.316 (31)	–	–	7.000 (***)	–
3, 2	–	8.000 (***)	–	–	–
4, 2	–	–	18.500 (***)	–	18.500 (***)
Degree-of-fit test by the statistical analysis of residuals					
R-factor (%)	0.44	0.6	0.84	0.27	0.29
s(A) (mAU)	1.62	2.18	3.06	1.01	1.08
s_k(A) (mAU), p	0.25, 3	0.25, 3	0.25, 3	0.19, 4	0.19, 4
$\bar{e}$	0.0001	0.0015	0.0022	0.0007	0.0008
$s(e)$	0.0002	0.0022	0.0031	0.0010	0.0011
$g_1(e)$	−0.42	−0.29	−0.34	−0.06	−0.12
$g_2(e)$	2.50	3.14	2.63	2.43	1.98
Model is	Rejected	Rejected	Rejected	Accepted	Rejected

The reliability of parameter estimates found is proven with goodness-of-fit statistics such as the Hamilton *R*-factor (%), the residual standard deviation $s_k(A)$ (mAU) and the standard deviation of absorbance after termination of the regression prooess, $s(A)$ (mAU); (***) means that the estimate of the standard deviation is too large.

process. It is usually compared with the standard deviation of absorbance calculated by the INDICES program [18], $s_k(A)$, 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 2). This proves that the $s_3(A)$ value is equal to 0.25 mAU and is quite close to the standard deviation of absorbance when the minimization process terminates, $s(A) = 1.21$ mAU. Although this statistical analysis of residuals [22] gives the most rigorous test of the degree-of-fit, realistic empirical limits must be used. For example, when $s_{inst}(A) \leq s(A) \leq 0.003$, the goodness-of-fit is still taken as acceptable, whereas $s(A) > 0.010$ indicates that a good fit has not been obtained. Alternatively, the statistical measures of residuals e can be calculated: the residual mean (known as the bias) $\bar{e}$ should be a value close to zero; the mean residual $|\bar{e}|$ and the residual standard deviation $s(e)$ should be close to the absorbance standard deviation $s_{inst}(A)$; the skewness $g_1(e)$ should be close to zero for a symmetric distribution; the kurtosis $g_2(e)$ should be close to 3 for a Gaussian distribution; a Hamilton *R*-factor of relative fit, expressed as a percentage ($R \times 100\%$), of <0.5% is taken as an excellent fit, but >2% is a poor one. The statistical measures of all residuals e proves that the minimum of the eliptic hyperparaboloid U is reached (Table 2): the residual mean $\bar{e} = -6.00 \times 10^{-12}$ proves that there is no bias or systematic error in spectra fitting. The mean residual $|\bar{e}| = 0.86$ mAU and the residual standard deviation $s(e) = 1.21$ mAU have sufficiently low values. The skewness $g_1(e) = -0.12$ is quite close to zero and proves a symmetric distribution of the residuals set, while the kurtosis $g_2(e) = 2.64$ is close to 3 proving a Gaussian distribution. The Hamilton *R*-factor of relative fitness is 0.33%, proving an excellent achieved fitness, and therefore the parameter estimates may be considered as suitably reliable.

The fifth diagnostic, the spectra deconvolution in Fig. 3, shows the deconvolution of the experimental spectrum into spectra for the individual, variously protonated species. Spectrum deconvolution seems to be quite useful tool in the proposal of a strategy for efficient experimentation. Such a spectrum provides sufficient information for a regression analysis which monitors at least two species in equilibrium

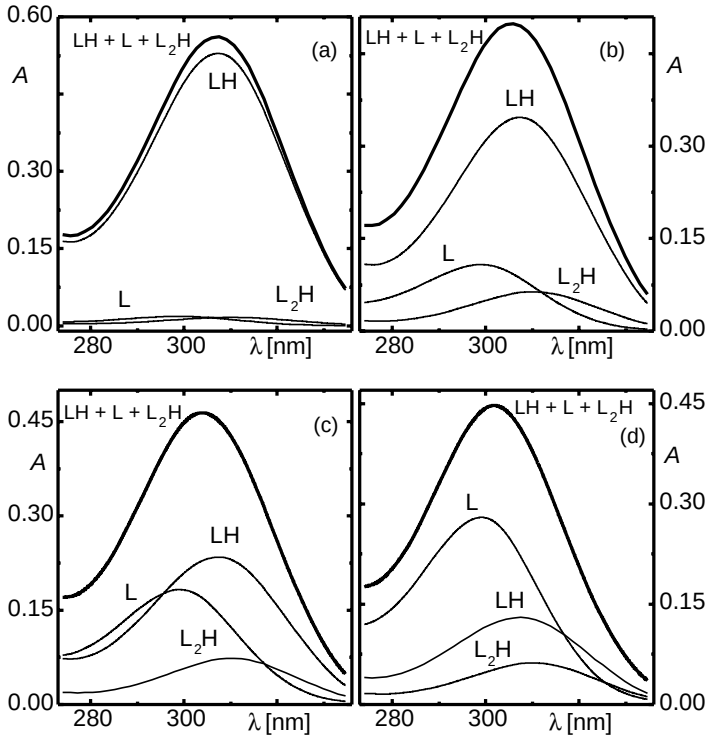


Fig. 3. Deconvolution of the experimental spectrum of ambroxol into spectra for the individual variously protonated species in solution for pH equal to: (a) 8.4, (b) 8.0, (c) 7.6, and (d) 6.6.

where none of them is a minor species. The minor species has a relative concentration in a distribution diagram of less than 5% of the total concentration of the basic component c_L. When, on the other hand, only one species is prevalent in solution, the spectrum yields quite poor information in a regression analysis, while the parameter estimate is rather unsure, and is definitely not reliable enough.

In searching for the best chemical model of protonation equilibria, 10 various hypotheses of the stoichiometric indices q and r of L_qH_r acid were tested in order to find that which best represented the data (Table 2). The criteria of resolution used for 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

Table 3
Dependence of the mixed dissociation constants of ambroxol on ionic strength using regression analysis of pH-spectrophotometric data with SQUAD(84), with the standard deviations of the parameter estimates in the last valid digits in brackets

Determined chemical model at 25 °C contains L, LH, L_2H						
Ionic strength	0.006	0.019	0.033	0.046	0.059	0.072
$\log \beta_{11}$	7.968 (9)	8.035 (15)	8.050 (9)	7.933 (9)	8.029 (11)	7.908 (15)
$\log \beta_{21}$	11.640 (17)	11.670 (38)	11.509 (21)	11.480 (18)	11.626 (27)	11.546 (27)
Goodness-of-fit test						
R-factor (%)	0.33	0.41	0.38	0.42	0.34	0.5
$s_k(A)$ (mAU)	0.25	0.29	0.25	0.23	0.23	0.23
$s(A)$ (mAU)	1.21	1.57	1.55	1.7	1.39	2.03
Determined chemical model at 37 °C contains L, LH, L_2H						
Ionic strength	0.006	0.019	0.033	0.046	0.059	0.072
$\log \beta_{11}$	8.194 (11)	8.184 (14)	8.160 (9)	8.192 (17)	8.190 (22)	8.200 (19)
$\log \beta_{21}$	11.860 (33)	11.917 (36)	11.850 (26)	12.018 (43)	11.830 (58)	12.004 (48)
Goodness-of-fit test						
R-factor (%)	0.56	0.54	0.47	0.69	0.51	0.54
$s_k(A)$ (mAU)	0.2	0.21	0.3	0.2	0.23	0.24
$s(A)$ (mAU)	1.87	1.98	1.92	2.73	2.02	2.10

The reliability of parameter estimates found is proven with goodness-of-fit statistics such as the Hamilton *R*-factor (%), the residual standard deviation $s_k(A)$ (mAU) and the standard deviation of absorbance after terminatiori of the regression process, $s(A)$ (mAU) at 25 °C (upper part) and 37 °C (lower part).

Table 4
Dependence of the mixed dissociation constants of antazoline on ionic strength using regression analysis of pH-spectrophotometric data with SQUAD(84) with the standard deviations of the parameter estimates in the last valid digits in brackets

Determined chemical model contains L, LH, LH2 at 25 °C									
Ionic strength	0.010	0.010	0.070	0.089	0.127	0.271	0.402	0.794	0.925
$pK_{a,1}$	9.778 (25)	9.721 (26)	9.516 (36)	9.535 (29)	9.478 (30)	9.559 (20)	9.459 (23)	9.275 (11)	9.297 (9)
Goodness-of-fit test									
R-actor (%)	0.19	0.25	0.34	0.33	0.29	0.25	0.35	0.24	0.25
$s_k(A)$ (mAU)	0.13	0.2	0.27	0.22	0.16	0.23	0.21	0.15	0.27
$s(A)$ (mAU)	0.64	0.81	1.22	1.13	1.03	0.90	1.25	0.91	0.90
Determined chemical model contains L, LH, LH_2 at 25 °C									
Ionic strength	0.010	0.030	0.071	0.141	0.271	0.402	0.663		
$pK_{a,2}$	7.694 (34)	7.626 (53)	7.546 (52)	7.530 (46)	7.524 (41)	7.449 (50)	7.460 (47)		
Goodness-of-fit test									
R-factor (%)	0.25	0.40	0.34	0.28	0.25	0.35	0.35		
$s_k(A)$ (mAU)	0.2	0.29	0.27	0.31	0.23	0.21	0.43		
$s(A)$ (mAU)	0.81	1.38	1.22	0.99	0.90	1.25	1.26		
Determined chemical model contains L, LH, LH_2 at 37 °C									
Ionic strength	0.010	0.170	0.206	0.411	0.467	0.491	0.571	0.598	
$pK_{a,1}$	9.532 (35)	9.315 (28)	9.298 (24)	9.206 (20)	9.211 (16)	9.206 (18)	9.188 (24)	9.178 (14)	
Goodness-of-fit test									
R-factor (%)	0.54	0.45	0.54	0.45	0.33	0.54	0.34	0.45	
$s_k(A)$ (mAU)	0.17	0.19	0.19	0.23	0.21	0.28	0.25	0.16	
$s(A)$ (mAU)	1.60	1.43	1.67	1.46	1.14	1.65	1.31	1.55	
Determined chemical model contains L, LH, LH_2 at 37 °C									
Ionic strength	0.010	0.170	0.337	0.467	0.571	0.598			
$pK_{a,2}$	7.679 (43)	7.361 (49)	7.051 (45)	7.000 (53)	7.003 (55)	7.058 (34)			
Goodness-of-fit test									
R-factor (%)	0.54	0.30	0.44	0.34	0.52	0.45			
$s_k(A)$ (mAU)	0.17	0.19	0.18	0.21	0.25	0.16			
$s(A)$ (mAU)	1.60	1.03	1.46	1.14	1.61	1.55			

The reliability of parameter estimates found is proven with goodness-of-fit statistics such as the Hamilton *R*-factor (%), the residual standard deviation $s_k(A)$ (mAU) and the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU) at 25 °C (upper part) and 37 °C (lower part).

Table 5
Dependence of the mixed dissociation constants of naphazoline on ionic strength using regression analysis of pH-spectrophotometric data with SQUAD(84), with the standard deviations of the parameter estimates in the last valid digits in brackets

Determined chemical model contains L, LH at 25 °C							
Ionic strength	0.009	0.026	0.038	0.050	0.062	0.074	0.086
$pK_{a,1}$	10.767 (5)	10.767 (6)	10.761 (7)	10.736 (6)	10.757 (9)	10.735 (9)	10.725 (5)
Goodness-of-fit test							
R-factor (%)	0.42	0.30	0.28	0.25	0.37	0.36	0.26
$s_k(A)$ (mAU)	0.28	0.31	0.17	0.24	0.42	0.43	0.19
$s(A)$ (mAU)	1.40	1.05	1.00	0.88	1.28	1.25	0.88
Determined chemical model contains L, LH at 37 °C							
Ionic strength	0.009	0.021	0.034	0.046	0.058	0.074	0.086
$pK_{0,1}$	10.582 (4)	10.569 (30)	10.553 (8)	10.551 (8)	10.540 (7)	10.544 (8)	10.522 (5)
Goodness-of-fit test							
R-factor (%)	0.37	0.37	0.37	0.35	0.27	0.34	0.34
$s_k(A)$ (mAU)	0.33	0.25	0.33	0.33	0.24	0.30	0.26
$s(A)$ (mAU)	1.22	1.40	1.31	1.24	0.97	1.20	1.14

The reliability of parameter estimates found is proven with goodness-of-fit statistics such as the Hamilton *R*-factor (%), the residual standard deviation $s_k(A)$ (mAU) and the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU) at 25 °C (upper part) and 37 °C (lower part).

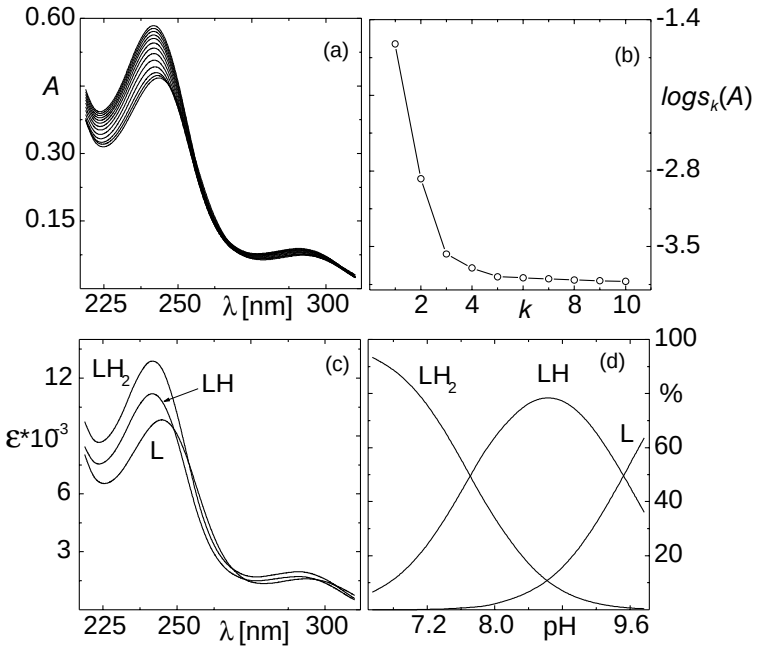


Fig. 4. Estimation of protonation constants and molar absorptivities of antazolin at 25 °C and ionic strength $I = 0.070$: (a) scree plot for determination of the number of light-absorbing species in mixture $k^* = 3$ and $s_3^*(A) = 0.27$ mAU, (b) goodness-of-fit scatter plot: $s(e)$ and $|\bar{e}|$ bar line for each spectrum, (c) the spectra of molar absorptivities vs. wavelengths for all of the variously protonated species, (d) distribution diagram of the relative concentrations of all of the variously protonated species.

parameters if they were both realistic and positive; and (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 parameter estimates were unsatisfactory.

Using the experimental and evaluation strategy, the protonation equilibria of ambroxol (Table 3 and Figs. 1–3), antazoline (Table 4 and Fig. 4), naphazoline (Table 5 and Fig. 5), oxymethazoline (Table 6 and Fig. 6) and ranitidine (Table 7 and Fig. 7) were also examined. To test the

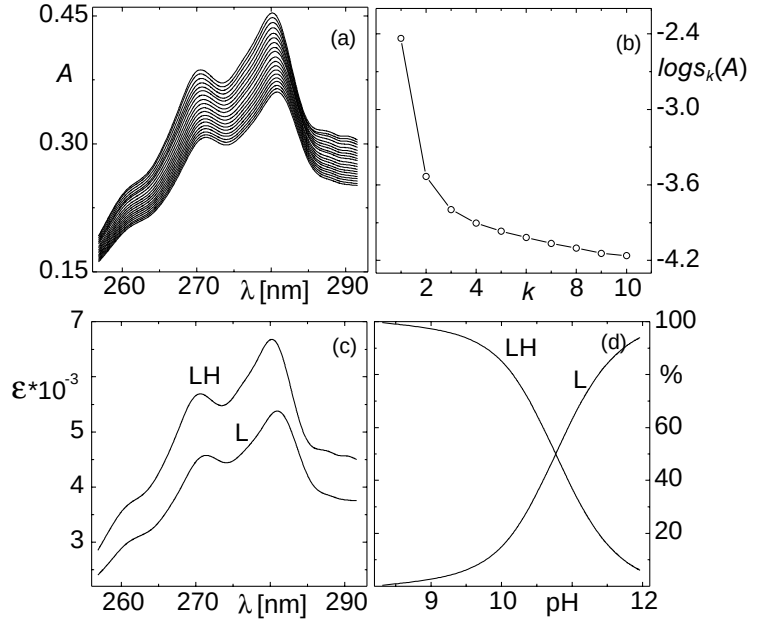


Fig. 5. Estimation of the protonation constants and molar absorptivities of naphazoline at 25 °C and ionic strength $I = 0.009$: (a) scree plot for determination of the number of light-absorbing species in mixture $k^* = 2$ and $s_2^*(A) = 0.28$ mAU, (b) goodness-of-fit scatter diagram plots $s(e)$ and $|\bar{e}|$ bar line for each spectrum, (c) the spectra of molar absorptivities vs. wavelengths for all of the variously protonated species, (d) distribution diagram of the relative concentrations of all of the variously protonated species.

Table 6
Dependence of the mixed dissociation constants of oxymetazoline on ionic strength using regression analysis of pH-spectrophotometric data with SQUAD(84), with the standard deviations of the parameter estimates in the last valid digits in brackets

Determined chemical model contains L, LH, LH_2 at 25 °C					
Ionic strength	0.009	0.023	0.079	0.135	0.228
$pK_{a,2}$	11.998 (9)	11.984 (7)	11.806 (6)	11.787 (6)	11.759 (9)
$pK_{a,1}$	10.623 (16)	10.595 (13)	10.408 (14)	10.549 (13)	10.577 (17)
Goodness-of-fit test					
R-factor (%)	0.19	0.24	0.25	0.24	0.12
$s_k(A)$ (mAU)	0.13	0.22	0.15	0.17	0.14
$s(A)$ (mAU)	0.84	0.89	0.89	0.72	0.4
Determined chemical model contains L, LH, LH_2 at 37 °C					
Ionic strength	0.012	0.066	0.108	0.176	0.262
$pK_{a,2}$	11.784 (29)	11.740 (23)	11.564 (16)	11.637 (37)	11.665 (32)
$pK_{a,1}$	10.526 (76)	10.457 (77)	10.133 (77)	10.076 (30)	9.799 (83)
Goodness-of-fit test					
R-factor (%)	0.48	0.65	0.48	0.51	0.38
$s_k(A)$ (mAU)	0.11	0.12	0.12	0.15	0.16
$s(A)$ (mAU)	1.56	1.2	1.13	1.31	1.07

The reliability of parameter estimates found is proven with goodness-of-fit statistics such as the Hamilton *R*-factor (%), the residual standard deviation $s_k(A)$ (mAU) and the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU) at 25 °C (upper part) and 37 °C (lower part).

reliability of protonation/dissociation constants at different ionic strength the goodness-of-fit test with the use of statistical analysis of the residuals was applied, and results appear in Tables 3–7. For all five drugs studied the most efficient tools, such as the Hamilton *R*-factor, the mean residual and the standard deviation of residuals were applied: as the *R*-factor in all cases reaches a value of less then 0.5% an excellent fitness and reliable parameter estimates are indicated. The standard deviation of absorbance $s(A)$ after termination of the minimization process is always better than 2 mAU, and the proposal of a good chemical model and reliable parameter estimates are proven.

Another problem concerns small differences of molar absorptivities in the variously protonated species within a spectrum (Figs. 2c, 4c, 5c, 6c and 7c). It may happen that non-linear regression fails when small differences of absorbance are of the same magnitude as the instrumental noise, $s_{inst}(A)$.

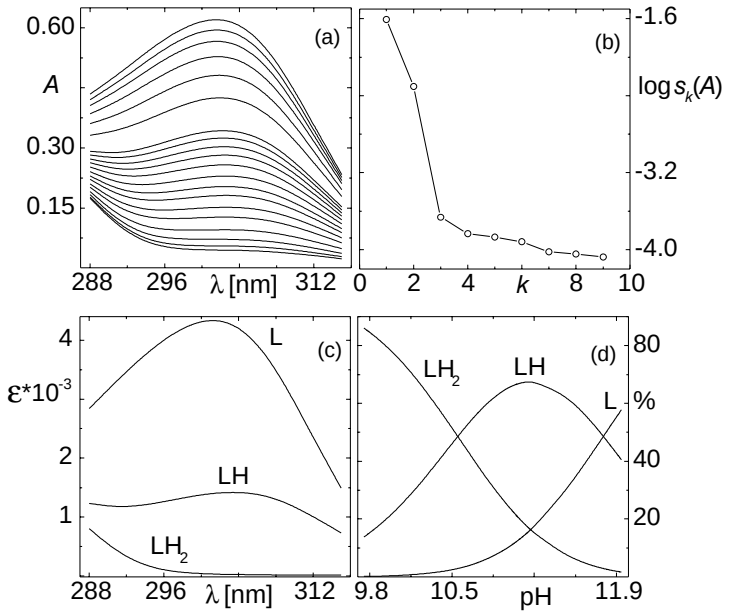


Fig. 6. Estimation of the protonation constants and molar absorptivities of oxymethazoline at 25 °C and $I = 0.023$: (a) scree plot for determination of the number of light-absorbing species in mixture $k^* = 3$ and $s_3^*(A) = 0.22$ mAU, (b) goodness-of-fit scatter plot: $s(e)$ and $|\bar{e}|$ bar line for each spectrum, (c) the spectra of molar absorptivities vs. wavelengths for all of the variously protonated species, (d) the distribution diagram of the relative concentrations of all of the variously protonated species.

Table 7
Dependence of the mixed dissociation constants of ranitidine on ionic strength using regression analysis of pH-spectrophotometric data with SQUAD(84), with the standard deviations of the parameter estimates in the last valid digits in brackets

Determined chemical model contains L, LH at 25 °C							
Ionic strength	0.009	0.021	0.079	0.116	0.195	0.292	0.456
$pK_{a,1}$	1.961 (1)	1.933 (2)	2.020 (1)	2.089 (1)	2.115 (1)	2.160 (1)	2.231 (1)
Goodness-of-fit test							
R-factor (%)	0.31	0.12	0.2	0.21	0.13	0.12	0.16
$s_k(A)$ (mAU)	0.12	0.11	0.14	0.13	0.15	0.13	0.13
$s(A)$ (mAU)	1.40	0.51	0.77	0.81	0.50	0.46	0.57
Determined chemical model contains L, LH at 37 °C							
Ionic strength	0.015	0.057	0.112	0.232	0.298	0.379	0.486
$pK_{a,1}$	1.828 (1)	1.889 (1)	2.030 (1)	2.002 (1)	2.044 (1)	2.064 (1)	2.114 (1)
Goodness-of-fit test							
R-factor (%)	0.43	0.17	0.32	0.21	0.24	0.41	0.12
$s_k(A)$ (mAU)	0.12	0.16	0.13	0.16	0.12	0.13	0.11
$s(A)$ (mAU)	1.59	0.74	1.14	1.26	0.82	1.33	0.21

The reliability of parameter estimates found is proven with goodness-of-fit statistics such as the Hamilton *R*-factor (%), the residual standard deviation $s_k(A)$ (mAU) and the standard deviation of absorbance after termination of the regression process, $s(A)$ (mAU) at 25 °C (upper part) and 37 °C (lower part).

Surprisingly, at the pH of the typical absorption conditions of ambroxol, pH = 7.7, almost one-third of the drug exists in the form of dimer. As the molecular weight of ambroxol is 378.11, its dimer already exceeds the (approximate) limit for molecular weight above which the size of molecule does play a role in absorption (ca. $M_r \geq 500$).

Naphazoline and antazoline are weak bases which are less protonated and thus, in theory, should be better absorbed at the pH of tears (pH ≈ 7.4) than of nasal mucosa (pH ≈ 5.5). On the other hand, from the stability point of view, the higher is the pH of the liquid preparation, the more susceptible are both drugs to hydrolysis. This is particularly true in the case of antazolin, which undergoes hydrolysis to *N*-benzylanilinoacetylethylene diamine in aqueous formulations. As the absorption of antazoline like most H_1—antagonists is generally good, antazoline should be formulated in a preparation whose pH compromises the best stability with the best and most comfortable perception after application. In general, for weak bases, the best stability can be expected at pH equal to or less than the half value of p*K*. In the case of antazoline, it would be a pH ranging from 3.9 to 4.8, which is too low with respect to the site of application

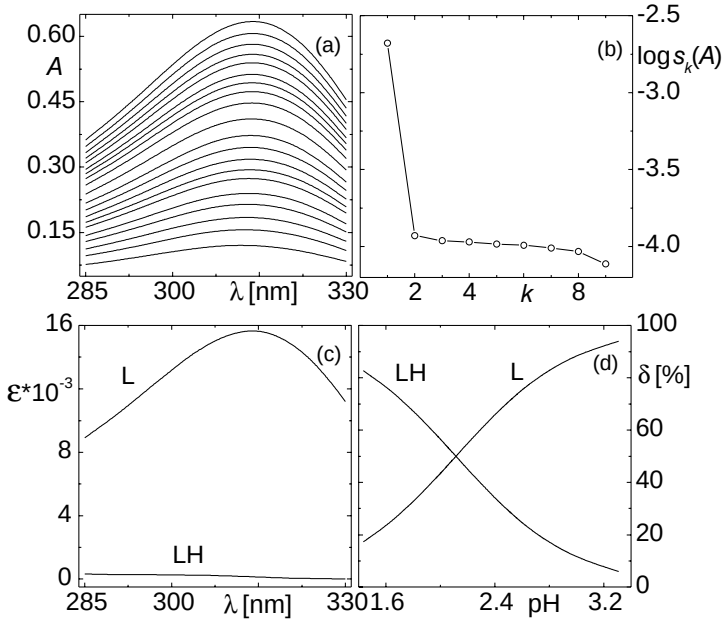


Fig. 7. Estimation of the protonation constants and molar absorptivities of ranitidin at 25 °C and $I = 0.009$: (a) the scree plot for determination of the number of light-absorbing species in mixture $k^* = 2$ and $s_2^*(A) = 0.12$ mAU, (b) the goodness-of-fit scatter plot: $s(e)$ and $|\bar{e}|$ bar line for each spectrum, (c) the spectra of molar absorptivities vs. wavelengths for all of the variously protonated species, (d) the distribution diagram of the relative concentrations of all of the variously protonated species.

Table 8
Thermodynamic dissociation constants for ambroxol, antazoline, naphazoline, oxymetazoline and ranitidine at two selected temperatures

		Value at 25 °C	Value at 37 °C
Ambroxol	$pK^{T}_{a,1}$	8.05 (6)	8.25 (4)
	$\log \beta^{T}_{21}$	11.67 (6)	11.83 (8)
Antazoline	$pK^{T}_{a,1}$	7.79 (2)	7.83 (6)
	$pK^{T}_{a,2}$	9.74 (3)	9.55 (2)
Naphazoline	pK^{T}_{a}	10.81 (1)	10.63 (1)
Oxymethazoline	$pK^{T}_{a,1}$	10.62 (2)	10.77 (7)
	$pK^{T}_{a,2}$	12.03 (3)	11.82 (4)
Ranitidine	$pK^{T}_{a,1}$	1.89 (1)	1.77 (1)

considered. Nevertheless, from the graph of occurrence of different protonated forms of antazoline at different pH, it can be stated that an anthazoline formulation should have pH under 6.4–6.8, where double-protonated species of antazoline dominate. The dissociation constant of antazoline was found electrochemically [21] $pK_a = 10.10$ at 25 °C.

The unknown parameter pK^{T}_{a} was estimated by applying a Debye–Hückel equation to the data in Tables 3–7 according to the regression criterion, Table 8 shows point estimates of the thermodynamic dissociation constants of five drugs at two temperatures. Because of the narrow range of ionic strengths the ion-size parameter *å* and the salting-out coefficient *C* could not be estimated.

5. Conclusions

When drugs are poorly soluble then instead of a potentiometric determination of dissociation constants, pH-spectrophotometric titration may be used with the non-linear regression of the absorbance response-surface data. The reliability of the dissociation constants of five drug acids (i.e. ambroxol, antazoline, naphazoline, oxymetazoline and ranitidine) 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.

Acknowledgements

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