

# Reliability of protonation constants and chemical model of drugs using SQUAD(84) and SPECFIT/32 regression analysis of pH-UV/VIS spectra

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The resolving power of multicomponent spectral analysis and the computation reliability of the stability constants and molar absorptivities determined for five variously protonated anions of physostigmine salicylate by the SQUAD(84) and SPECFIT/32 programs has been examined with the use of simulated and experimental spectra containing overlapping spectral bands. The reliability of the dissociation constants of drug were proven with goodness-of-fit tests and by examining the influence of pre-selected noise level  $s_{\text{inst}}(A)$  in synthetic spectra regarding the precision  $s(pK)$  and also accuracy of the estimated dissociation constants. Precision was examined as the linear regression model  $s(pK) = \beta_0 + \beta_1 s_{\text{inst}}(A)$ . In all cases the intercept  $\beta_0$  was statistically insignificant. When an instrumental error  $s_{\text{inst}}(A)$  is small and less than 0.5 mAU, the parameters' estimates are nearly the same as the bias  $\Delta pK = pK_{a,\text{calc}} - pK_{a,\text{true}}$  is quite negligible. In all four dissociation constants the bias seems to be quite small even though for  $pK_{a4}$  it is a little bit higher, *i.e.* +0.05 for  $s_{\text{inst}}(A)$  about 1.0 mAU. In the interval of  $s_{\text{inst}}(A)$  from 0.1 to 1.0 mAU all four dissociation constants  $pK_i$  are accurate enough. Of the various regression diagnostics considered, the goodness-of-fit is the most efficient criterion of whether the parameters found adequately represent the data. The magnitude of instrumental error  $s_{\text{inst}}(A)$  only slightly affects the shape of a Cattell's scree graph  $s_k(A) = f(k)$  to determine the true number of light-absorbing species in the equilibrium mixture.

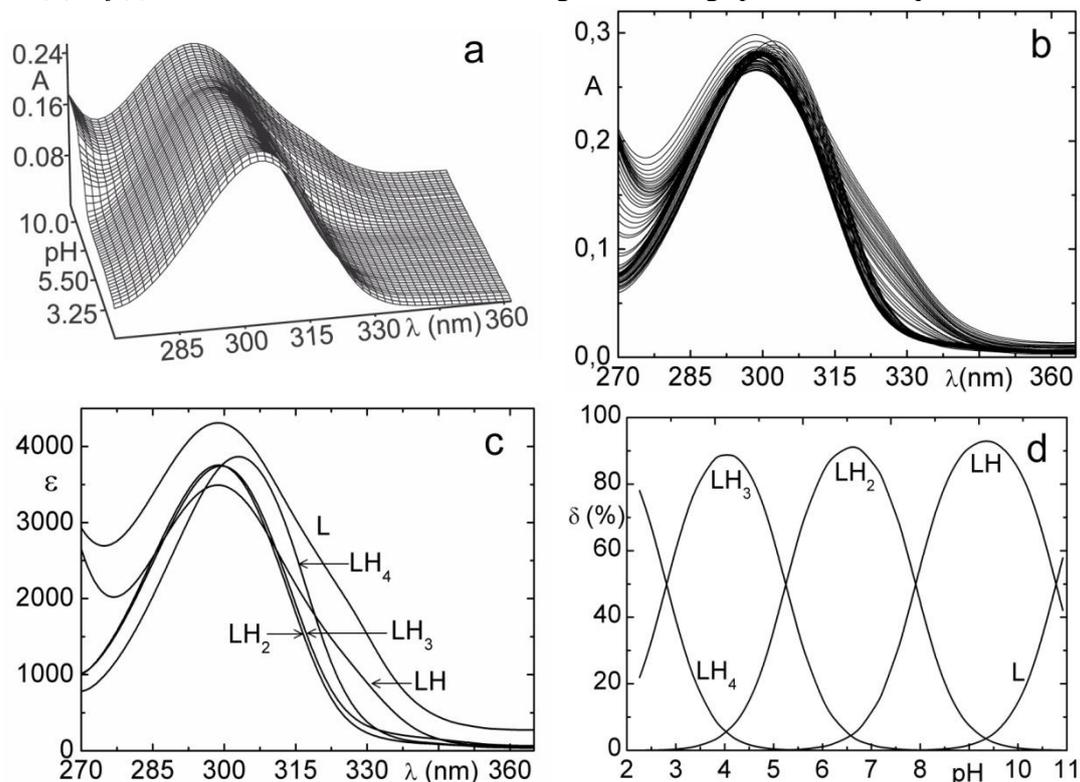


Fig. 1 (a) The 3D-absorbance-response-surface representing the measured multiwavelength absorption spectra of protonation equilibria for physostigmine salicylate depending on pH at 25°C, (b) Absorption spectra of  $7 \times 10^{-5}$  M physostigmine depending on pH at 25°C; (c) pure spectra profiles of molar absorptivities vs. wavelengths for the variously protonated species L, HL, H<sub>2</sub>L, H<sub>3</sub>L and H<sub>4</sub>L; (d) distribution diagram of the relative concentrations of all variously protonated species L, HL, H<sub>2</sub>L, H<sub>3</sub>L and H<sub>4</sub>L of physostigmine depending on pH at 25°C, (SPECFIT/32, ORIGIN). The charges of species are omitted for the sake of simplicity.

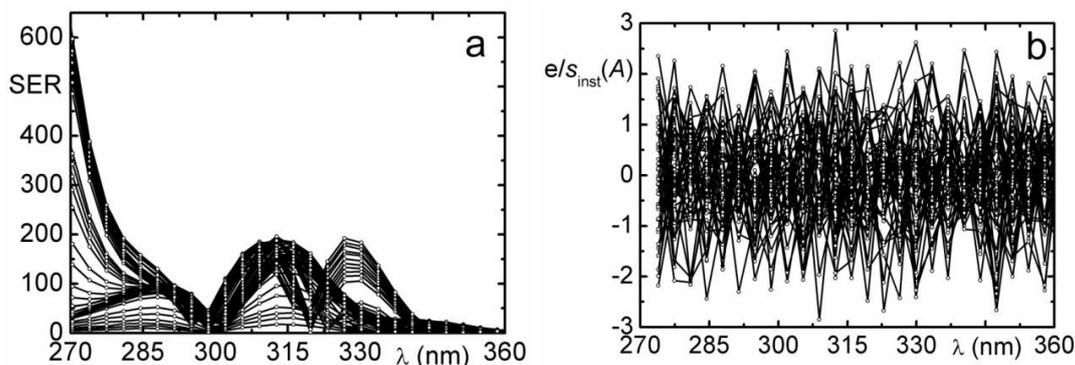


Fig. 2 (a) The plot of small absorbance changes in the spectrum of physostigmine salicylate means that the value of the absorbance difference for the  $j$ th-wavelength of the  $i$ th-spectrum  $\Delta_{ij} = A_{ij} - A_{i,\text{acid}}$  is divided by the instrumental standard deviation  $s_{\text{inst}}(A)$ , and the resulting ratios  $SER = \Delta/s_{\text{inst}}(A)$  are plotted in dependence of wavelength  $\lambda$  for all absorbance matrix elements, where  $A_{i,\text{acid}}$  is the limiting spectrum of the acid form of the drug measured. This ratio is compared with the limiting  $SER$  value for the physostigmine salicylate to test if the absorbance changes are significantly larger than the instrumental noise. (b) The plot of the ratio  $e/s_{\text{inst}}(A)$ , *i. e.* the ratio of the residuals divided by the instrumental standard deviation  $s_{\text{inst}}(A)$  depending on wavelength  $\lambda$  for all the residual matrix elements for physostigmine salicylate tests if the residuals are of the same magnitude as the instrumental noise, (SPECFIT/32, ORIGIN).

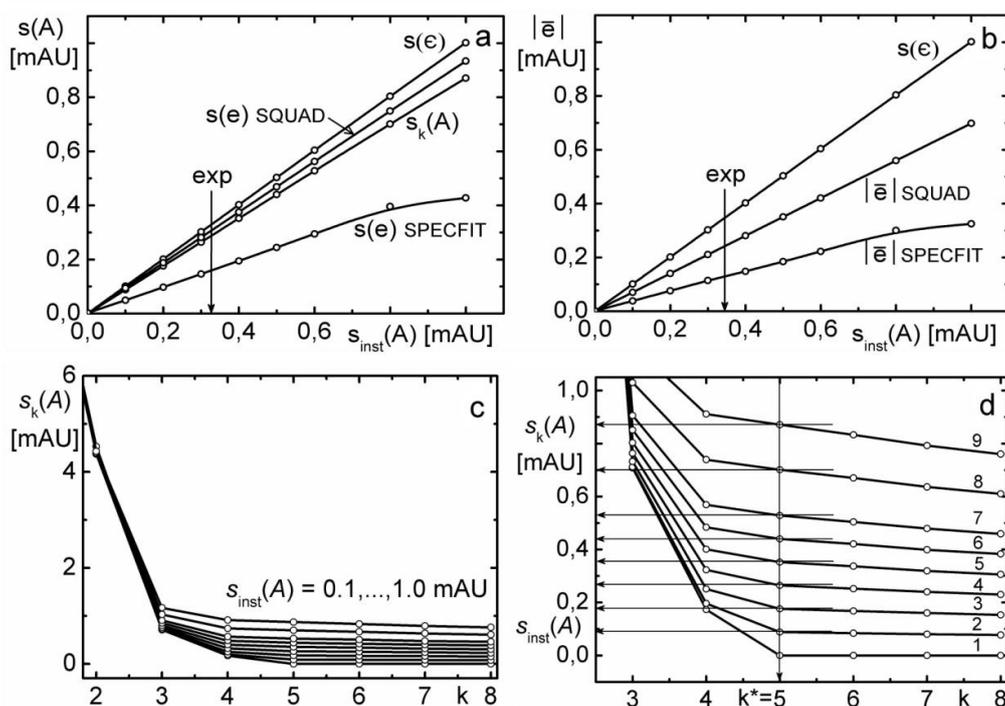


Fig. 3 The influence of the instrumental error  $s_{\text{inst}}(A)$  on a goodness-of-fit represented with the statistical analysis of random errors  $\epsilon$  and residuals  $e$  with the use of (a) standard deviation of random errors  $s(\epsilon)$  and of residuals  $s(e)$ , (b) the mean deviation  $|\bar{\epsilon}|$  and residuals  $|\bar{e}|$ , (c) The Cattel's scree plot of the residual standard deviation of absorbance  $s_k(A)$  depending on the number of the light-absorbing species in physostigmine salicylate equilibria mixture for nine various levels of instrumental noise (d). The detail view on the Cattel's scree plot enabling an evaluation from simulated spectra of the actual instrumental standard deviation  $s_{\text{inst}}(A)$  for five components  $k^* = 5$ , (ORIGIN).

## References:

- [1] M. Meloun, J. Havel, E. Högfeltd, Computation of solution equilibria : a guide to methods in potentiometry, extraction, and spectrophotometry, Ellis Horwood Chichester, England, 1988.
- [2] M. Meloun, Z. Ferenciková, M. Javůrek: Reliability of dissociation constants and resolution capability of SQUAD(84) and SPECFIT/32 in the regression of multiwavelength spectrophotometric pH-titration data, Spectrochimica Acta Part A 86 (2012) 305– 314.