A Search for the Protonation Model with Thermodynamic Dissociation Constants and (Extra)-Thermodynamics of the Nilotinib Hydrochloride (TASIGNA)

*Milan Meloun[1], Lucie Pilařová[1], Milan Javůrek[2] and Tomáš Pekárek[3]

[1]Department of Analytical Chemistry, University of Pardubice, CZ 532 10 Pardubice, Czechia,
[2]Department of Process Control, , University of Pardubice, CZ 532 10 Pardubice, Czechia,
[3]Zentiva k.s., U Kabelovny 130, CZ 102 37 Prague, Czechia,

***Corresponding author:** milan.meloun@upce.cz, **Phone:** +420466037026, **Fax:** +420466037068,

Abstract

Nilotinib hydrochloride (AMN107, TASIGNA, Novartis) treats adults with chronic myeloid leukemia CML, white blood cell cancer. It is a novel, orally active BCR-ABL tyrosine kinase inhibitor derived from aminopyrimidine more effective against CML cells than in Imatinib.

The nonlinear regression of the *A*-pH spectra with REACTLAB and SQUAD84 and of the pH-titration curve with ESAB determined four close pK_a in 11 steps of the proposed procedure. Prediction by MARVIN, PALLAS and ACD/Percepta shows the protonation sites.

A sparingly soluble Nilotinib forms LH^+, LH_2^{2+}, LH_3^{3+}, $LH_4^{4+}$with four pK^T_a:

(UV-metric spectra) pK^T_{a1} = 3.60±0.04, pK^T_{a2} = 4.42±0.07, pK^T_{a3} = 4.71±0.04, pK^T_{a4} = 4.84±0.03 at 25°C and pK^T_{a1} = 3.61±0.11, pK^T_{a2} = 4.29±0.18, pK^T_{a3} = 4.49±0.02, pK^T_{a4} = 5.05±0.03 at 37°C.

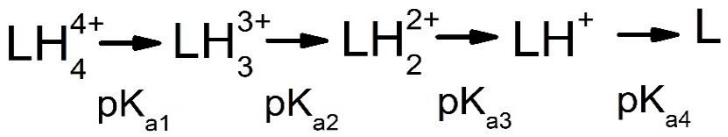
(pH-metric titration) pK^T_{a1} = 3.74±0.01, pK^T_{a2} = 4.05±0.01, pK^T_{a3} = 4.25±0.01, pK^T_{a4} = 4.91±0.20 at 25°C and pK^T_{a1} = 3.63±0.03, pK^T_{a2} = 3.96±0.03, pK^T_{a3} = 4.18±0.03, pK^T_{a4} = 4.81±0.05 at 37°C.

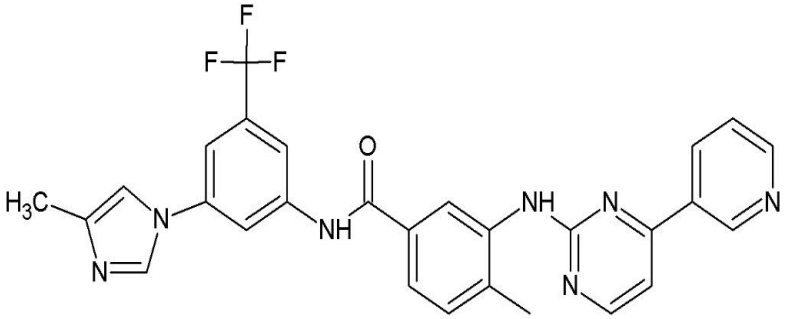
Positive enthalpy values ΔH^0 at 25°C show the dissociation process is endothermic and is accompanied by absorption of heat.

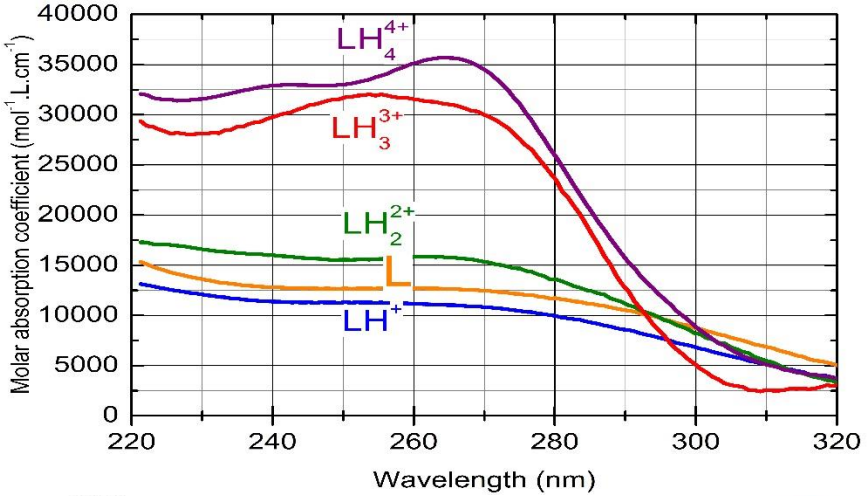
Positive value of the Gibbs free energy ΔG^0 at 25°C and 37°C indicated that the dissociation process was not proceeded spontaneously.

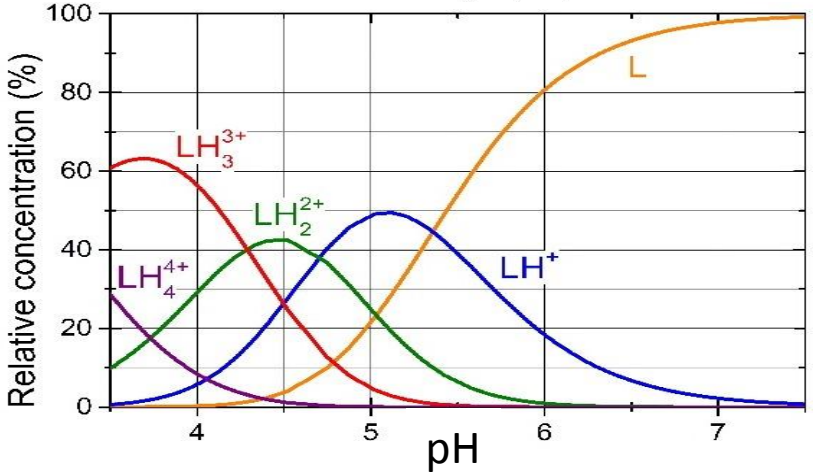
Negative entropy value of dissociation process ΔS^0 at 25°C and 37°C indicated the dissociation process is reversible.

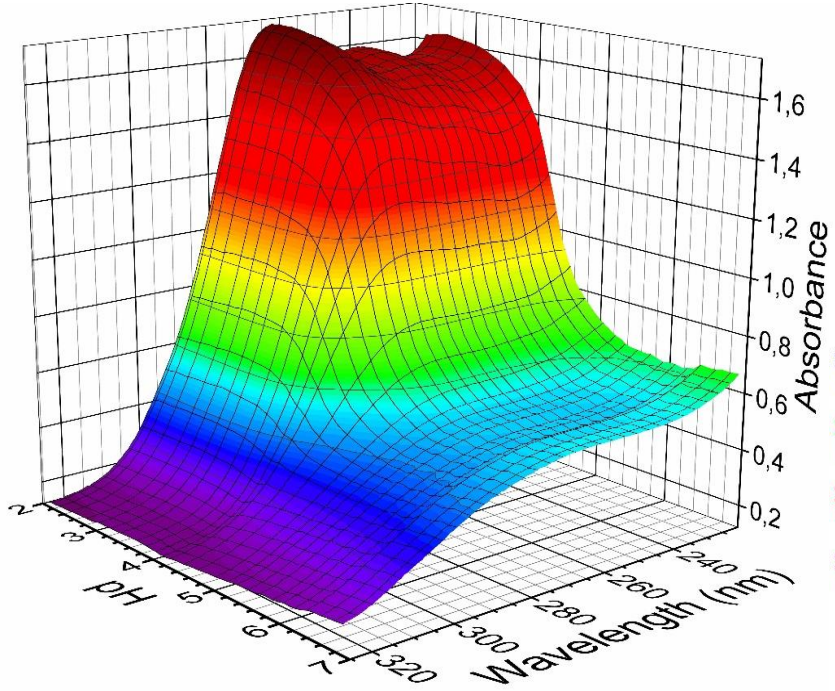
Nilotinib is BCR-ABL tyrosine kinase inhibitor treats adults with chronic myeloid leukemia, a type of white blood cell cancer

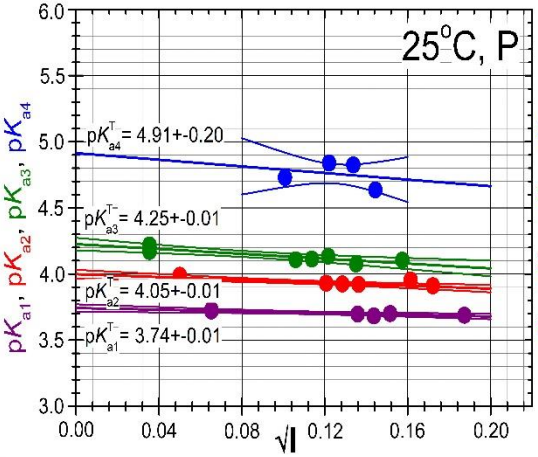


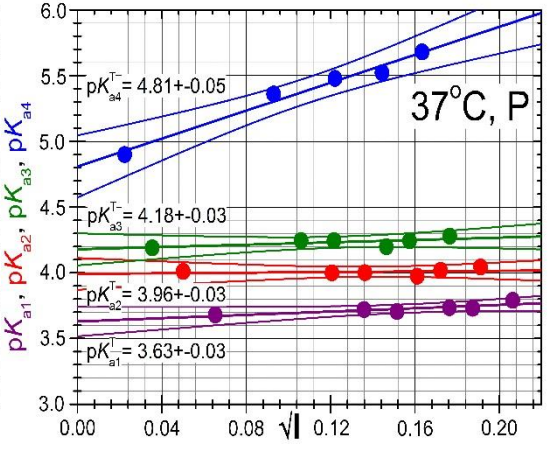








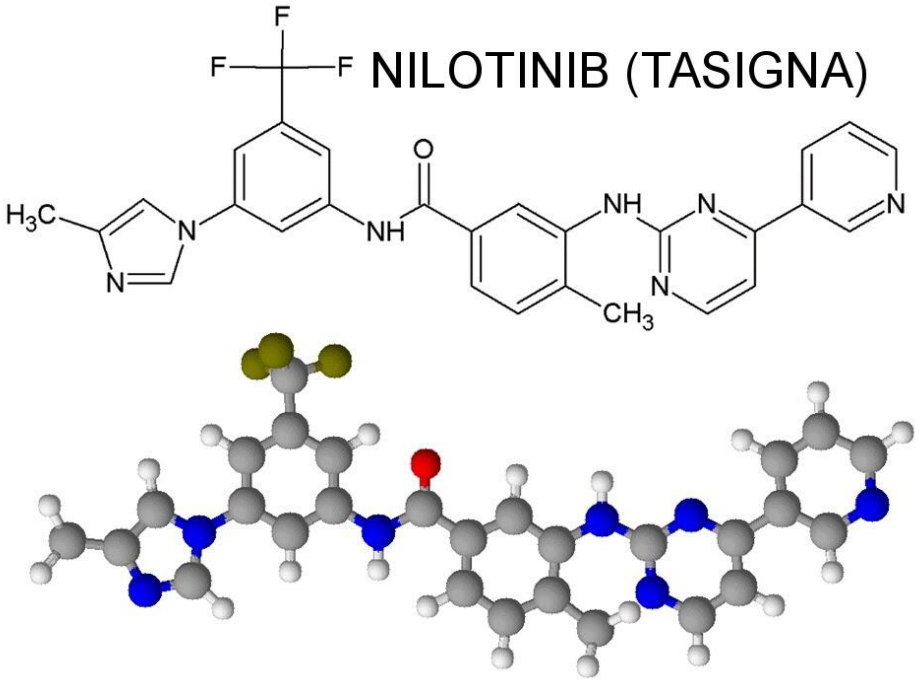


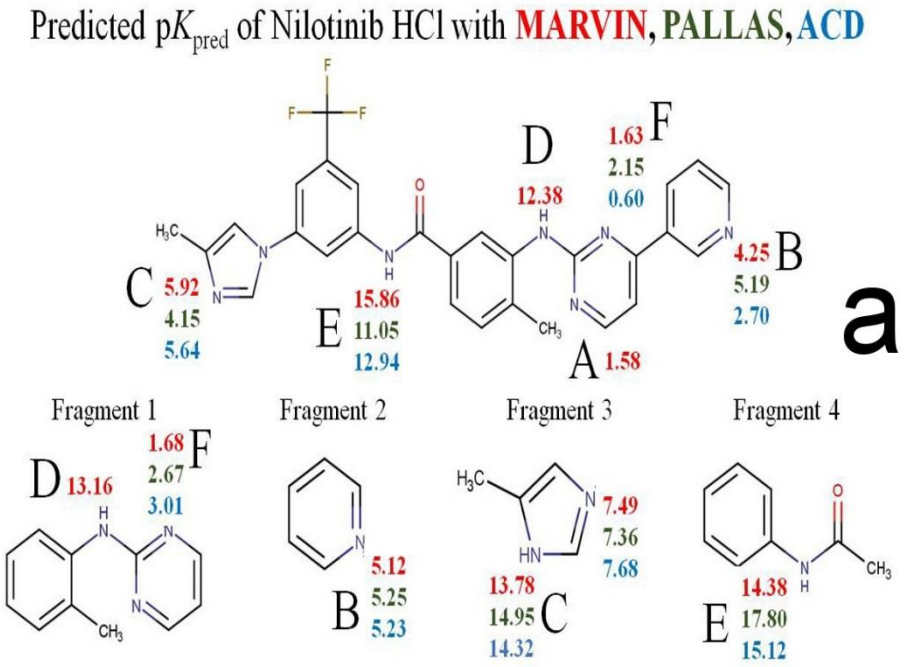


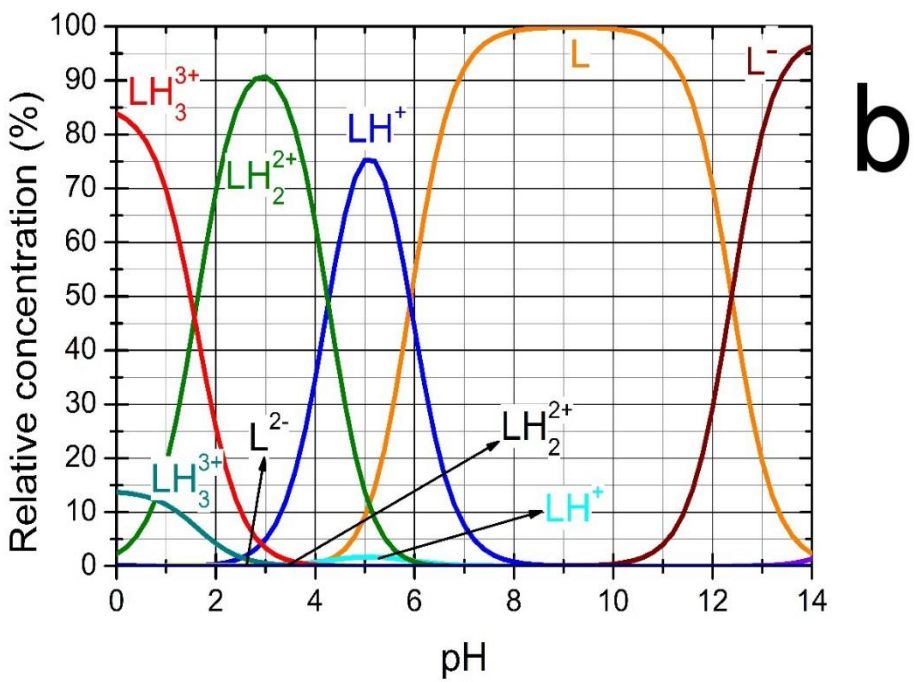
Prediction of pK_a

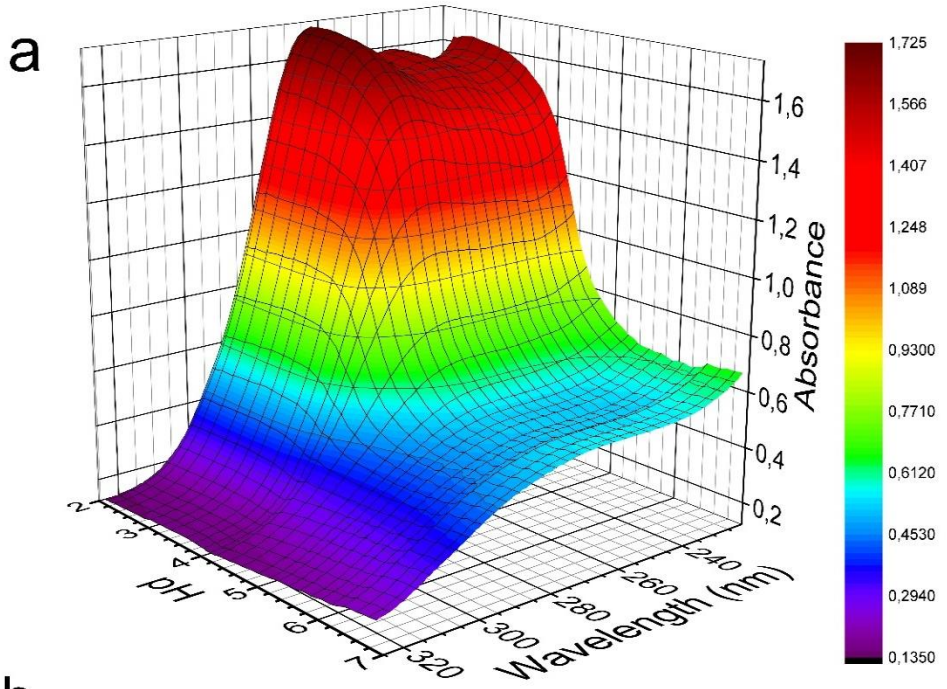
Graph a: Molecular structure of Nilotinib hydrochloride (insert) with highlighted protonation centers A, B, C, D, E and F, and predicted pK_a values using programs MARVIN, PALLAS and ACD/Percepta. Structure of auxiliary fragments 1 - 4 and their predicted pK_a.

Graph b: The distribution diagram of the relative concentrations [%] of variously protonated ions of Nilotinib hydrochloride for predicted dissociation constants.



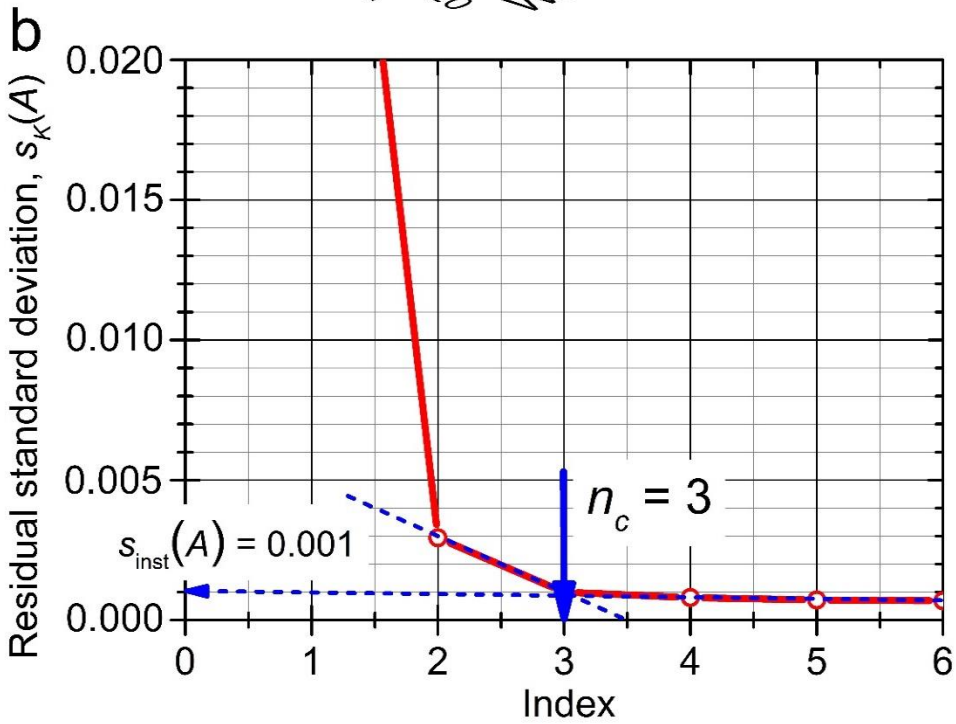






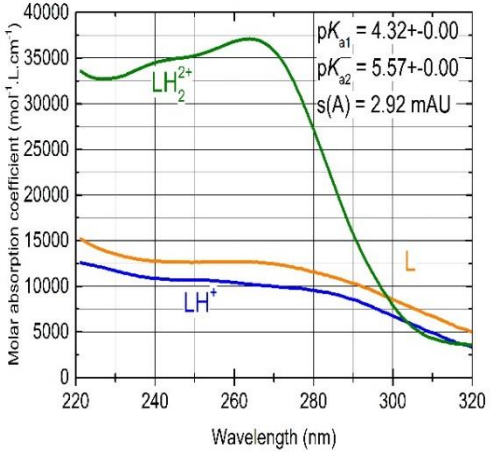
The absorbance responce surface

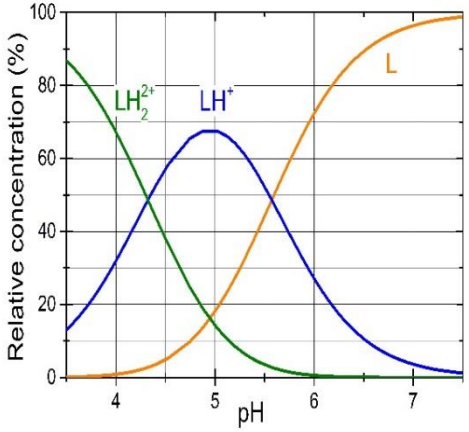
Graph a: The absorbance responce surface of Nilotinib hydrochloride in dependence on pH.

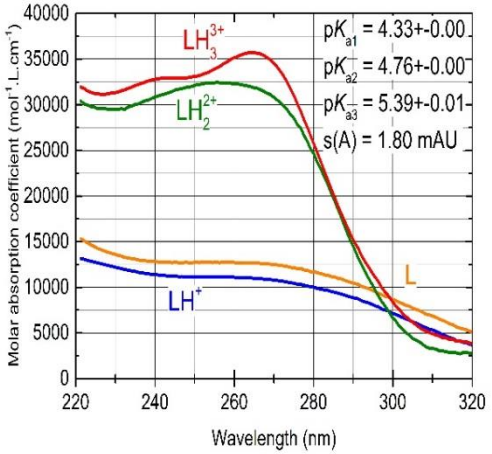


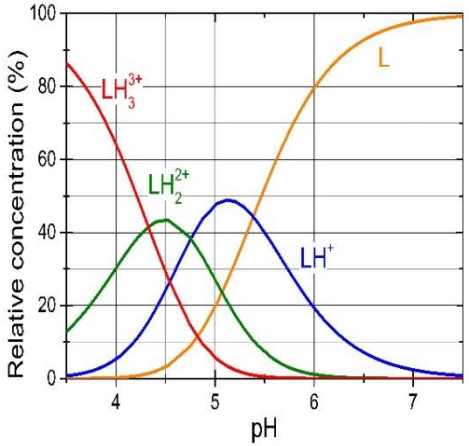
Number of species with FA

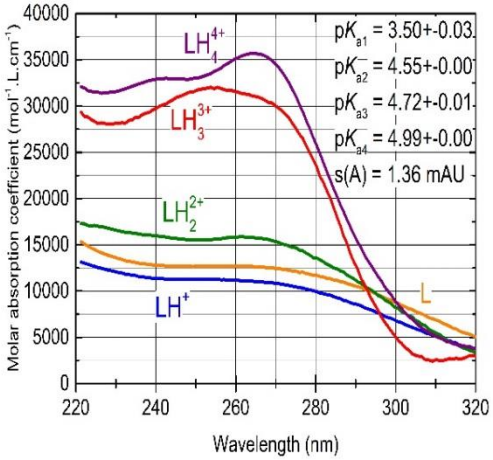
Graph b: Using Cattel index graph with the residual standard deviation $s_k(A)$, the rank of the absorbance matrix is $k^* = 3$ for Nilotinib hydrochloride or the number of species is equal to $n_c = 3$. (INDICES in S-PLUS), [42].

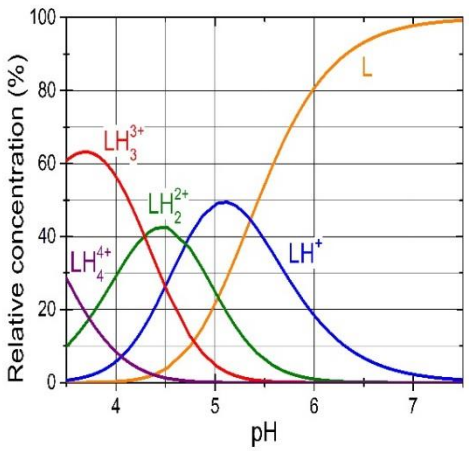












The search in protonation model building and testing

Building and testing of the best protonation model of Nilotinib hydrochloride in the pH range of 2 to 7

for two pK$_a$´s (***Upper 2 graphs***),

for three pK$_a$´s (***Middle 2 graphs***),

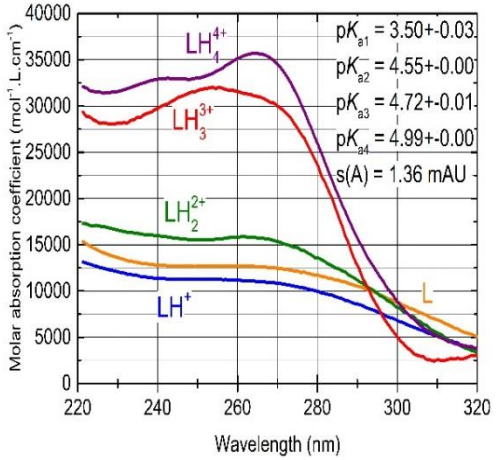
for four pK$_a$´s (***Lower 2 graphs***).

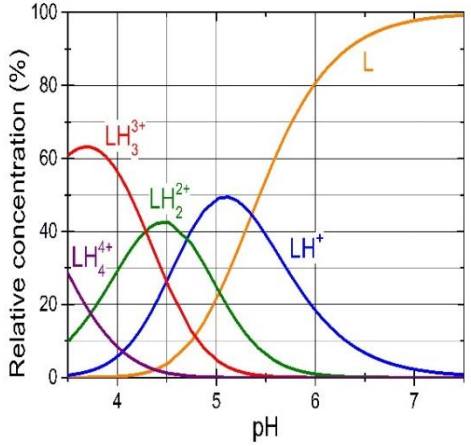
The spectra analysis of 1.0×10^{-4} mol. dm^{-3} Nilotinib hydrochloride at I = 0.0026 and 25°C.

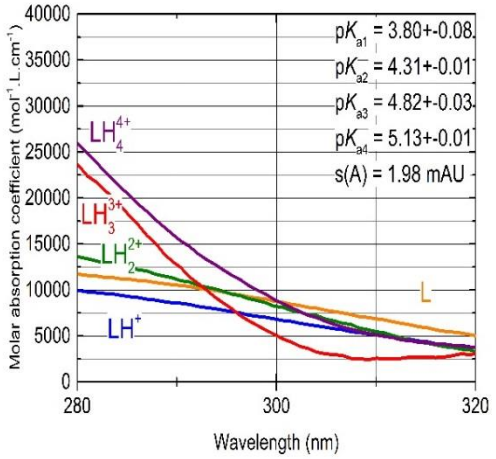
Left graphs: Pure spectral profiles of molar absorption coefficients versus wavelength (nm) for all the variously protonated ions of Nilotinib hydrochloride.

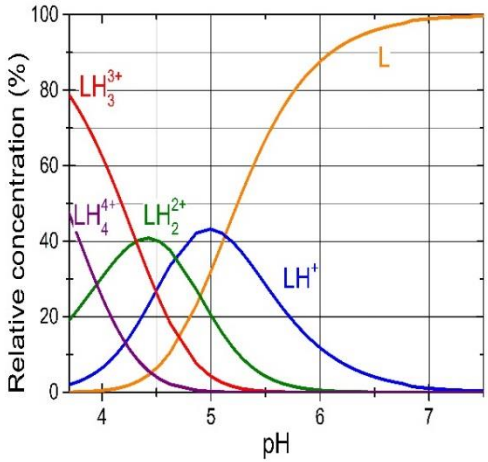
Right graphs: The distribution diagram of the relative concentrations of all of the variously protonated species in dependence on pH, (REACTLAB, ORIGIN 9).

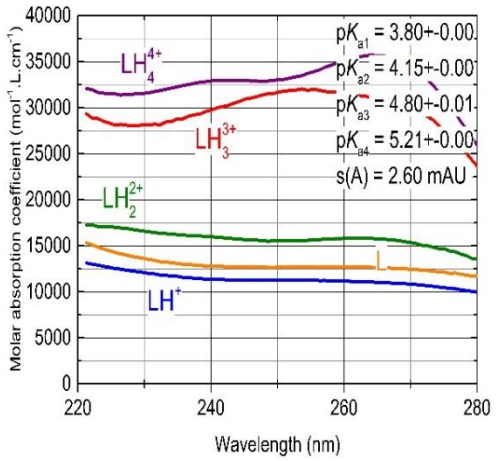
Search for the effective wavelength range

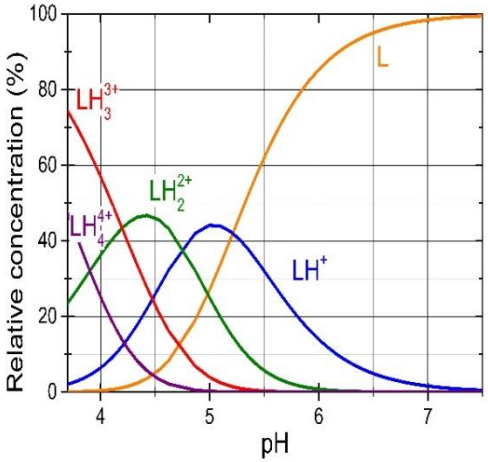












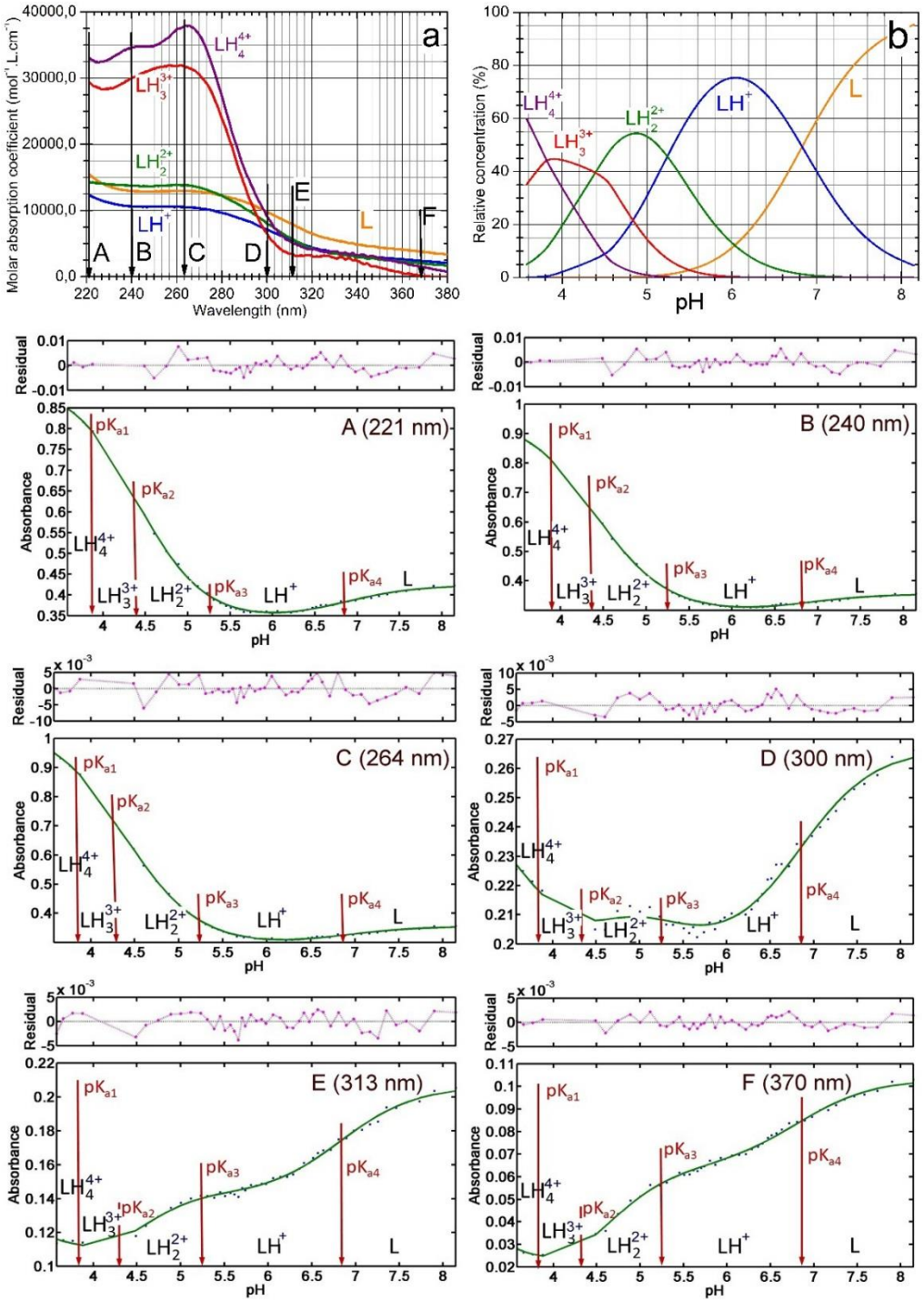
Search for the effective wavelength range to examine the position of ionizable groups and chromophores to find a sufficient absorbance change in spectrum for adjusted pH which allows a reliable determination of dissociation constants.

The protonation model of four dissociation constants was analyzed using

1) either the whole wavelength spectrum (***Upper 2 graphs***)
2) or by two separate absorption bands (***Middle*** and ***Lower 2 graphs***).

The best fitted spektra: in the 220 - 320 nm range, although pK_a estimates were similar for all three wavelength ranges.

The efficient wavelength range

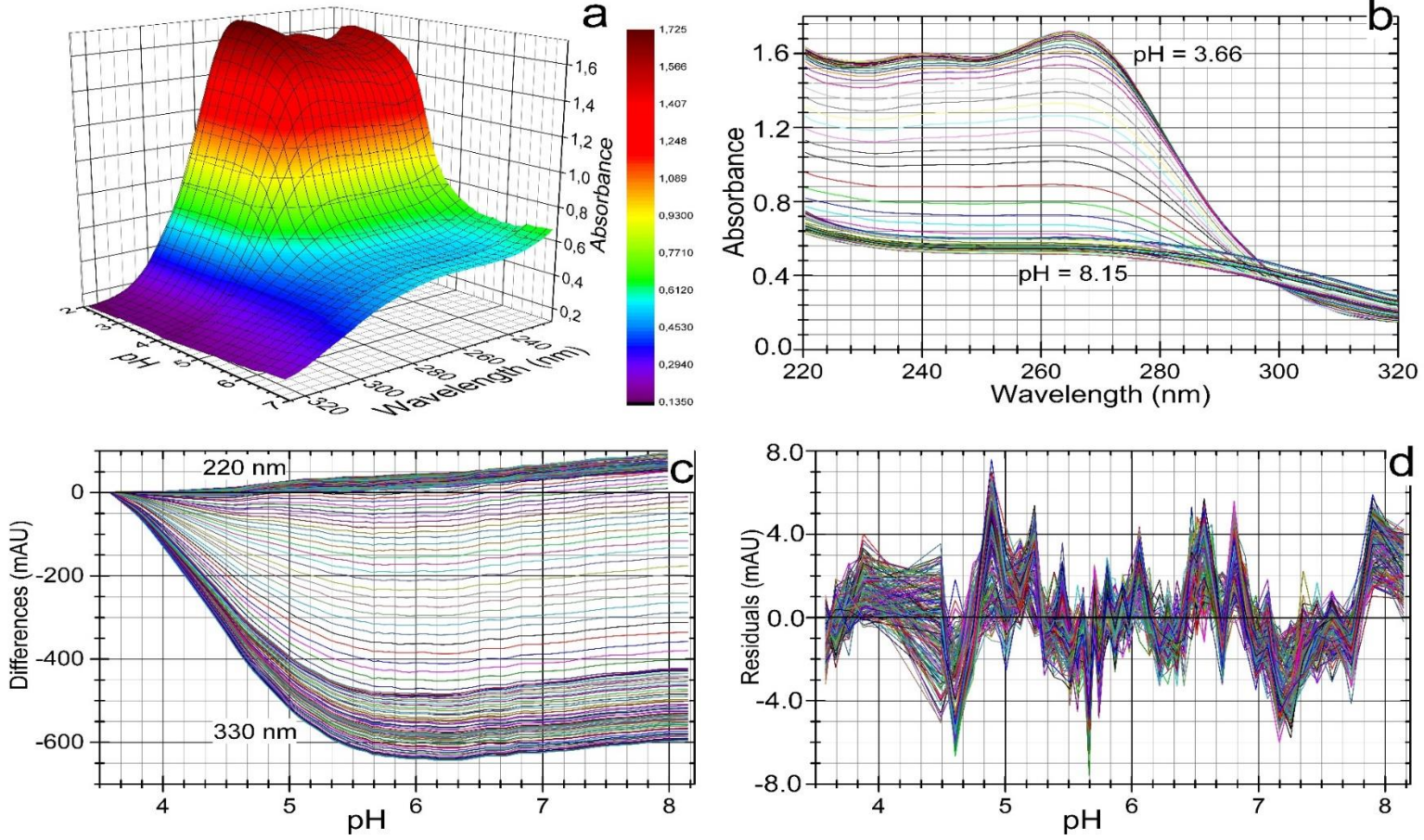


The adjusted pH did not cause the same absorbance change in the Nilotinib hydrochloride spectrum as some chromophores were slightly affected by pH:

Upper left graph: The spectrum of the molar absorption coefficient contains positions of six wavelengths **A** through **F** for which the A-pH curves were analyzed.

Upper right graph: The distribution diagram of the relative concentration of variously protonated species indicated a consecutive equilibrium with close dissociation constants.

Graphs A through F: The sensitivity of chromophores in the Nilotinib hydrochloride molecule to pH is analyzed.



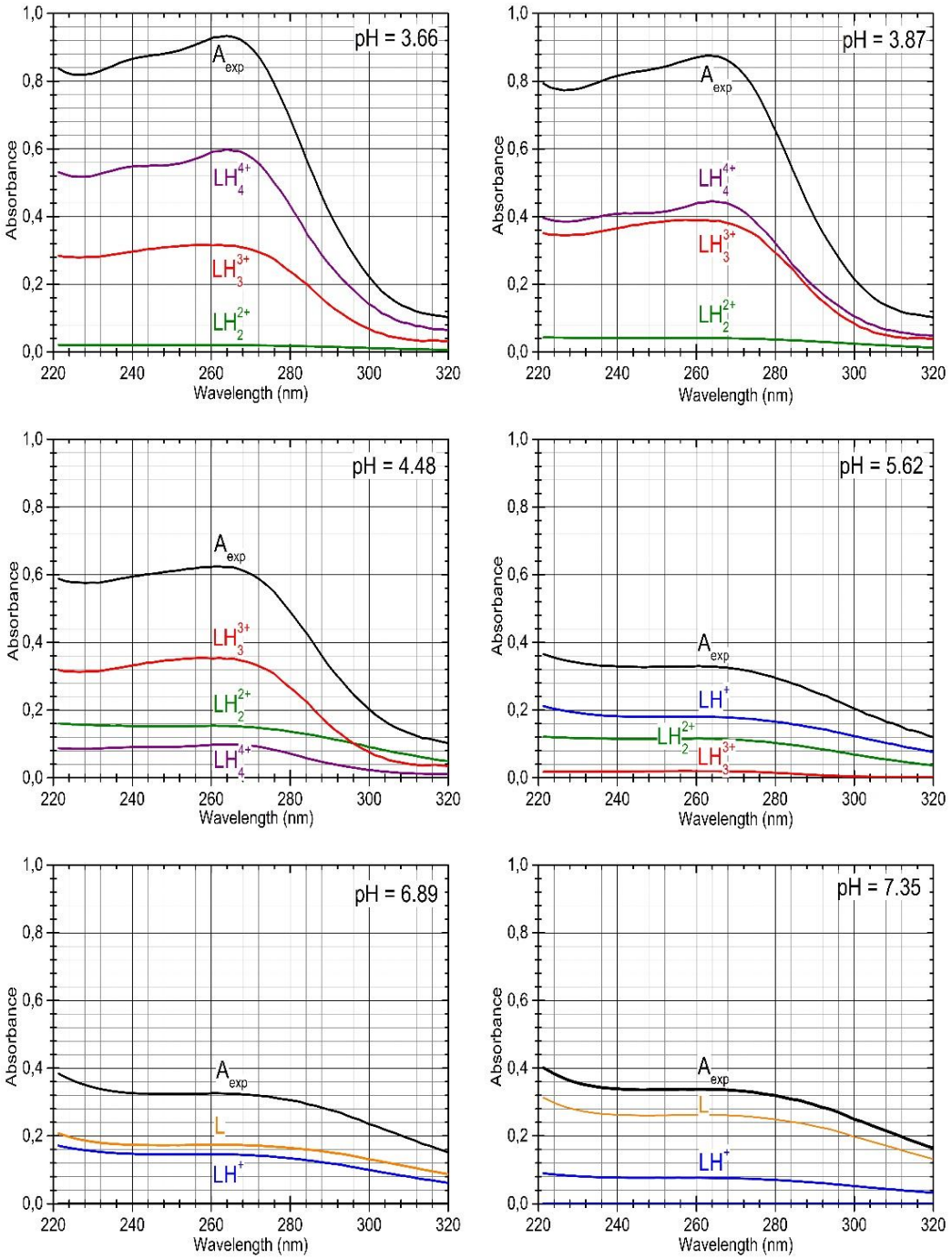
Analysis of the small changes in spectra at adjusted pH

Graph a: 3D-graph of the absorbance change in the Nilotinib spectrum at pH-titration.

Graph b: 2D-graph of the absorbance change in the spectra set from pH_1 = 3.66 to pH_n = 8.15,

Graph c: The graph of the absorbance Differences D_{ij} [mAU] in the Nilotinib spectrum from 220 to 320 nm within pH titration.

*Graph **d**:* Residuals ***e*** [mAU] show whether they were of the same size as the instrumental noise $s_{inst}(A)$, (REACTLAB, ORIGIN 9).

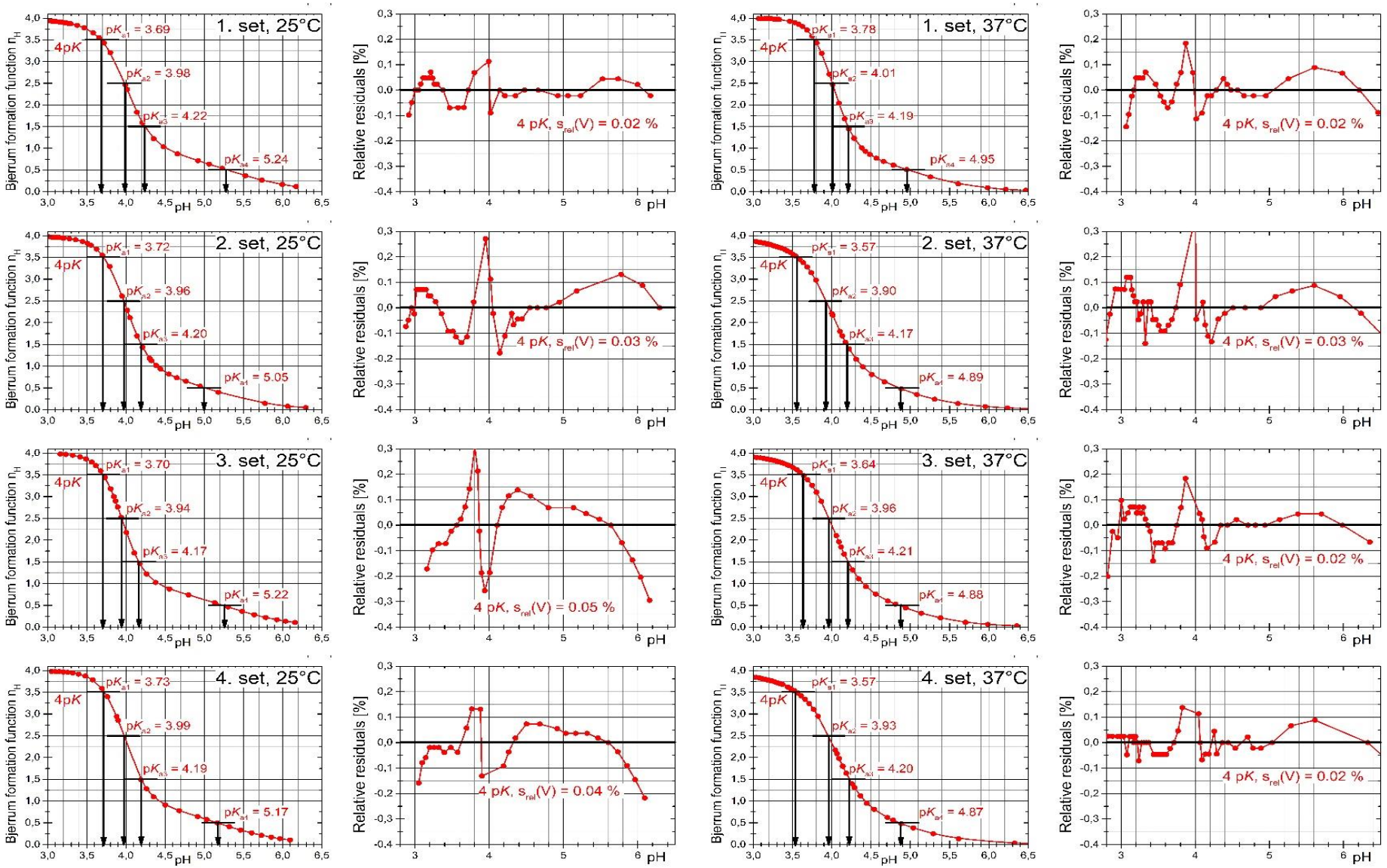


Spectra deconvolution

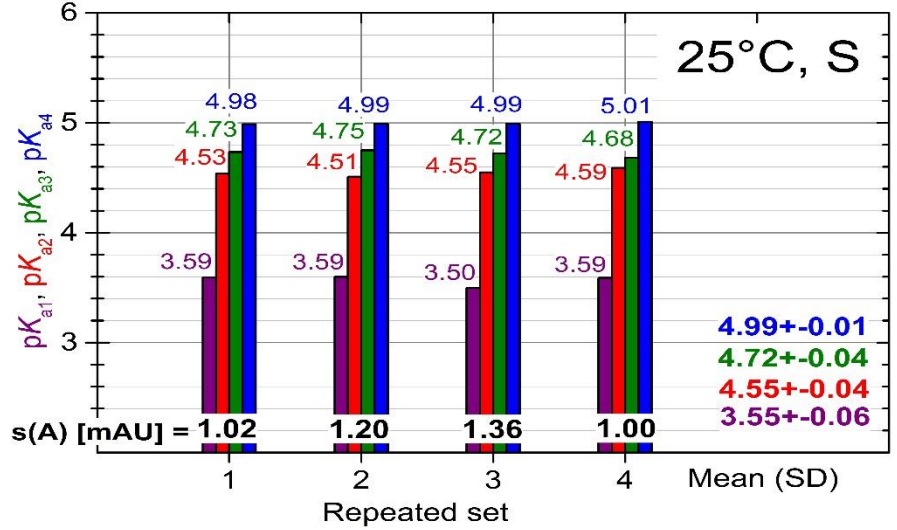
Deconvolution of each experimental spectrum A_{exp} of 1.0×10^{-4} mol. dm^{-3} Nilotinib hydrochloride at I = 0.0026 and 25°C into the spectra of the individual differently protonated ions L, LH^+, LH_2^{2+}, LH_3^{3+}, LH_4^{4+} in mixture for selected pH values:

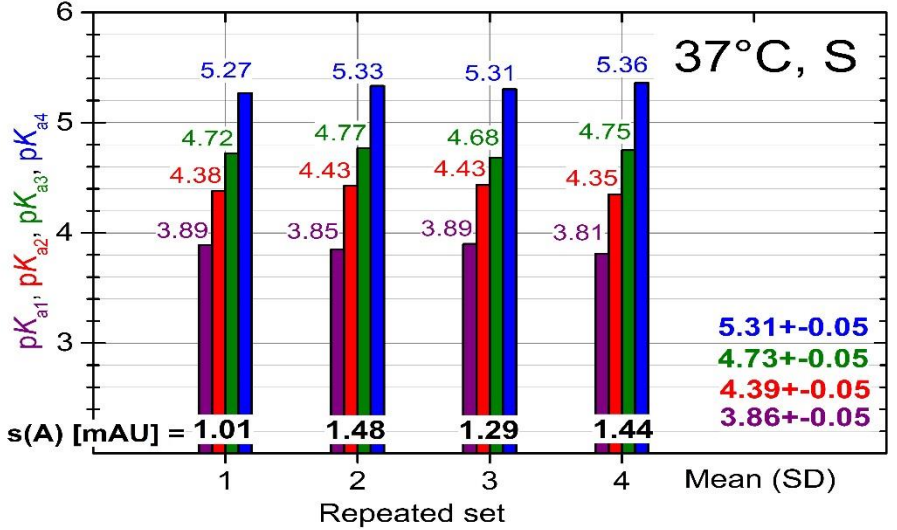
3.66,
3.87,
4.48,
5.62,
6.89,
7.35

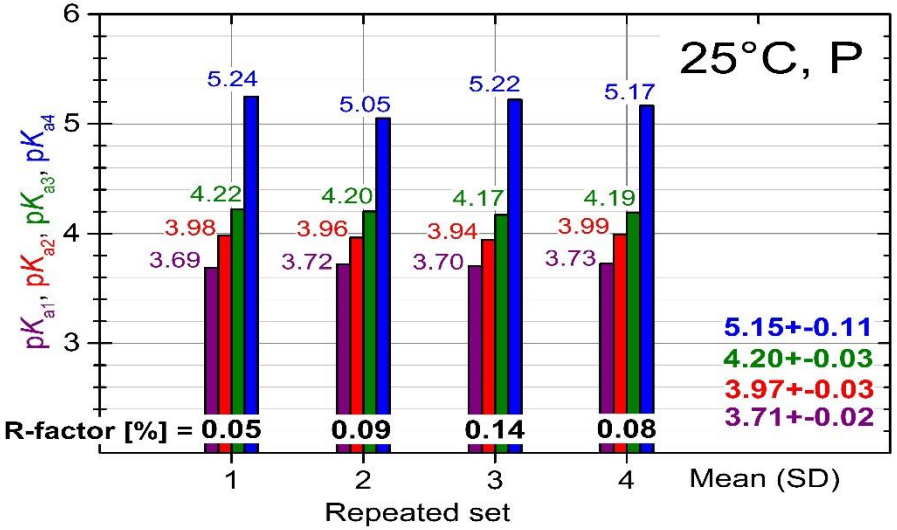
calculated by SQUAD84.

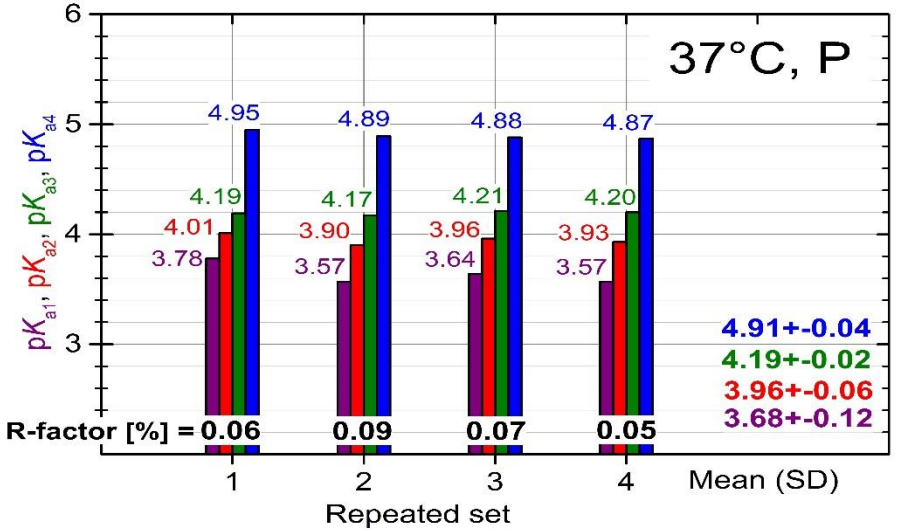


Bjerrum formation function: Reproducibility in the search for the protonation model analysing four pH potentiometric titration curves at 25°C and 37°C. Acidified Nilotinib hydrochloride was titrated with KOH and the Bjerrum protonation curve was plotted for four pK_a. The residual graphs show the best curve fitting of titration curves (ESAB, ORIGIN).

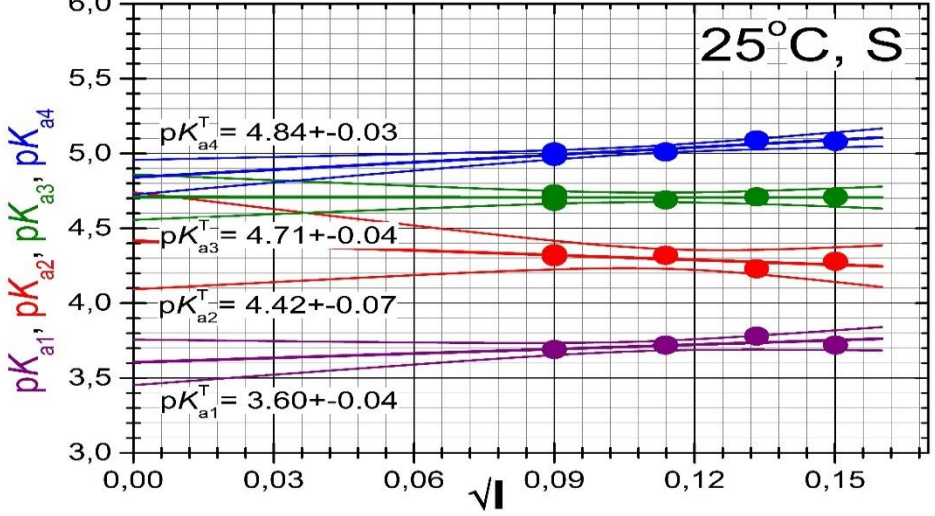


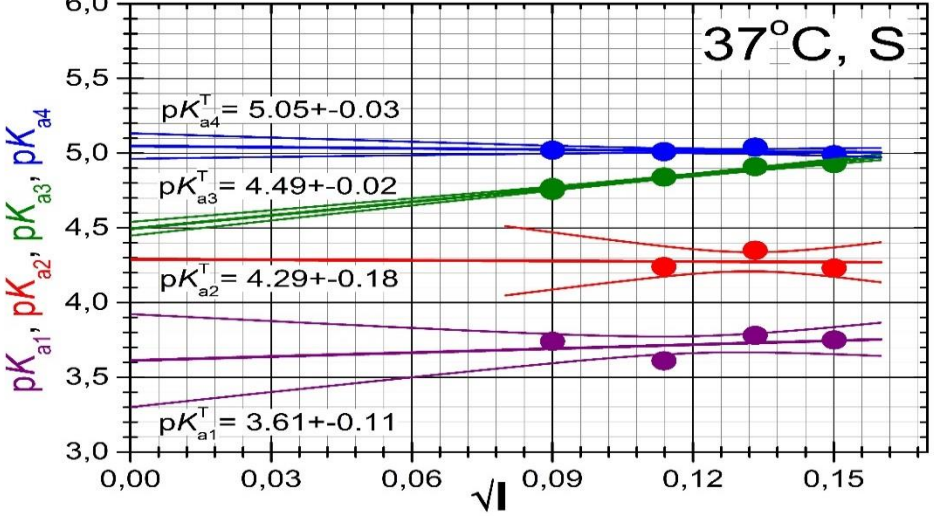


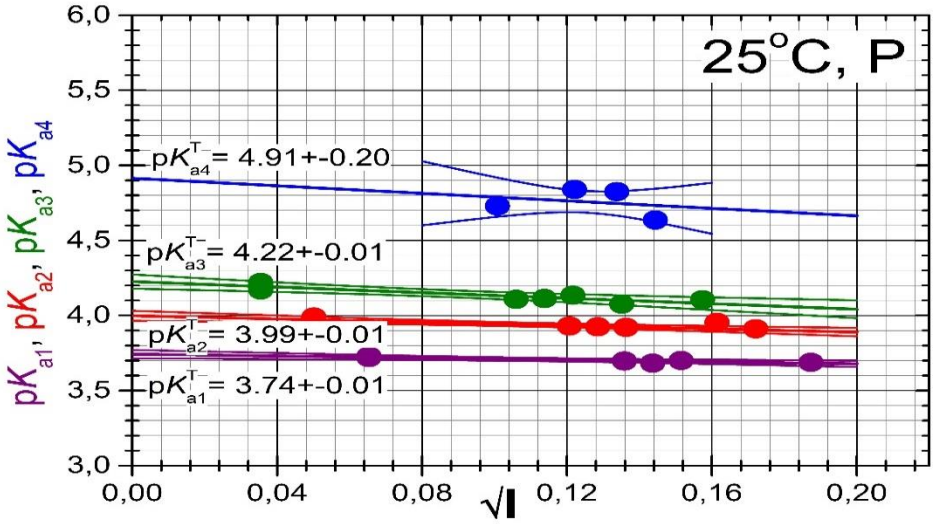


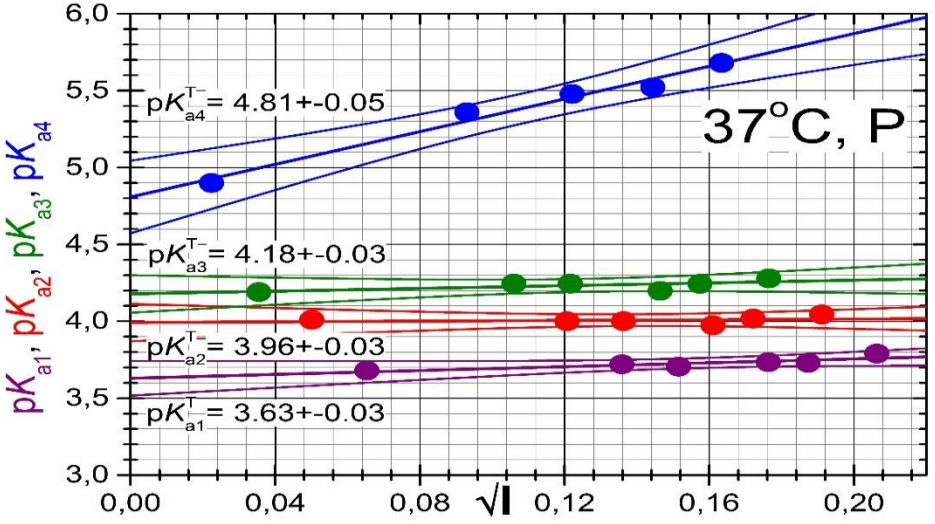


The reproducibility of Nilotinib dissociation constants of the four reproduced UV-metric spektra analysis (***graph 25°C, S*** and ***graph 37°C, S***) and four reproduced pH-metric titration curves (***graph 25°C, P*** **and** ***graph 37°C, P***) were in an agreement. Reproducibility of protonation model estimates with four dissociation constants was compared. The arithmetic mean of dissociation constants with its confidence interval was expressed on the base of the standard deviation *s*(*A*) and *s*(*V*), (REACTLAB, SQUAD84, ESAB, ORIGIN 9).

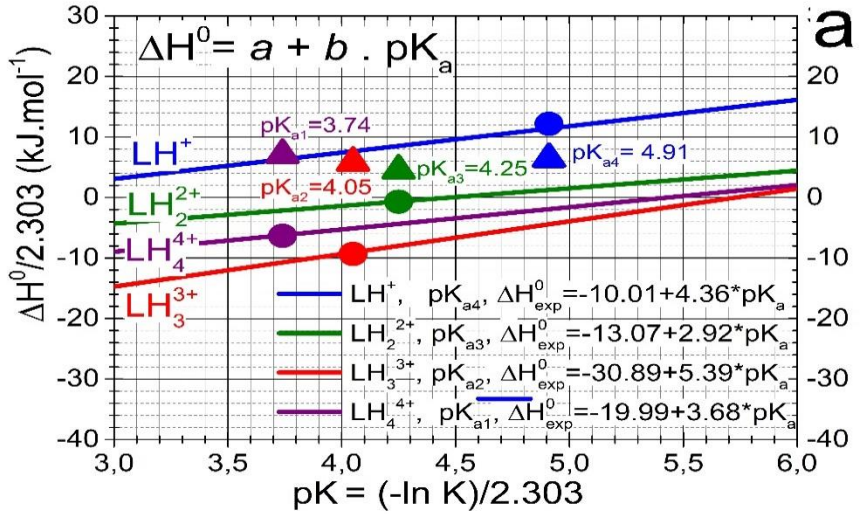


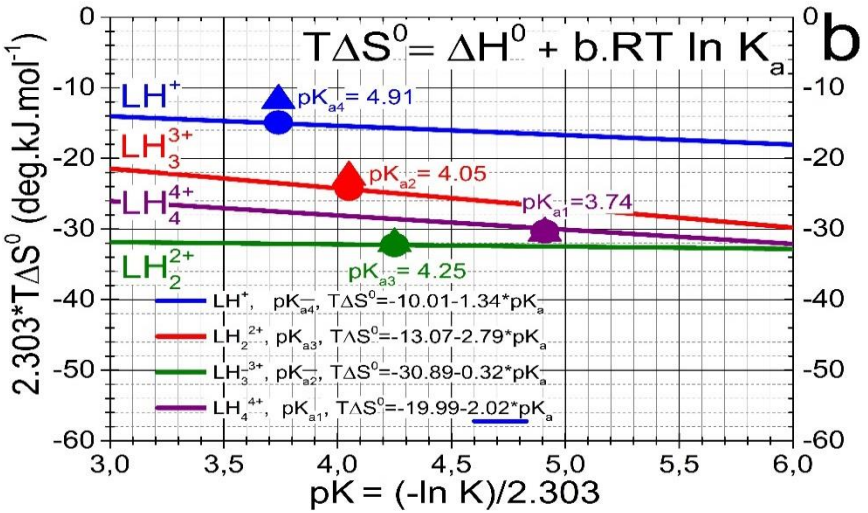


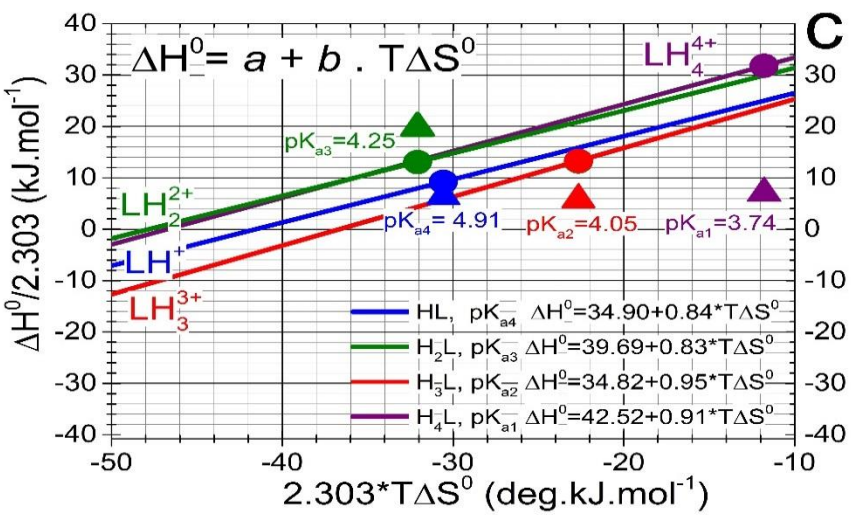


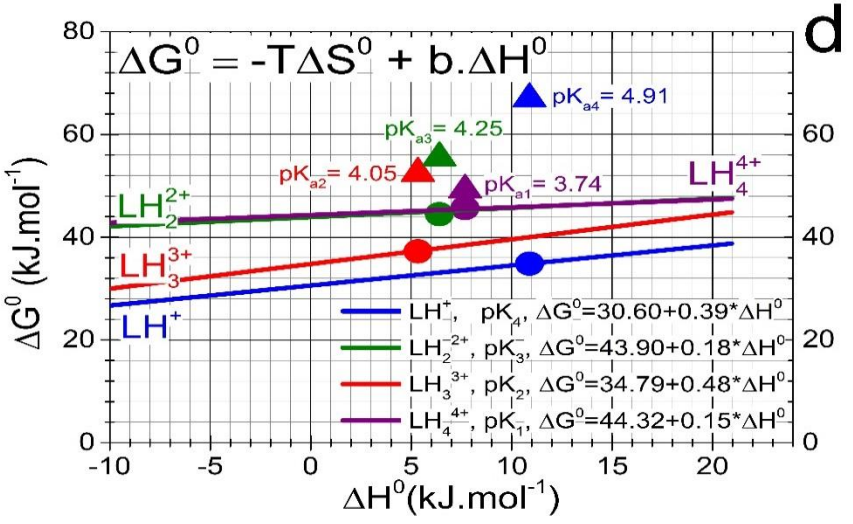


Thermodynamic dissociation constant pK^{T}_{a} **:** Dependence of the mixed dissociation constants of Nilotinib hydrochloride on the square-root of the ionic strength for the four dissociation constants leading to the pK^{T}_{a} using the spectra analysis (***graph 25°C, S*** and ***graph 37°C, S***) and pH-metric technique (***graph 25°C, P*** and ***graph 37°C, P***). The straight lines are drawn with their Working-Hotteling confidence bands (QCEXPERT).

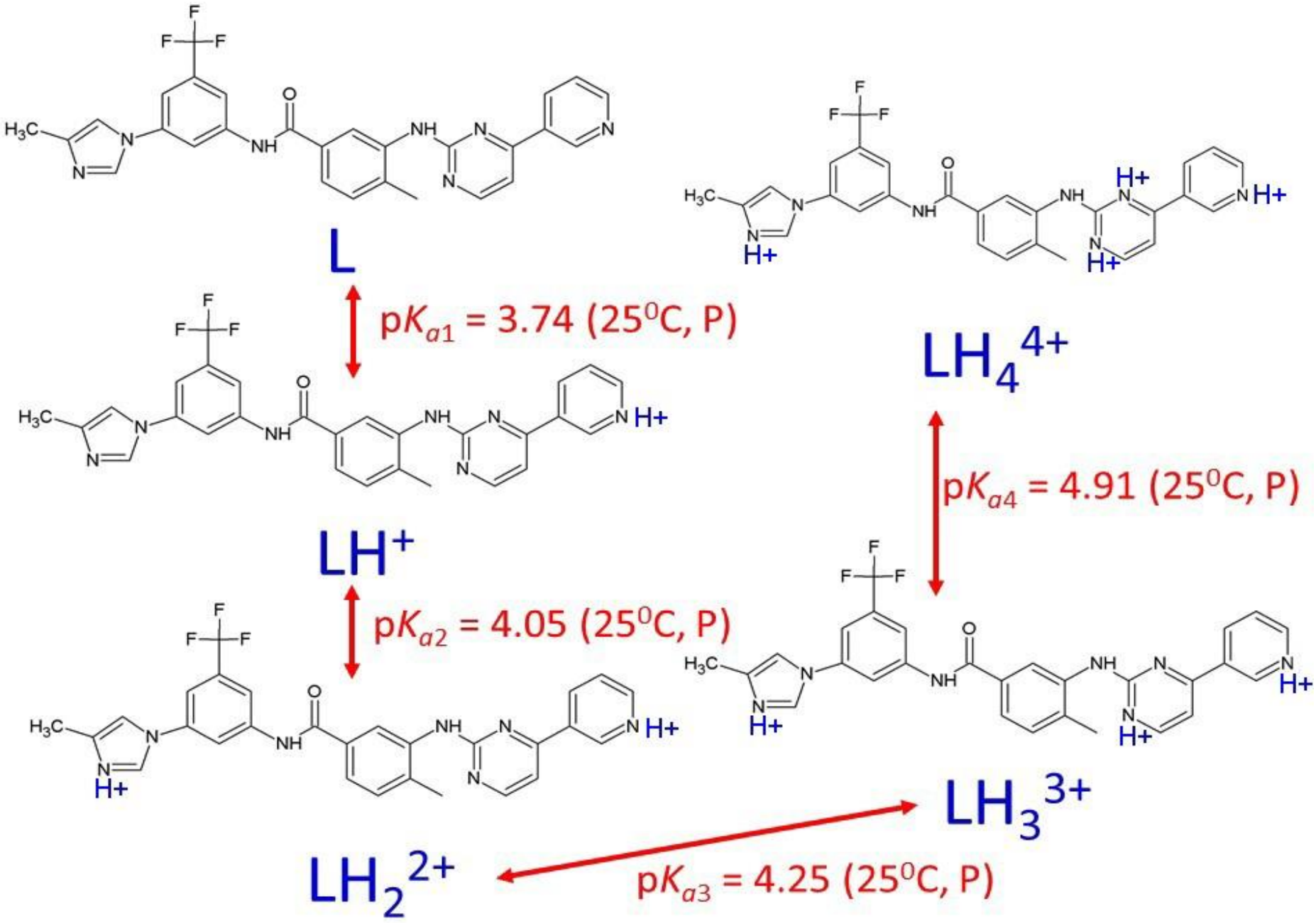








QSARs extra-thermodynamics concerning dissociation of four cations for the prediction of the actual thermodynamics of Nilotinib (***triangles***) in comparison with their experimental value (***full circles***): ***Graph a:*** $\Delta H^0 = a + b \,.\, pK_a$, ***Graph b:*** $T\Delta S^0 = \Delta H^0 + b \,.\, RT \ln K_a$, ***Graph c:*** $\Delta H^0 = \Delta G^0 + b \,.\, T\Delta S^0$, ***Graph d:*** $\Delta G^0 = -T\Delta S^0 + b \,.\, \Delta H^0$. Each figure contains four lines for cations LH^+, LH^{2+}, LH^{3+} and LH^{4+} to predict the thermodynamics of Nilotinib (*full circles*).



Protonation scheme of Nilotinib hydrochloride

Conclusion

(1)Spectrophotometric and potentiometric pH titration allowed measurement of up to four close dissociation constants of Nilotinib hydrochloride.

(2) Nilotinib hydrochloride was capable of protonation to produce in pure water four soluble species LH^{+}, LH_2^{2+}, LH_3^{3+}, LH_4^{4+}. The graph of the molar absorption coefficients of differently protonated species in relation to wavelength indicated that the spectrum of ε_L, ε_{LH}, ε_{LH2}, ε_{LH3}, ε_{LH4} were for two pairs of species correlated and values in each pair were almost the same.

(3) In the range of pH 2 to 7, four dissociation constants could be reliably estimated from the spectrum when the concentration of the sparingly soluble Nilotinib with SQUAD84 and REACTLAB $pK^{T}_{a1} = 3.60\pm0.04$, $pK^{T}_{a2} = 4.42\pm0.07$, $pK^{T}_{a3} = 4.71\pm0.04$, $pK^{T}_{a4} = 4.84\pm0.03$ at 25°C and $pK^{T}_{a1} = 3.61\pm0.11$, $pK^{T}_{a2} = 4.29\pm0.18$, $pK^{T}_{a3} = 4.49\pm0.02$, $pK^{T}_{a4} = 5.05\pm0.03$ at 37°C.

4) The four thermodynamic dissociation constants of Nilotinib hydrochloride were determined by regression analysis of potentiometric titration curves at a potentiometric concentration of 3×10^{-4} mol. dm^{-3} with ESAB, $pK^{T}_{a1} = 3.74\pm0.01$, $pK^{T}_{a2} = 4.05\pm0.01$, $pK^{T}_{a3} = 4.25\pm0.01$, $pK^{T}_{a4} = 4.91\pm0.20$ at 25°C and $pK^{T}_{a1} = 3.63\pm0.03$, $pK^{T}_{a2} = 3.96\pm0.03$, $pK^{T}_{a3} = 4.18\pm0.03$, $pK^{T}_{a4} = 4.81\pm0.05$ at 37°C.

(5) Prediction of the dissociation constants of Nilotinib hydrochloride was performed by the programs MARVIN, PALLAS and ACD/Percepta to determine protonation sites.

(6) **Positive enthalpy values ΔH^0** at 25°C show that the dissociation process is endothermic and is accompanied by absorption of heat. **Positive value of the Gibbs free energy ΔG^0** at 25°C and 37°C indicated that the dissociation process was not proceeded spontaneously. **Negative entropy value ΔS^0** of dissociation process ΔS^0 at 25°C and 37°C means the dissociation process is reversible.