The dissociation constants of the cytostatic bosutinib by nonlinear least-squares regression of multiwavelength spectrophotometric and potentiometric pH-titration data

*Milan Meloun[1], Veronika Nečasová[1], Milan Javůrek[2] and Tomáš Pekárek[3]
[1]Department of Analytical Chemistry, University of Pardubice,
CZ 532 10 Pardubice, Czech Republic,
[2]Department of Process Control, University of Pardubice,
CZ 532 10 Pardubice, Czech Republic,
[3]Zentiva k.s., U kabelovny 130,
CZ 102 37 Prague, Czech Republic

Logo: Meloun et al. Bosutinib

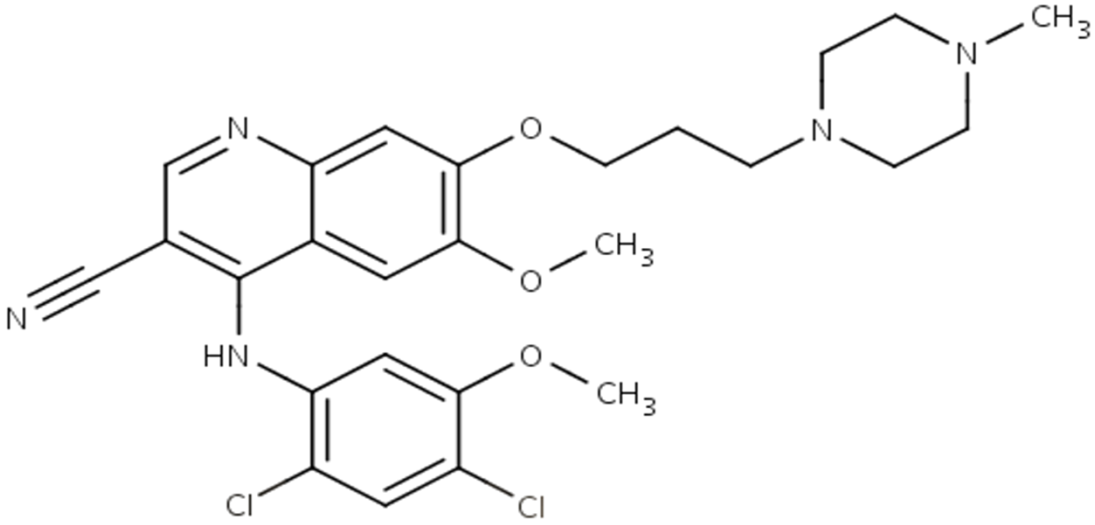
Summary

Bosutinib treats patients with positive chronic myeloid leukemia. Bosutinib exhibits four protonatable sites in a pH range from 2 to 11. Due to limited solubility of bosutinib the protonation in a mixed 3% to 6% aqueous-methanolic medium three dissociation constants can be reliably determined and after extrapolation to zero content of methanol they lead to at 25°C pK_{c1} = 3.43(12), pK_{c2} = 4.54(10), pK_{c3} = 7.56(07) and pK_{c4} = 11.04(05) and at 37°C pK_{c1} = 3.44(06), pK_{c2} = 5.03(08) pK_{c3} = 7.33(05) and pK_{c4} = 10.92(06). From the potentiometric pH-titration at 25°C the concentration dissociation constants were in a good agreement with those from spectra analysis.

Logo: Meloun et al. Bosutinib

Background

Bosutinib can be found under the trade name SKI-606 or Bosufilhas. A systematic title based (IUPAC) 4-[(2,4-dichloro-5-metoxyfenyl)amino)]-6-metoxy-7-[(3-(4-methylpipe-razin-1-yl)pro-poxy)]chinolin-3-carbonitril.
Bosutinib belongs cytostatics or anti-tumor drugs retard or arrest cell growth or even cause their disintegration. Bosutinib is designated for the treatment of adult patients with chronic, accelerated and blast phase Philadelphia chromosome positive chronic myeloid leukemia (Ph + CML). The term for this type of chronic leukemia means that the cancer progresses more slowly than acute forms of myeloid leukemia and refers to the type of cells affected by the type of cancer.



Methodology

The protonation between the anion L (the charges are omitted) of a drug and a proton H are considered to form a set of variously protonated species L, HL, H_2L, H_3L ...etc., with the general formula H_rL. The overall protonation constant of the protonated species, β_r, is then expressed as

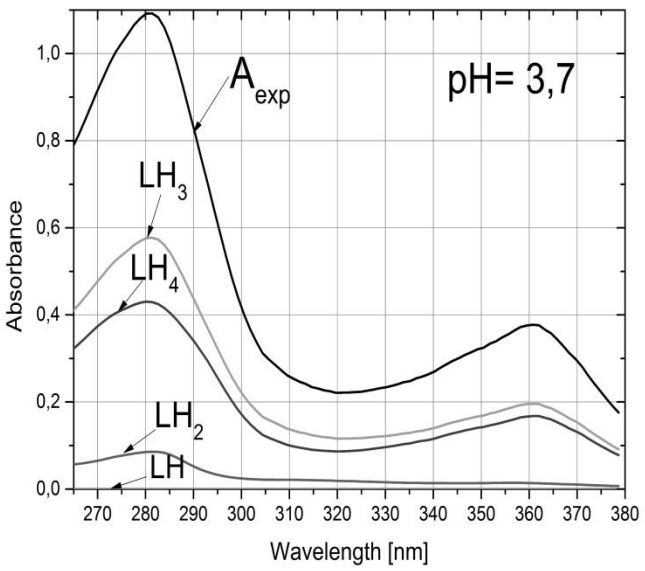
(1)

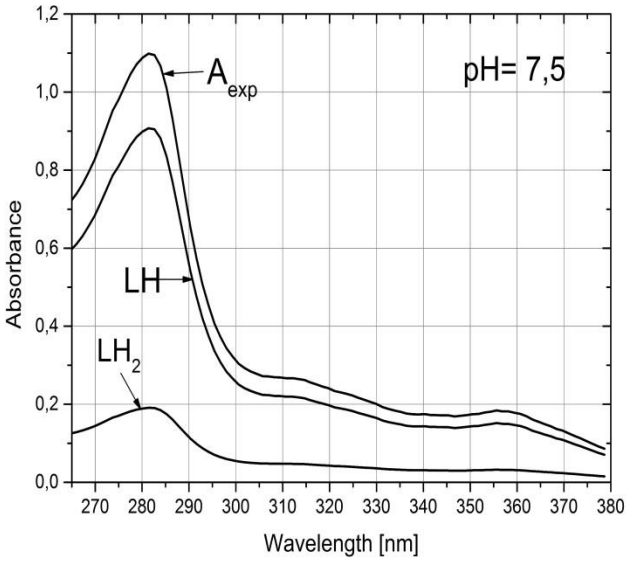
where the free concentration [L] = l, [H] = h and [H_rL] = c.

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

(3)

where $\varepsilon_{r,j}$ is the molar absorption coefficient of the H_rL species. The absorbance $A_{i,\ j}$ is an element of the absorbance matrix $\boldsymbol{A}$ of size ($n_s \times n_w$) 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.





Logo: Meloun et al. Bosutinib

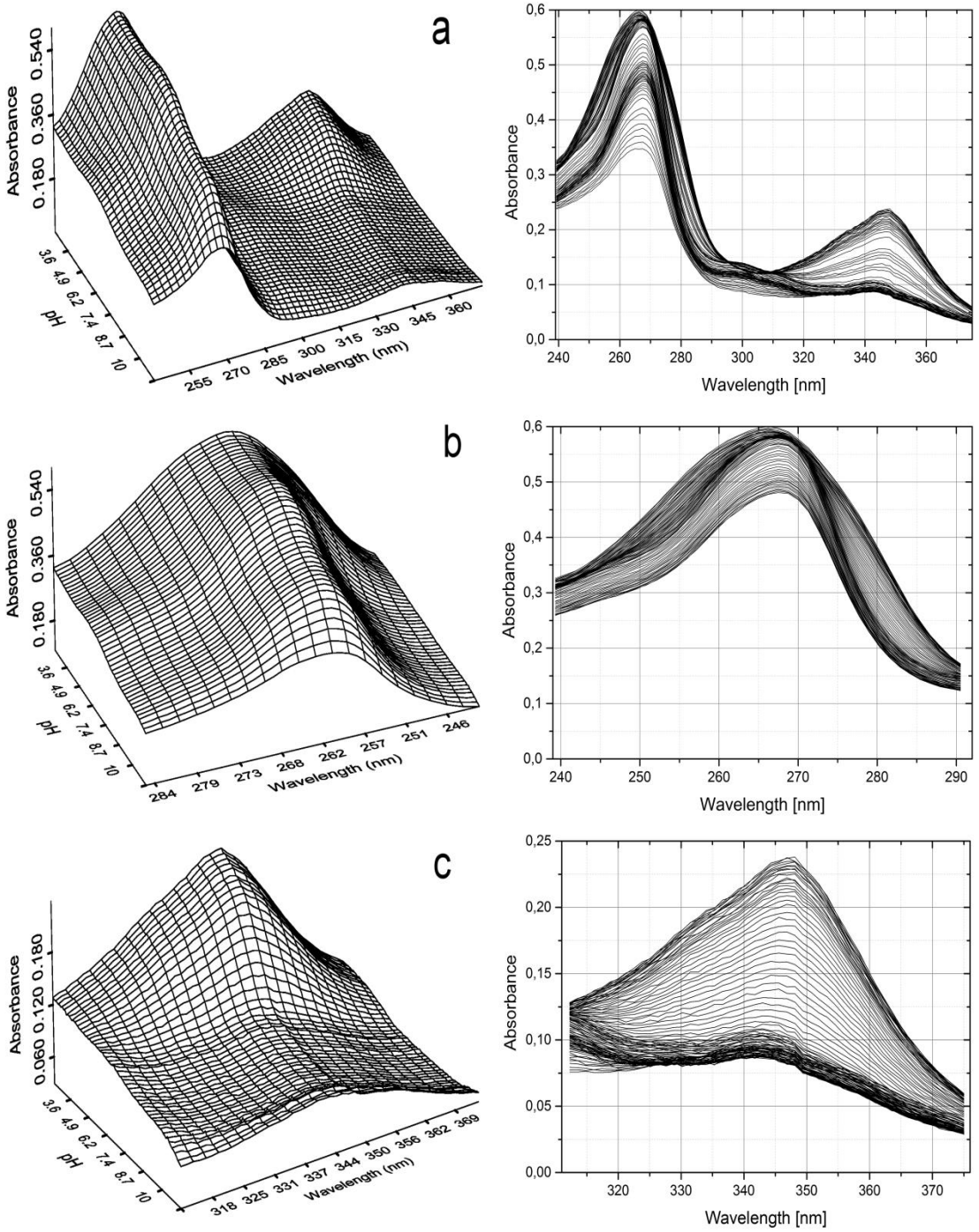
Results

The 3D-absorbance-response-surface (left) and 2D-graph (right) representing the measured multiwavelength absorption spectra for bosutinib according to pH then titrated with KOH:

(a) Spectrum range of 239.3 to 375.0 nm was divided into

(b) the first absorption band of a range of 239.3 to 290.5 nm and

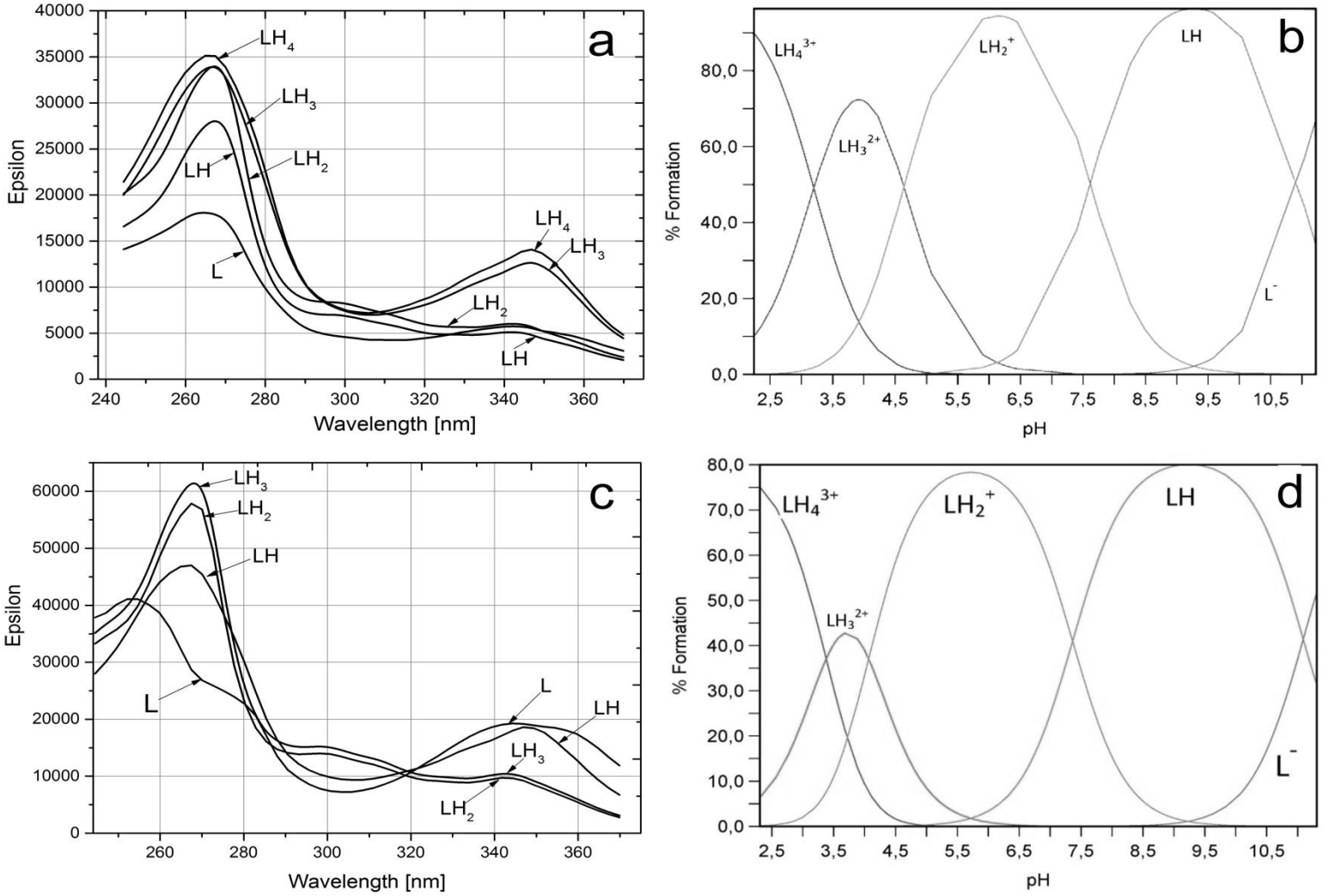
(c) the second absorption band of 312.3 to 375.0 nm (c) prior further regression analysis, (S-PLUS).



Logo: Meloun et al. Bosutinib

Results

The graph of molar absorption coefficients differently protonated particles bosutinib for two different contents of methanol solvent:
(a) 6% methanol and (c) 48% methanol and corresponding distribution diagram of relative concentration of variously protonated species for
(b) (b) 6% methanol and (d) 48% methanol.



Logo: Meloun et al. Bosutinib

Results

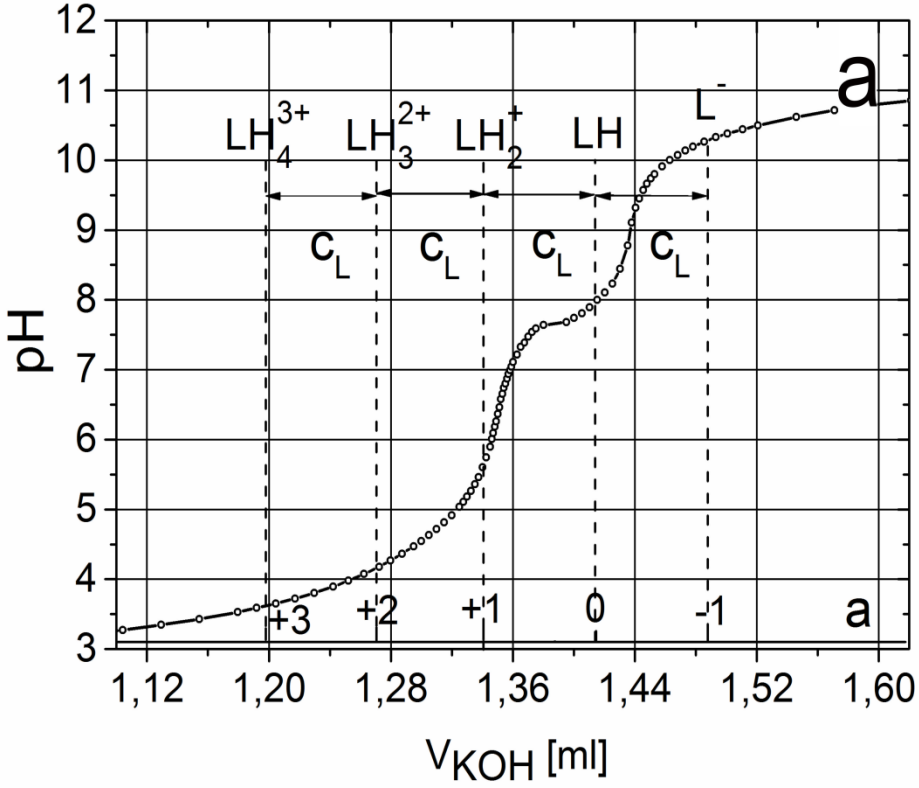
Protonation equilibria of bosutinib analyzed with the least-squares ESAB:

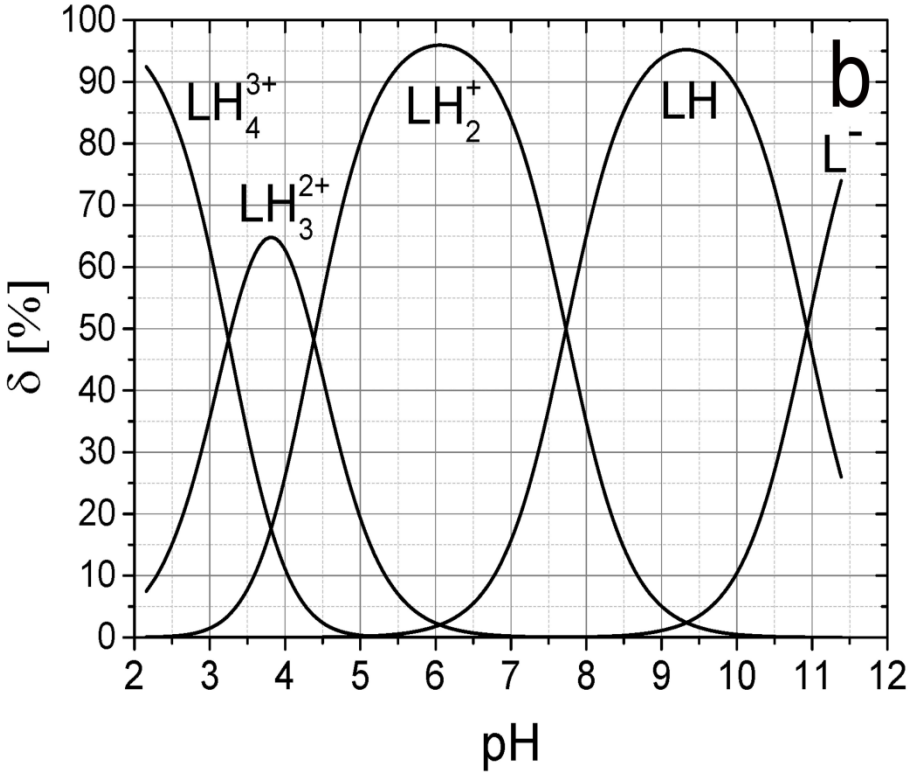
(a) Potentiometric titration curve of bosutinib with KOH in 3% methanol,

(b) Distribution diagram of relative presentation of variously pronated species of all variously protonated species L^-, HL, HL_2^+, LH_3^{2+} and LH_4^{3+} of bosutinib according to pH at 25ºC,

Estimates of parameters: pK_{a1} = 3.25(12), pK_{a2} = 4.38(10), pK_{a3} = 7.73(07), pK_{a4} = 10.94(03), V_0 = 15.12 ml, c_L = 0.0080(0028) mM, H_0 = 0.137(001) mM.

Statistical analysis of residuals: $E(e)$ = -0.025 j.pH, $|e|$ = 0.082 j.pH, $s(e)$ = 0.120 j.pH, M = 0.001 j.pH.





Conclusion

1) In pH 7 bosutinib occurs in water sparingly soluble LH being capable of protonation to better soluble LH_4^{3+}. The LH can be dissociated into still hardly water soluble anion L^-. Acid-base titration of LH_4^{3+} cation with KOH leads to eight species H_3O^+, OH^-, LH_4^{3+}, LH_3^{2+}, LH_2^+, LH, L^- and the cation K^+.

2) The molar absorption coefficients of variously protonated species on wavelength shows that cations LH_4^{3+} and LH_3^{2+} are of only a little different color and dissociation of the chromophore LH_4^{3+} to LH_3^{2+} has little influence on the shape of the spectrum. The same is true for the chromophore LH to LH_2^+, while protonation of chromophore LH_2^+ to LH_3^{2+} has greater influence on chromophores.

3) With increasing methanol content in solvent the dissociation apparently lessens and the percentage of species LH_3^{2+} decreases and in solution the species LH prevails.

4) With a low methanol content of the solvent 3% to 6% in the range of pH 2 to 7 three dissociation constants can be reliably estimated from the spectra. Three concentration dissociation constants $pK_{c1} = 3.41$, $pK_{c2} = 4.69$ and $pK_{c3} = 6.4$ still could be distinguished.

Logo: Meloun et al. Bosutinib