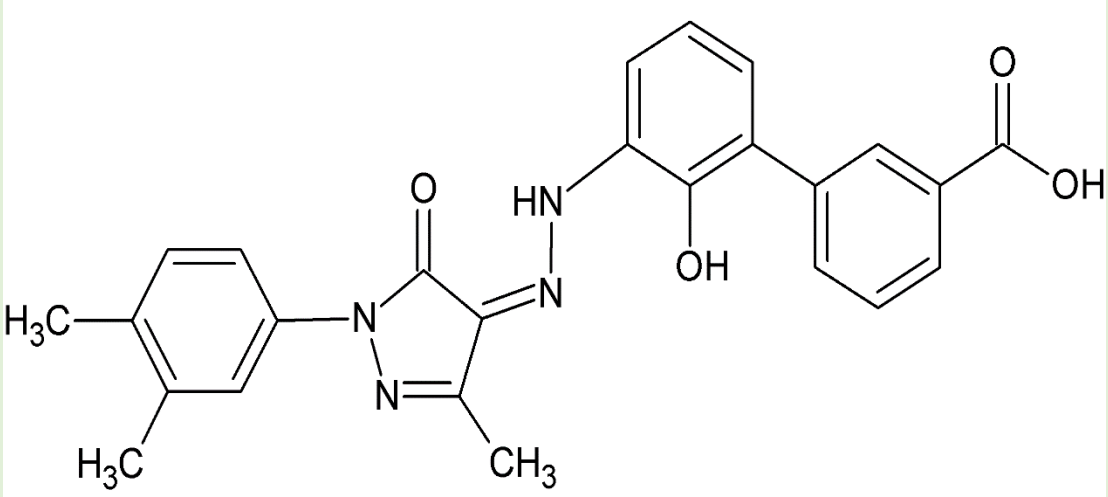
Overlapping pK_a of the multiprotic hemostyptic Eltrombopag using UV/VIS multiwavelength spectroscopy and potentiometry

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

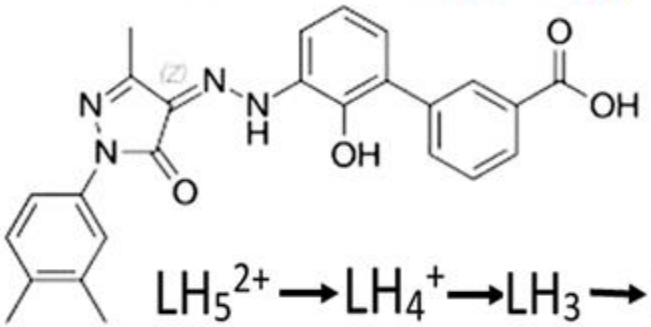
[1]Department of Analytical Chemistry, University of Pardubice, CZ 532 10 Pardubice, Czech Republic,
[2]Zentiva k.s., U kabelovny 130, CZ 102 37 Prague, Czech Republic
[3]Department of Process Control, University of Pardubice, CZ 532 10 Pardubice, Czech Republic

***Corresponding author:** milan.meloun@upce.cz, **orcid.org**/0000-0003-2209-8865,
Phone: +420466037026, **Fax:** +420466037068,



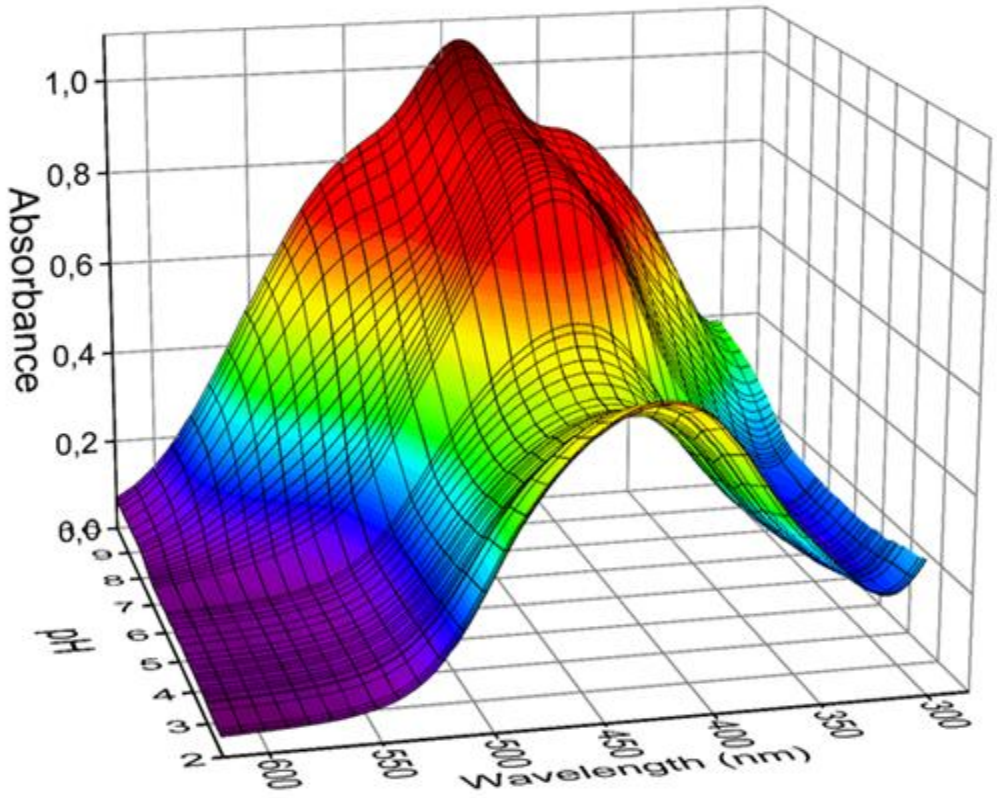
Graphical abstract

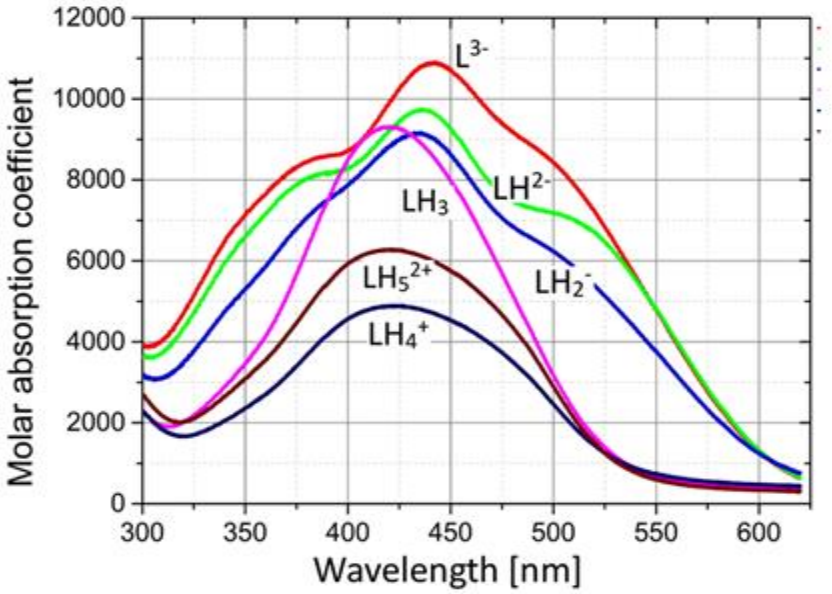
Eltrombopag

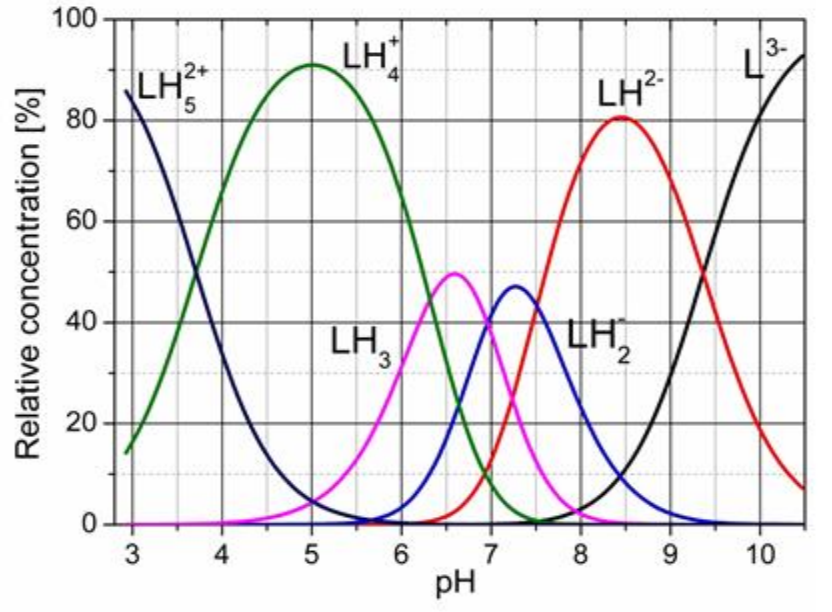


25C
pK1 = 2,69
pK2 = 6.97
pK3 = 7.13
pK4 = 7.65
pK5 = 8.30

$LH_5^{2+} \rightarrow LH_4^{+} \rightarrow LH_3 \rightarrow LH_2^{-} \rightarrow LH^{2-} \rightarrow L^{3-}$





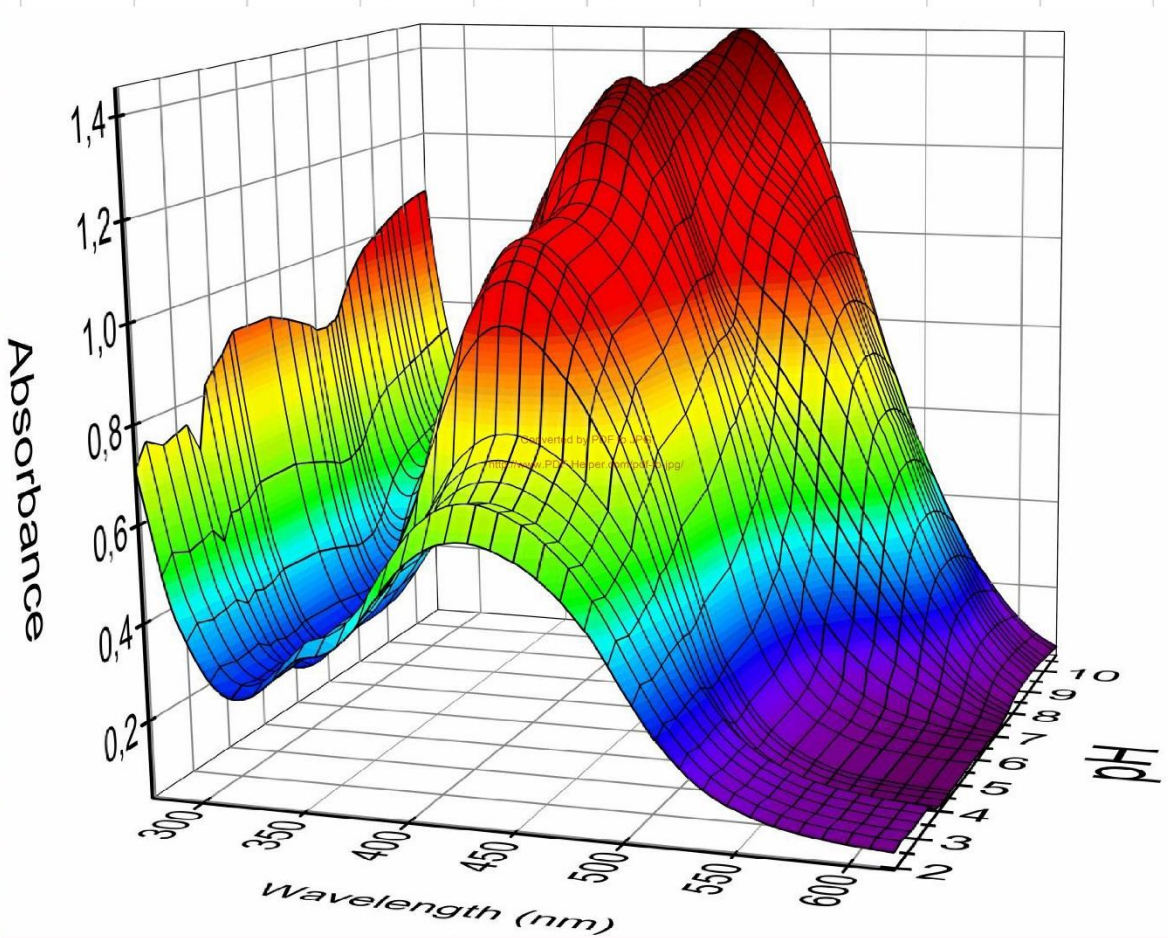


Abstract

pH-potentiometric and WApH-spectrophotometric titrations of the multiprotic hemostyptic Eltrombopag for dissociation constants determination were compared. Hemostyptic and hemostatic Eltrombopag treats low blood platelet counts in adults with chronic immune idiopathic thrombocytopenia ITP. Eltrombopag exhibits five protonatable sites in a pH range of 2 to 10, where only two p*K* are well separated ($\Delta pK > 3$), while the other three are near dissociation constants of overlapping equilibria. According to MARVIN prediction, in the neutral medium Eltrombopag occurs in the slightly water soluble form LH_3 that can be protonated to the soluble species LH_4^+ and LH_5^{2+} The molecule LH_3 can be dissociated to still difficultly soluble species LH_2^-, LH^{2-} and L^{3-}. Due to limited solubility of Eltrombopag above pH 9.5 the protonation was studied up to pH 10. Five dissociation constants can be reliably determined with REACTLAB and SQUAD84 leading to the same value. From a dependence on ionic strength the thermodynamic dissociation constants were estimated at 25°C: $pK_{a1}^T = 2.69$, $pK_{a2}^T = 6.97$, $pK_{a3}^T = 7.13$, $pK_{a4}^T = 7.65$, $pK_{a5}^T = 8.30$. Since pH above 10 and pH down 5 occurs in a titrated solution the very fine precipitate of Eltrombopag which is initially forming a slight opalescence, this part of the potentiometric titration curve pH over 9 and pH below 5 was not taken into regression analysis to estimate $pK_{a2} = 6.59(01)$, $pK_{a3} = 7.56(04)$, $pK_{a4} = 8.48(59)$, $pK_{a5} = 9.29(34)$ at 25°C with ESAB.

Absorbance matrix represents absorbance response surface

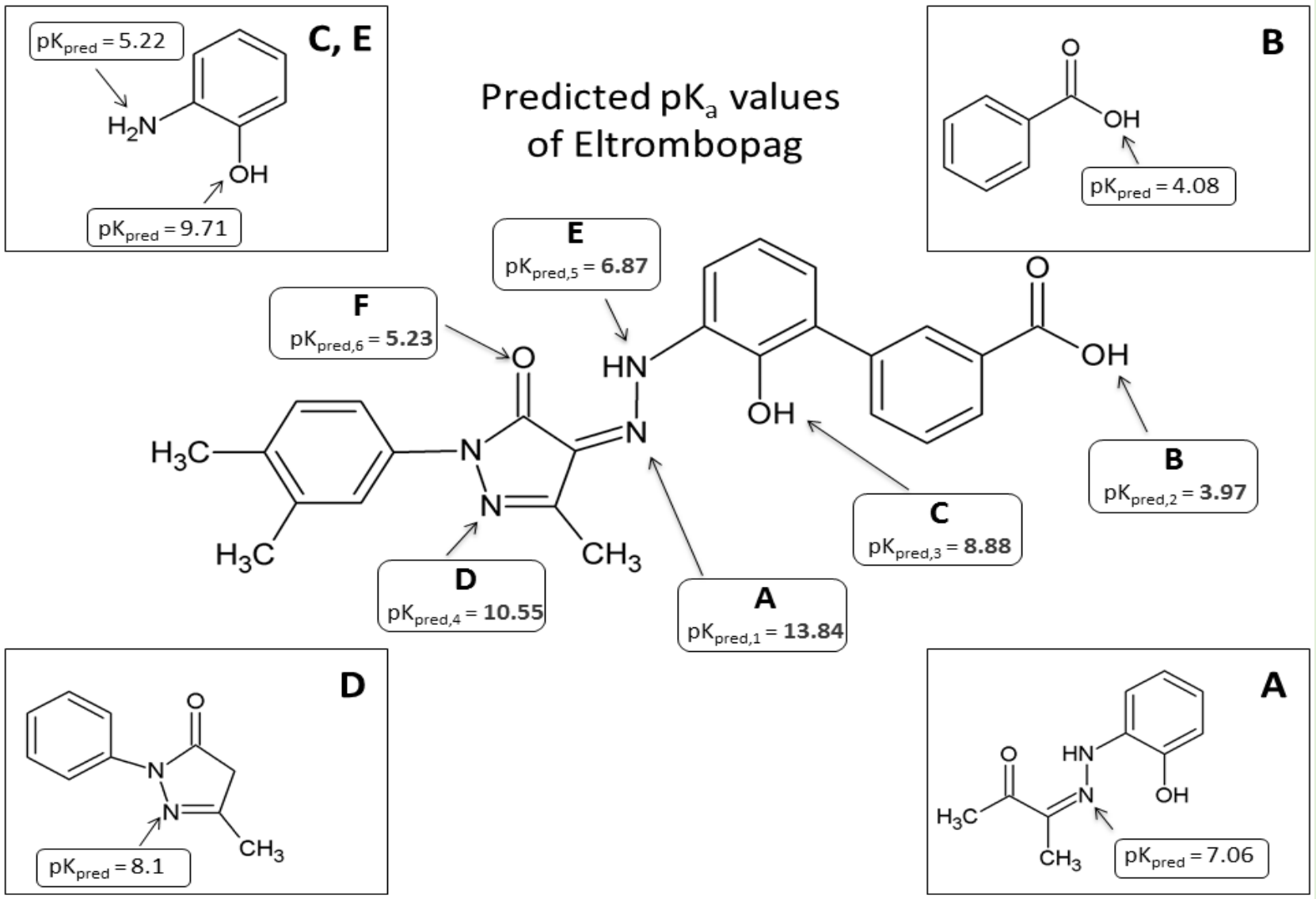
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
1		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
2		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
3		0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0,00012	0
4		5,89E-11	6,55E-11	7,4E-11	8,43E-11	9,93E-11	1,15E-10	1,43E-10	1,79E-10	2,08E-10	2,92E-10	3,76E-10	5,9E-10	8,65E-10	1,43E-09	2,46E-09	4,
5	620	0,066281	0,0660											,066307	0,065693	0,064557	0,
6	618,2933	0,07098	0,0707											,071705	0,07096	0,069156	0,
7	616,5867	0,076998	0,0767											0,07737	0,076179	0,074827	0,
8	614,88	0,0827	0,0821											,082973	0,08218	0,080321	0,
9	613,1733	0,088067	0,0877											,088829	0,087585	0,085476	0
10	611,4667	0,093924	0,0937											,094395	0,093248	0,091654	0
11	609,76	0,100489	0,1000											,101312	0,099714	0,097717	0,
12	608,0533	0,107856	0,1076											,108431	0,107309	0,104608	0,
13	606,3467	0,115731	0,1155											,116377	0,114708	0,112404	0
14	604,64	0,123624	0,1231											,124896	0,12306	0,120198	0,
15	602,9333	0,131834	0,131											,133274	0,131207	0,127895	0,
16	601,2267	0,141228	0,1409											,141995	0,14043	0,137107	0,
17	599,52	0,150712	0,1506											,151861	0,149511	0,146172	0,
18	597,8133	0,160403	0,1603											,162022	0,159357	0,155193	0,
19	596,1067	0,170607	0,1705											,172177	0,169688	0,165364	0,
20	594,4	0,181168	0,1812											,182669	0,180324	0,175931	0,
21	592,6933	0,192735	0,1928											,194171	0,19183	0,187038	0,
22	590,9867	0,20458	0,2047											,206109	0,203401	0,198588	0,
23	589,28	0,217138	0,2172											,218592	0,215676	0,209979	0,
24	587,5733	0,229659	0,2299											,231515	0,228194	0,222252	0,
25	585,8667	0,242903	0,2434											,244926	0,241359	0,235576	0
26	584,16	0,256376	0,256											,258478	0,254997	0,24894	0,
27	582,4533	0,269297	0,2700											,271924	0,268054	0,2615	0,
28	580,7467	0,282567	0,2833											,285227	0,281027	0,273915	0
29	579,04	0,296353	0,2975											,299052	0,294511	0,286896	0,
30	577,3333	0,311322	0,3125											0,31405	0,309667	0,301628	0,
31	575,6267	0,326319	0,3276											,329617	0,324183	0,31591	0,



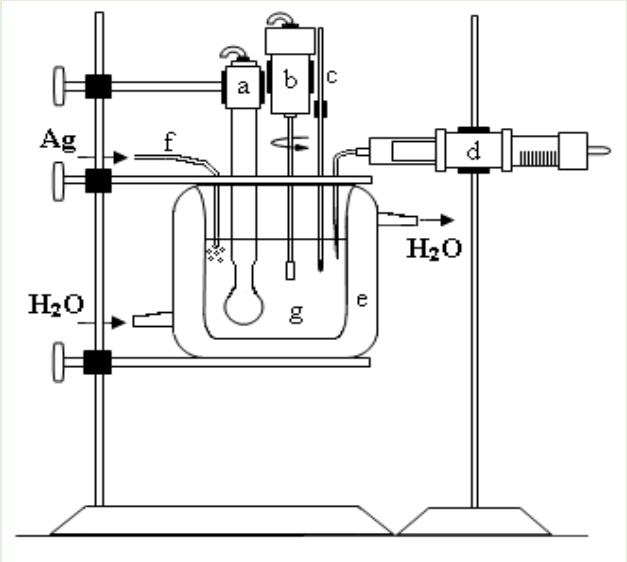
INPUT: pH-spektra Eltrombopagu pro SQUAD(84) a REACTLAB

Prediction of protonation equilibria of Eltrombopag

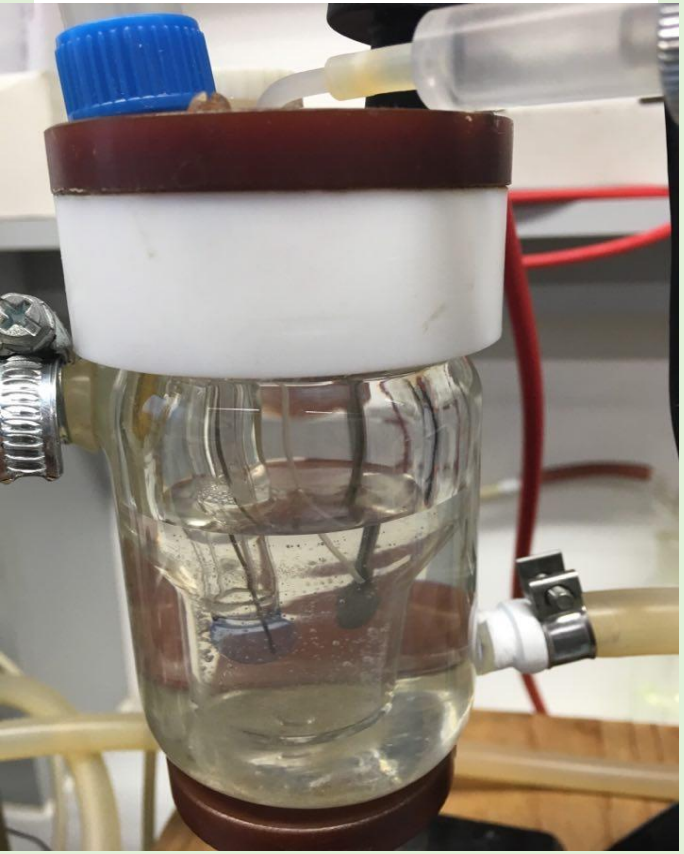
The whole molecule of Eltrombopag was subdivided into four auxiliary fragments containing functional groups on which protonation occurred. These predicted pK_a values served to compare with predicted values throughout the structure of the Eltrombopag molecule

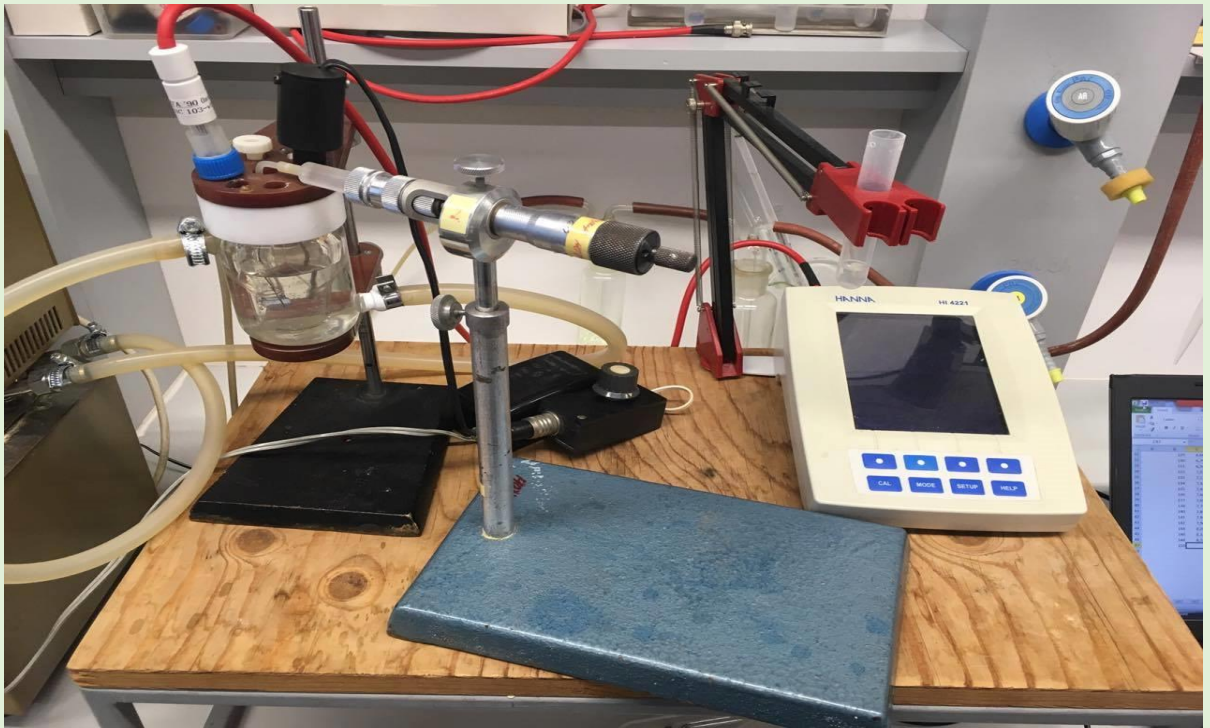


Experimental equipment

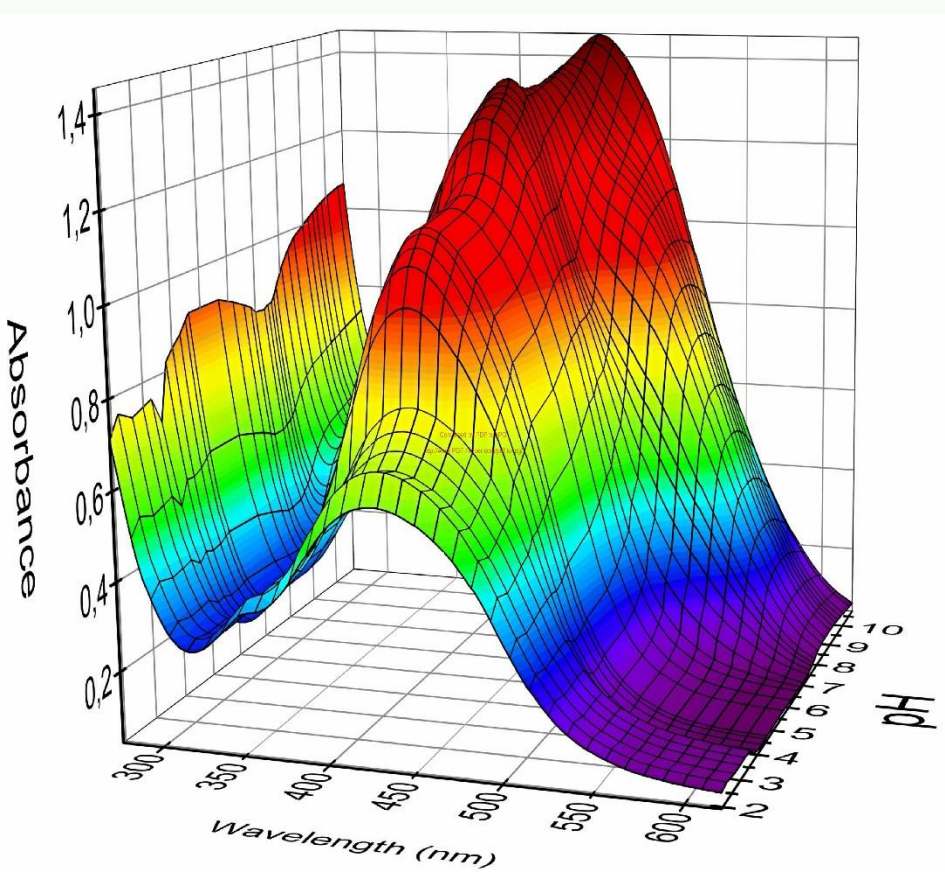


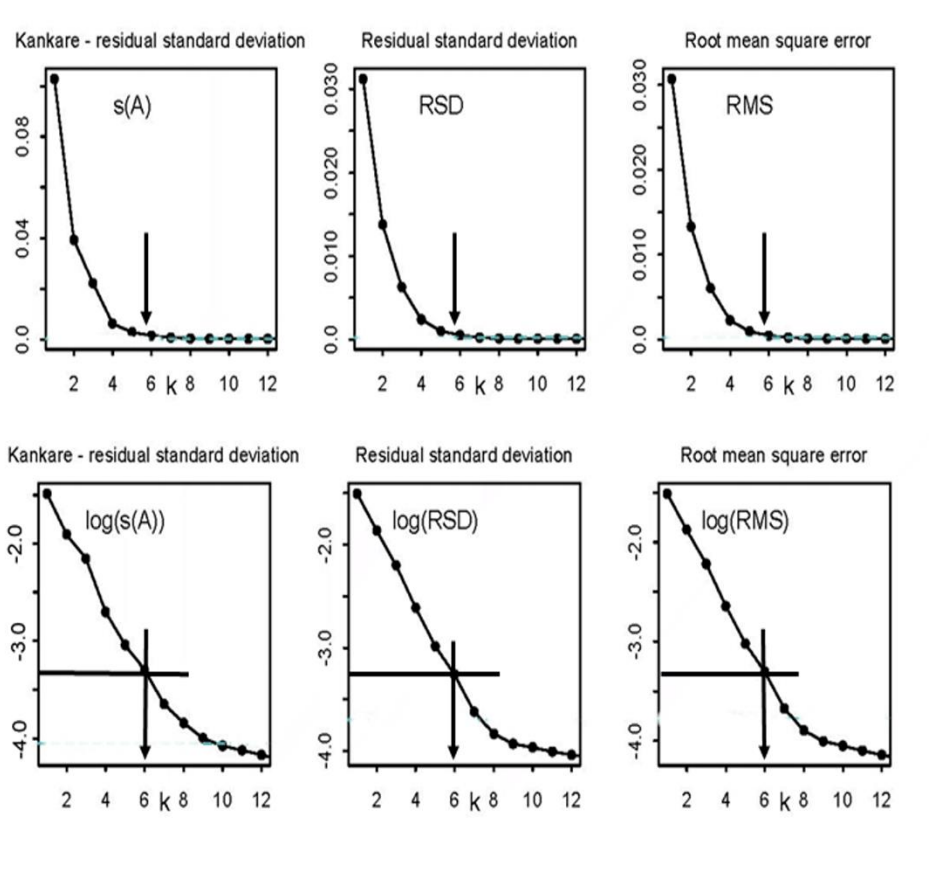
- Glass electrode HC103 (THETA ´90) – high precision
- Digital pH-metr HANNA HI 3220 (measurement pH in a range -2.00 to 20.00 with the precision ± 0.002 pH)
- Thermostat ED-5 (JULABO), thermometer
- Input of argon using polyethylene tube to keep carbondioxide-free solution
- Piston microburette for very precise dosing of KOH solution or HCl (±0,1μL)





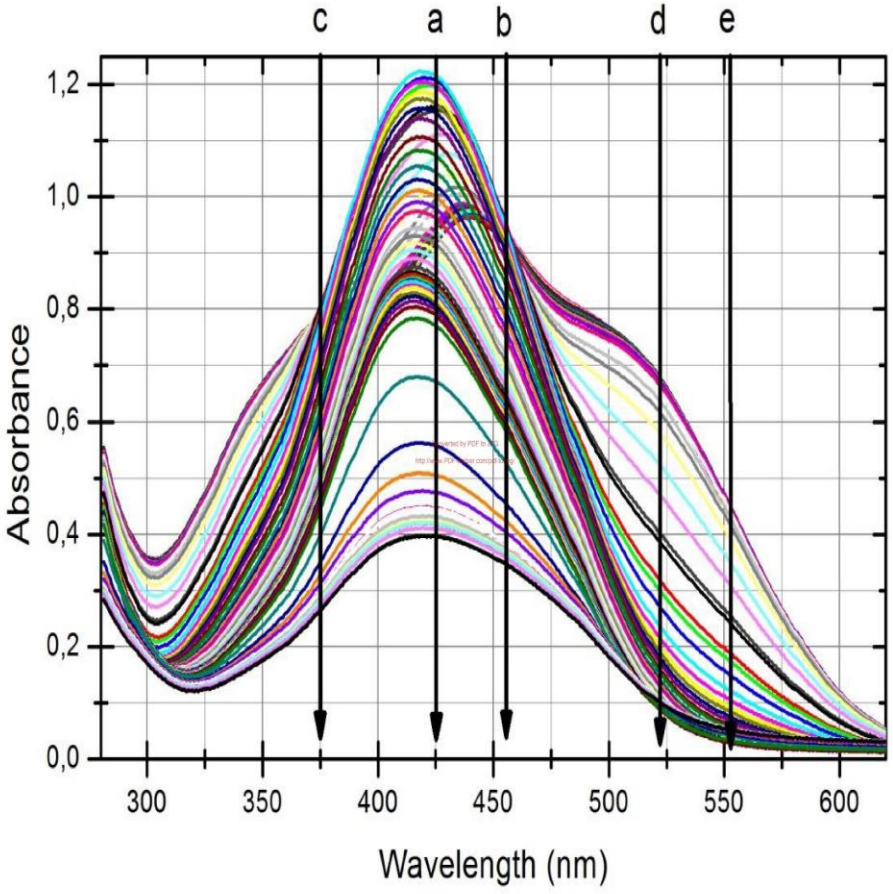
Determination of the number of light-absorbing species

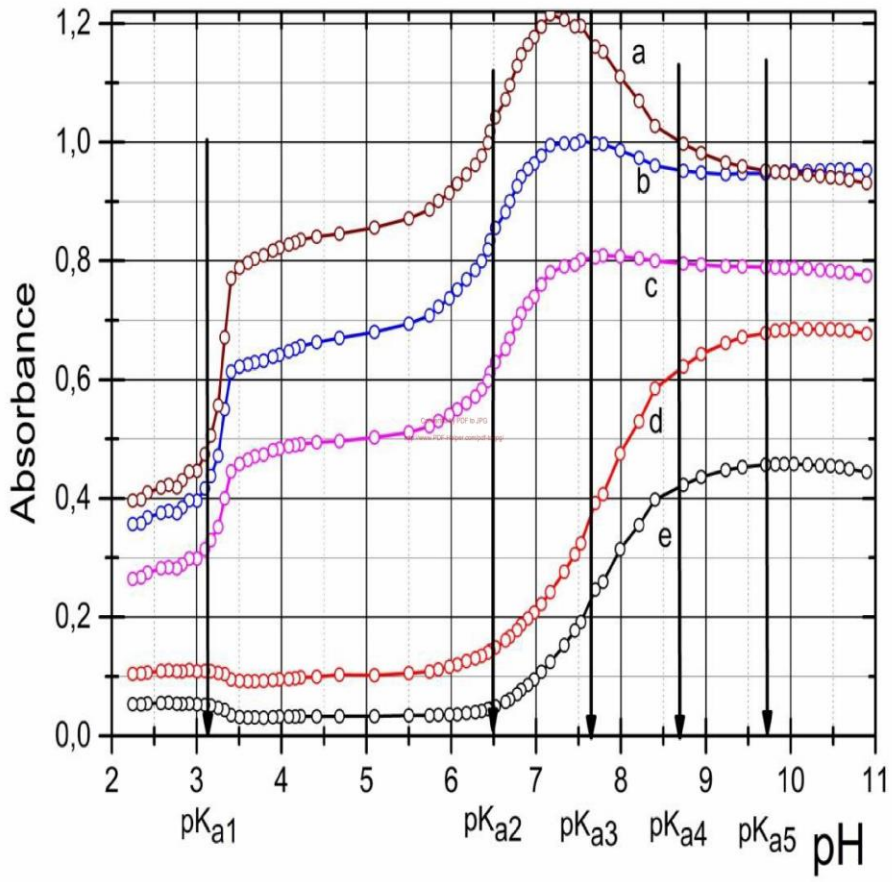




The 3D-absorbance-response-surface concerning 71 measured absorption spectra of protonation equilibria for 9,5 . 10^{-5} M Eltrombopag in dependence on pH at 25°C. The Cattel's scree plot of the Wernimont-Kankare procedure for the determination of the rank of the absorbance matrix of Eltrombopag $k^* = 6$ leads to six light-absorbing species in the mixture, $n_c = 6$, with the use of Kankare´s $s(A)$, RSD and RSM.

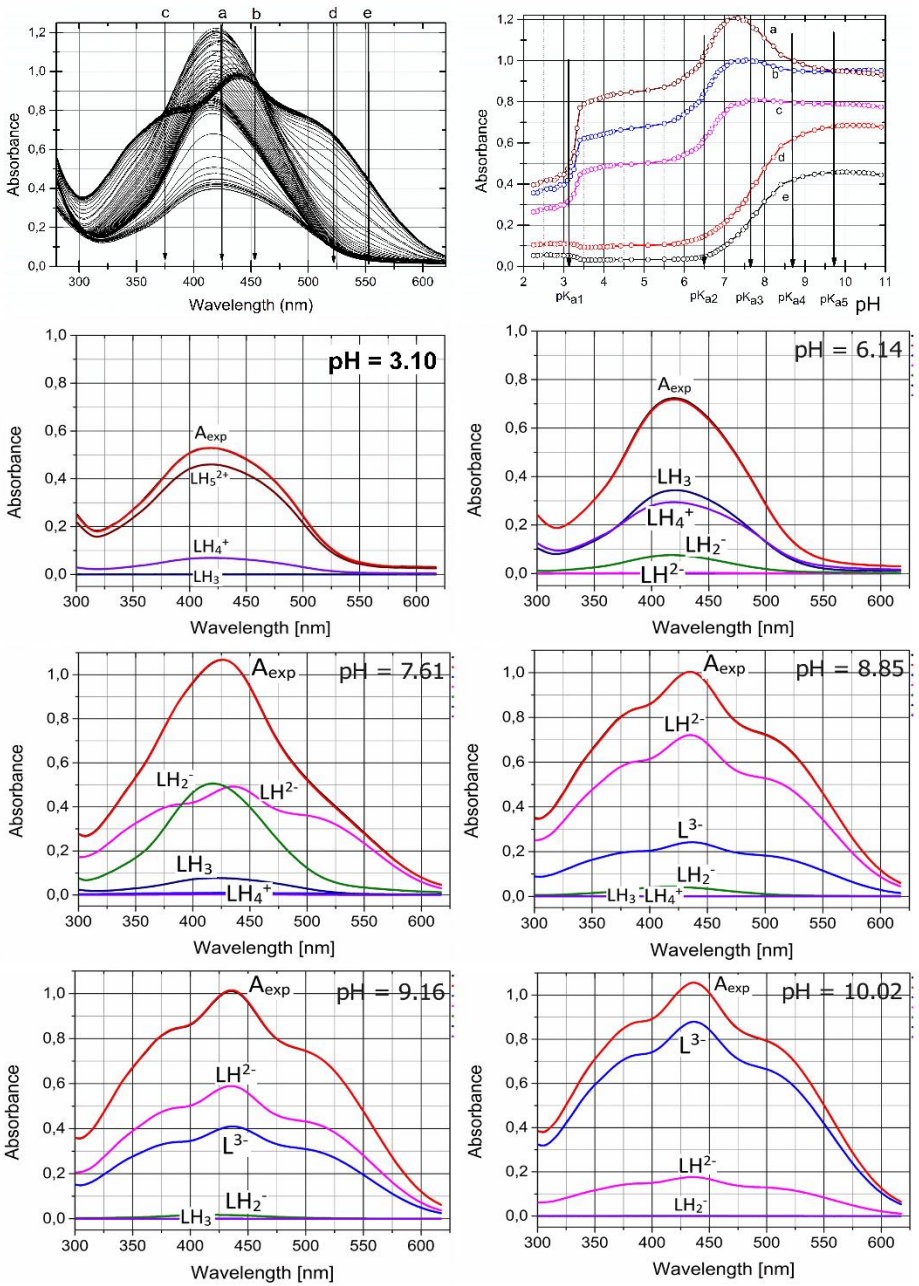
Analysis of A-pH curves at efficient wavelengths





In a spectra set the five analytical wavelengths ***a*** through ***e*** were selected at which the absorbance-pH curves were plotted. Six following figures from pH = 3.10 through pH = 10.02 show the consecutive deprotonation response in spectra, when each spectrum was deconvoluted on the spectrum of differently protonated species in mixture of Eltrombopag.

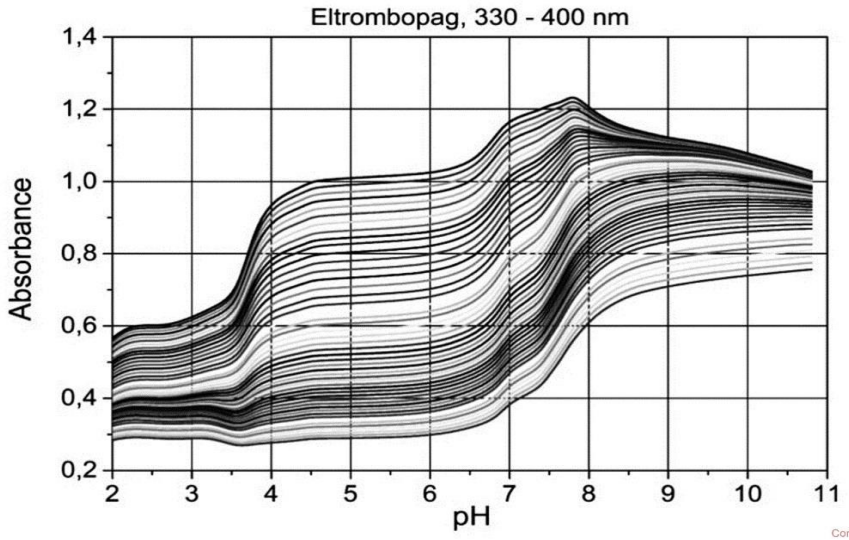
Protonation equilibria at the response wavelengths

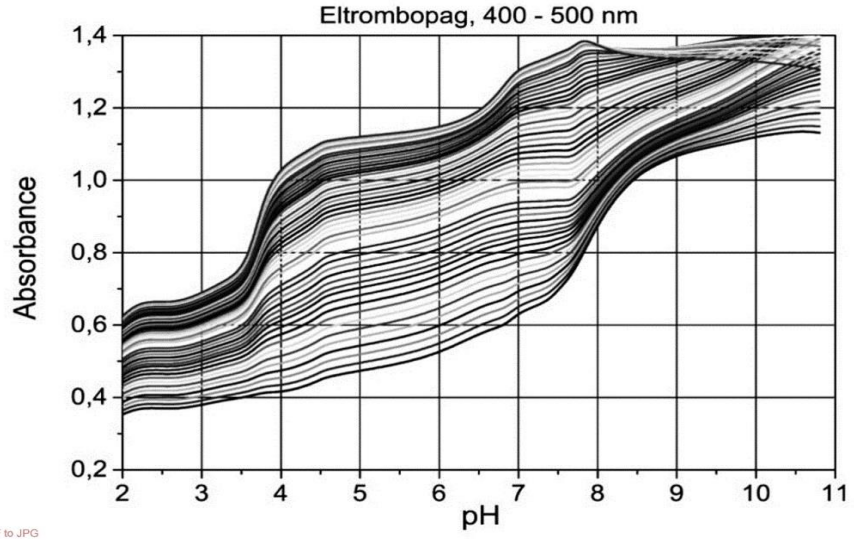


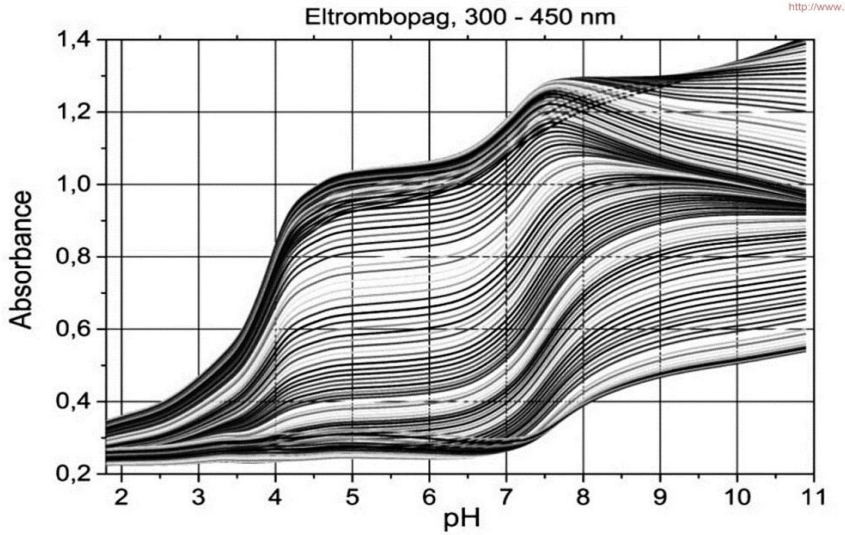
In a spectra set the five analytical wavelengths ***a*** through ***e*** were selected at which the absorbance-pH curves were plotted. Six following figures from pH = 3.10 through pH = 10.02 show the consecutive deprotonation response in spectra, when each spectrum was deconvoluted on the spectrum of differently protonated species in mixture of Eltrombopag. At pH = 3.10 the species LH_5^{2+} accompanied species LH_4^{+} predominates in the solution. At pH = 6.14 together with the species LH_3 two species LH_5^{2+}, LH_4^{+} exhibit absorption bands at the same wavelength of absorption maximum λ_{max}. At pH = 7.61 the experimental spectrum is decomposed to three absorption bands concerning the species LH_3 which dissociate to species LH_2^{-} and LH^{2-}. At pH = 8.85 and 9.16 the species L^{3-} occurs with species LH_2^{-} and LH^{2-}, and concentration of L^{3-} in the solution increases up to pH = 10.02

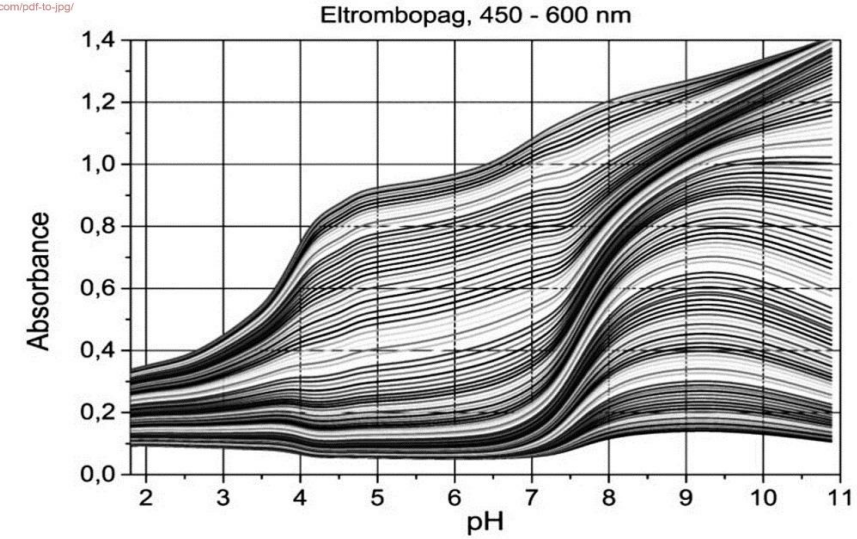
Search for a range of efficient wavelengths

Inputs of the absorbance data matrix show four regions of selected wavelengths of the 2D-absorbance-response spectra set for 9.5×10^{-5} M Eltrombopag in dependence on pH at 25℃

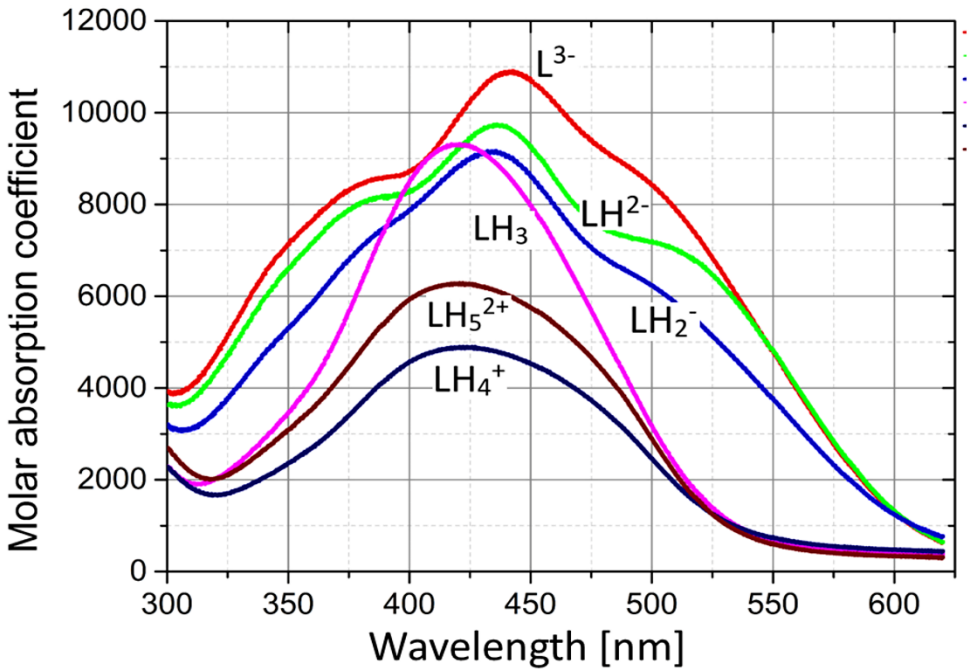




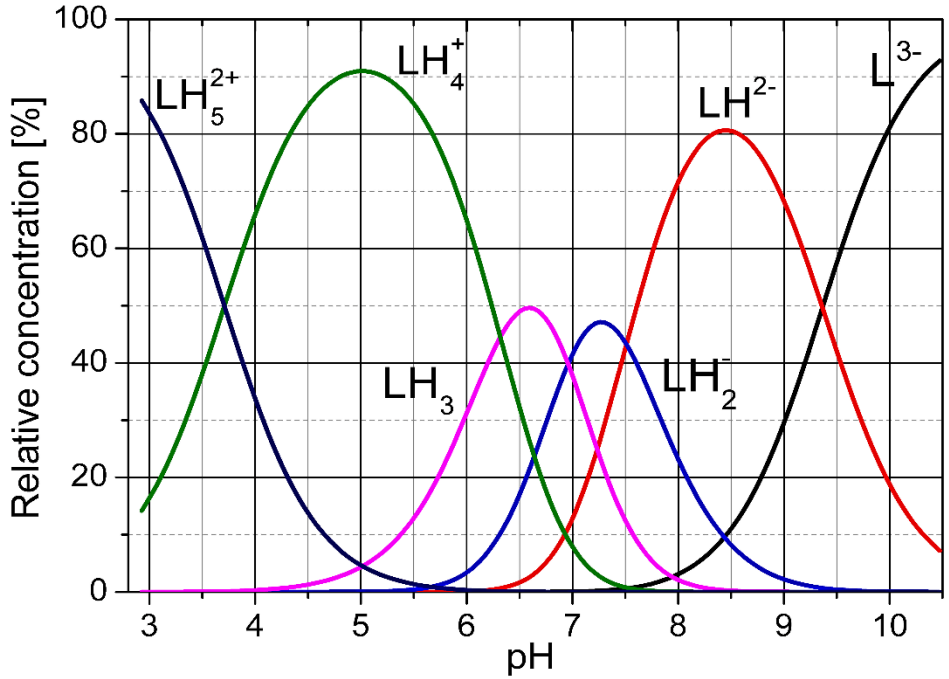




Resulting graphs of protonation equilibria

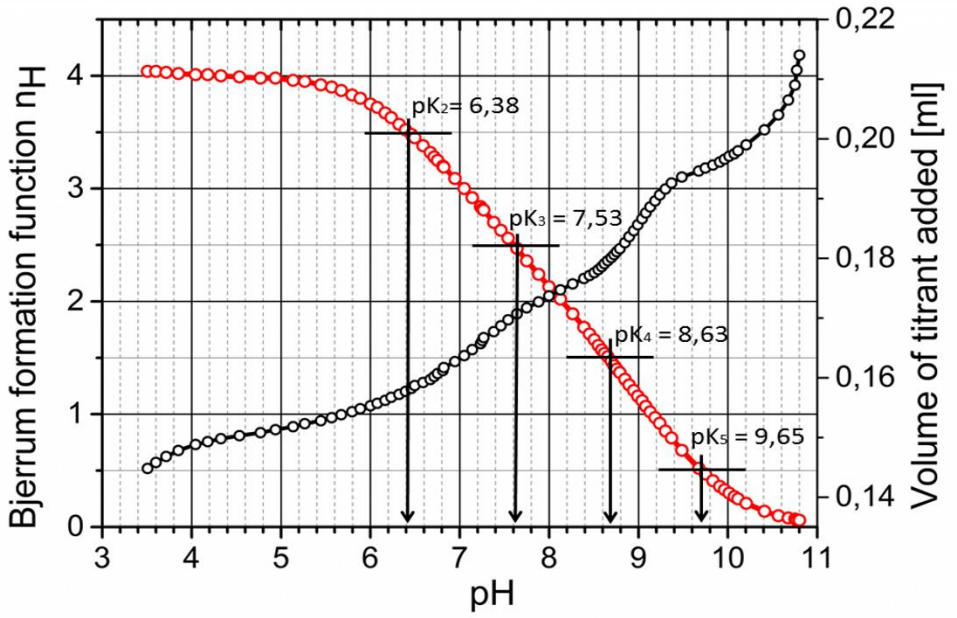


The graph of molar absorption coefficients for six variously protonated species of Eltrombopag *versus* wavelength.

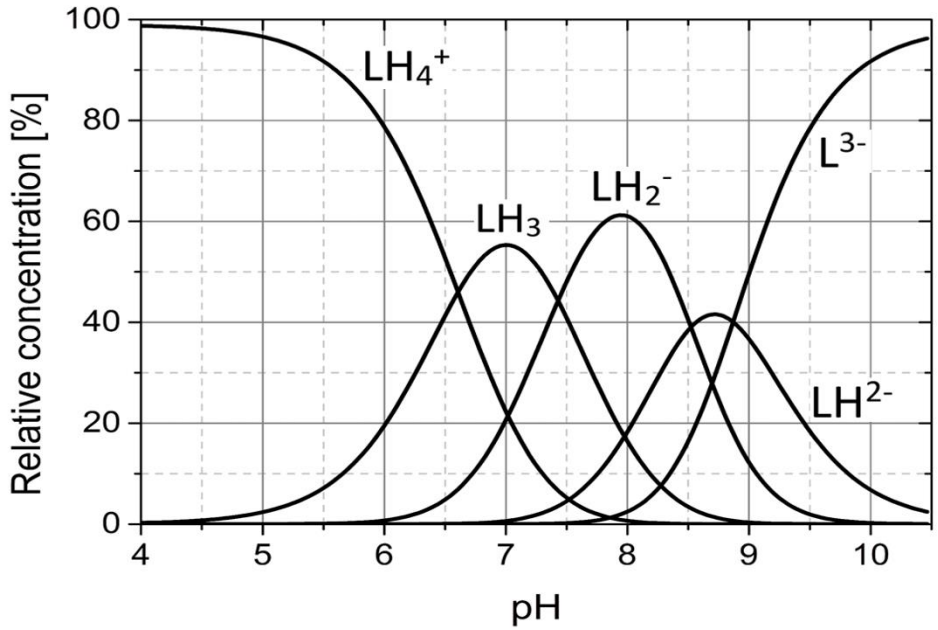


Corresponding distribution diagram of the relative concentration of six variously protonated species for Eltrombopag, (SPECFIT, ORIGIN)

Protonation equilibria of Eltrombopag analyzed with ESAB

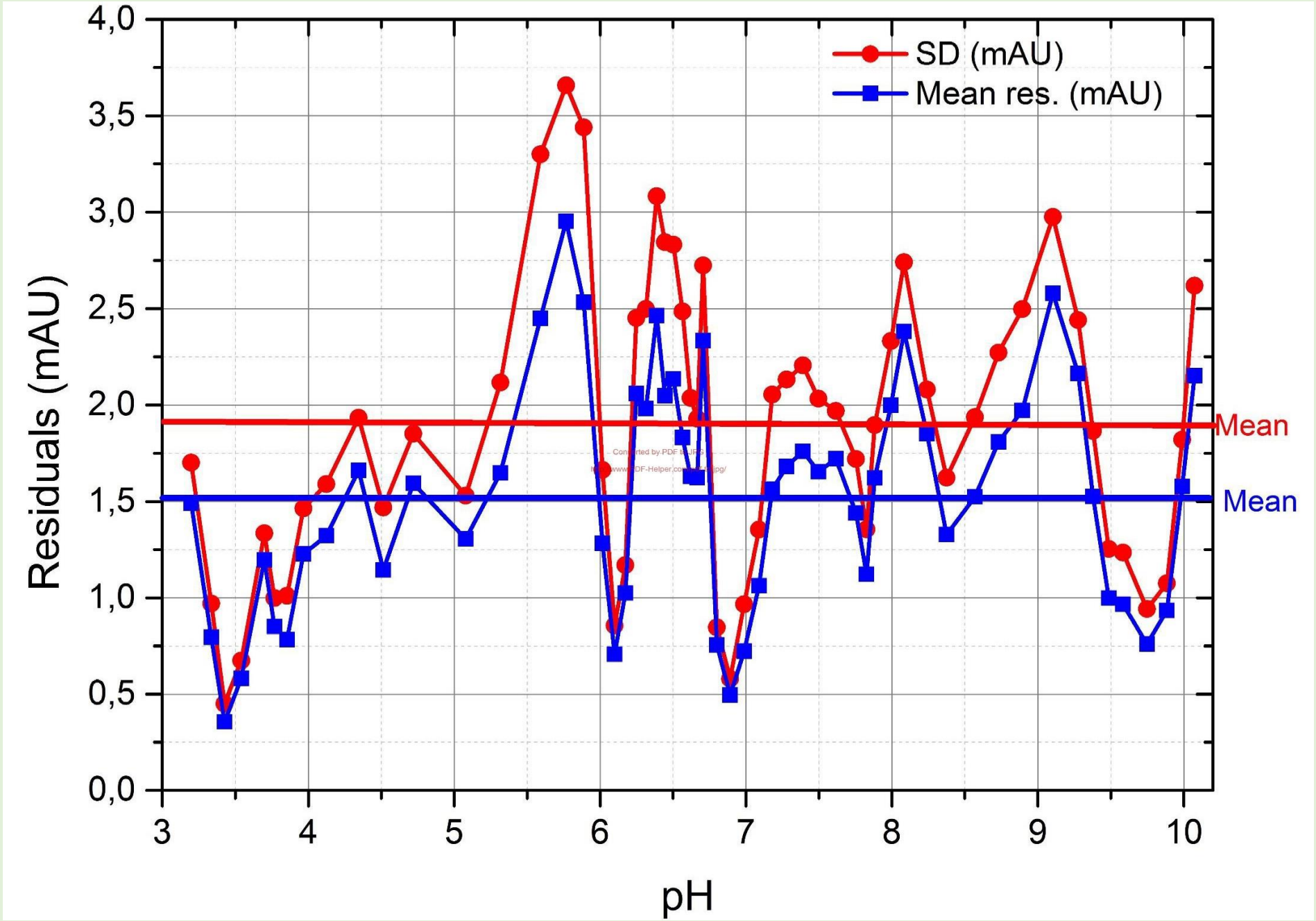


The pH-potentiometric titration curve of acidified Eltrombopag plus HCl titrated with KOH is plotted with the Bjerrum protonation function indicating pK values.



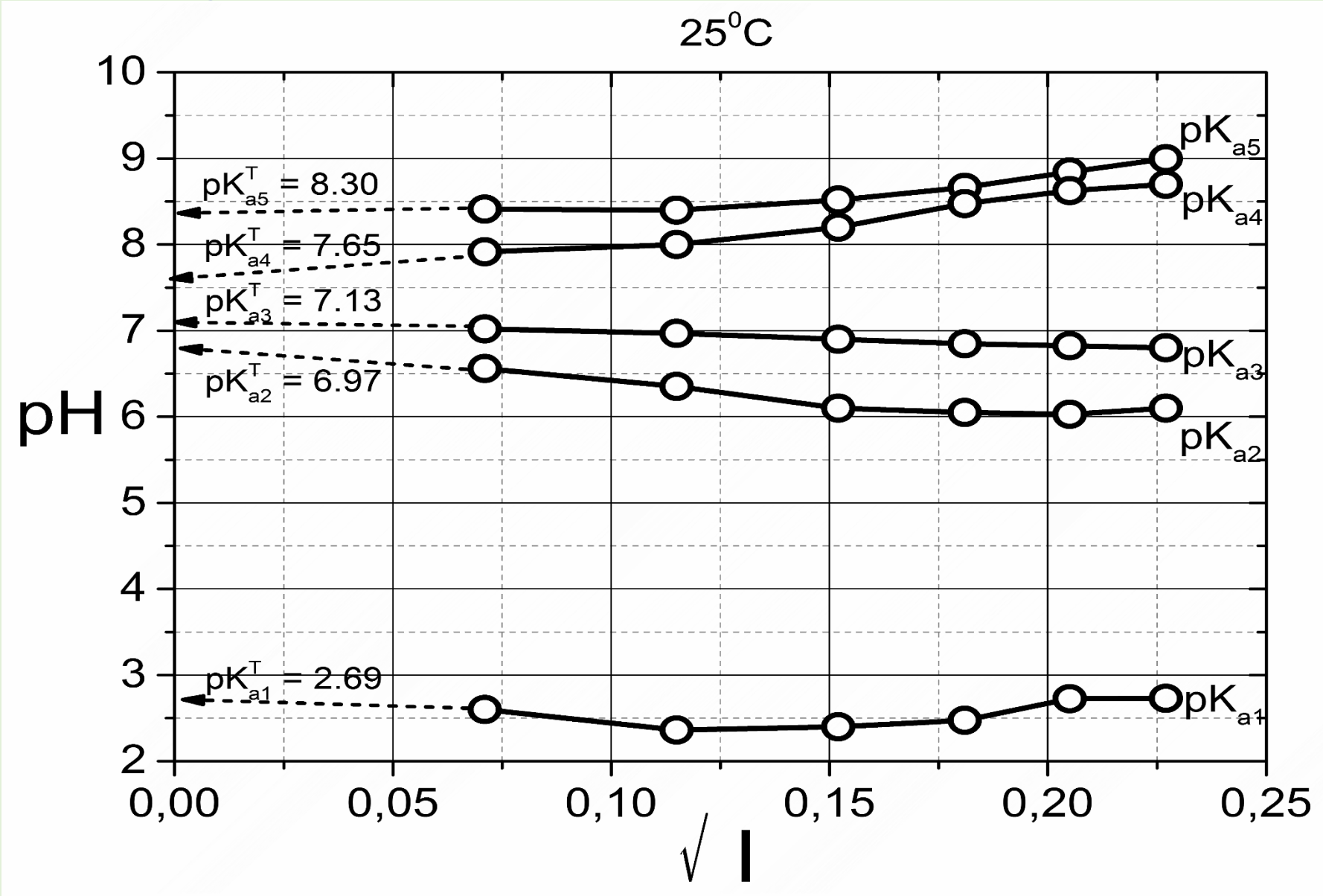
The distribution diagram of a relative presentation of variously protonated species L^{3-}, LH^{2-}, LH_2^-, LH_3 and LH_4^+ of Eltrombopag in dependence on pH at 25ºC, (ESAB, HYPERQUAD, ORIGIN)

Reliability of estimated pK_a´s using the goodness-of-fit test



Thermodynamic dissociation constants of Eltrombopag

Dependence of the mixed dissociation constants of Eltrombopag on the square root of the ionic strength for five dissociation constants at 25°C.



Conclusion

Spectrophotometric and potentiometric pH-titration allowed the measurement of five dissociation constants of Eltrombopag, but worse solubility at pH above 9 at Eltrombopag concentration of micromoles also pH down 5 limits an estimation of the pK_a higher than 10 and in potentiometry lower than 5.

1) In the neutral pH the Eltrombopag occurs in water sparingly soluble form LH_3, which is capable of protonation to form still soluble species LH_4^+. The species LH_3 can be dissociated into water soluble species L^{3-}. Acid-base titration of the triprotic molecule LH_3 with KOH leads to a mixture of six species H_3O^+, OH^-, LH_3, LH_2^-, LH^{2-}, L^{3-} and the potassium species K^+.

2) In the range of pH 2 to 10 five dissociation constants can be reliably estimated from the spectra when concentration of Eltrombopag is less than 10^{-4} M at an ionic strength I = 0.005 can be reliably determined with REACTLAB and SQUAD84 reaching the similar values with both programs. From a dependence on ionic strength the thermodynamic dissociation constants were estimated at 25°C pK_{a1}^T = 2.69, pK_{a2}^T = 6.97, pK_{a3}^T = 7.13, pK_{a4}^T = 7.65, pK_{a5}^T = 8.30

3) Four dissociation constants of Eltrombopag in concentration of 5 micromoles were determined by regression analysis of potentiometric titration curves without adjusting the ionic strength I = 0.005 and using ESAB and HYPERQUAD: pK_{a2} = 6.59(01), pK_{a3} = 7.56(04), pK_{a4} = 8.48(59), pK_{a5} = 9.29(34) at 25°C (Table 3). The standard deviations in the last valid unit number are in the brackets.

4) Prediction of the dissociation constants of Eltrombopag was performed using MARVIN program to specify protonation locations to give the values.