DETERMINATION OF NUMBER OF SPECIES IN EQUILIBRIUM MIXTURE

Zuzana Ferenčíková, Milan Meloun
University of Pardubice, Department of Analytical chemistry,Studenská 573, Pardubice, Czech Republic
zuzka.ferencikova@seznam.cz

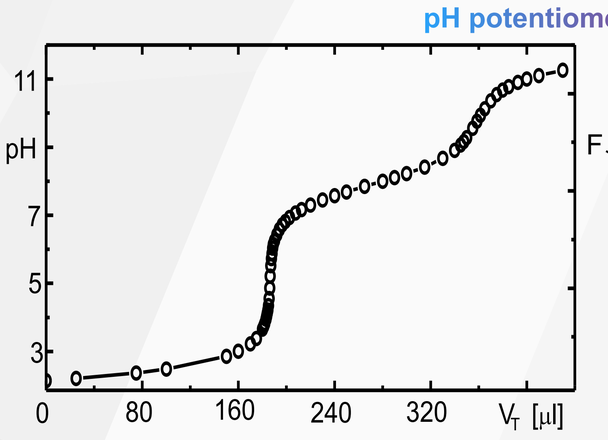
Summary
Protonation constants are very important both in the analysis of drug and in the interpretation of their mechanism of action, as they are key parametrs for predicting the extend of the ionozation of the drug molecule in solution at different pHs. The methodology of determination consists of several steps. The first step is the prediction of pK using MARVIN and SPARC programs based on quantum mechanics calculations. The next step is the determination of number of species in equilibrium mixture using algorithm INDICES containing several methods of factor analysis. Then we have to prove the chemical model using some different method like statistical analysis of proposed chemical model. On the basis of goodness-of-fit test we can decide which model is the best one. If we know the number of species we can determine not only the dissociation constants but also the distribution for each variously protonated species. Sitagliptin and methotrexate are demonstrated.

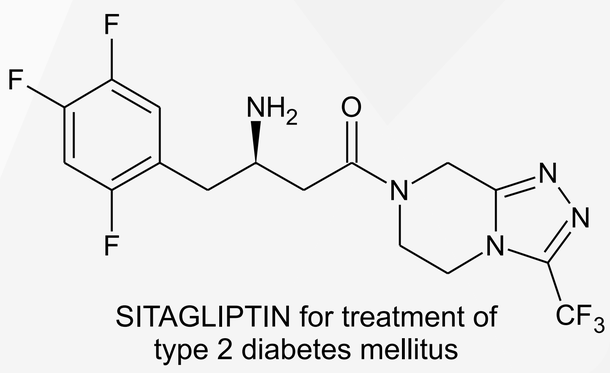
Theory
Potentiometry and spectrophotometry are often used to study solution equilibria. In the study of solution equilibria, it is not only necessary to determine the stability (protonation or dissociation) constant of each species, but also to find out how many such species are present in equilibrium mixture. The expression “chemical model” means the number of species in mixture, their stoichiometry, values of protonation constants and values of other physical constants (e.g. molar absorption coefficient). Consider the complex-forming equilibria of components L and H with the general reaction equation $qL + rH \leftrightarrow L_qH_r$ and the corresponding overall stability constants $\beta_{qr} = [L_qH_r]/([L]^q[H]^r)$.

Results and disscusion
Sitagliptin was measured using potentiometric titration. The existence of dimers was observed therefore several measurements at different concetrations of L and at different concentrations of KCl were made. The best chemical model was searched for each concetration of L and KCl. Statistical analysis of proposed models proved that the formation of dimers depends on both the concentration of L and the concentration of KCl.

Methotrexate was measured using spectrophotometric titration. This drug contains several centers of dissociation therefore the factor analysis (FA) was aplicated to the absorbance- response surface. Then the statistical analysis of proposed chemical models was used to prove results of FA. The results show that methotrexate contains four variosly protonated species in equilibrium mixture.

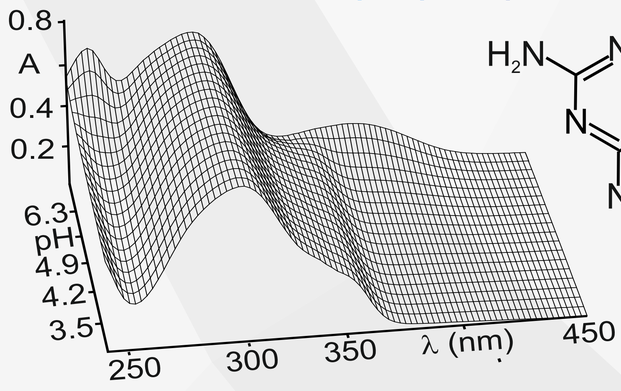
pH potentiometric titration

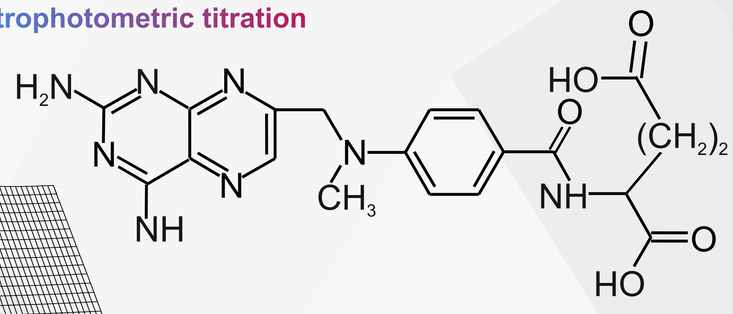




SITAGLIPTIN for treatment of type 2 diabetes mellitus

pH-spectrophotometric titration



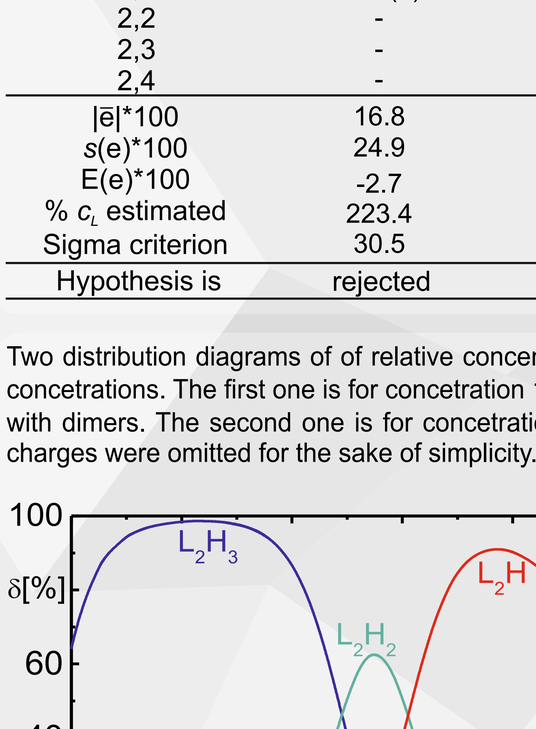


METHOTREXATE for chemotherapy of various neoplastic diseases

The equation for potentiometric titration curve must be transformed into the form of normalized variables $Z = f(\mathrm{pH})$ where the free equilibrium concestration of component H is usually measured potentiometrically and Z represents the average number of component H bound per H.

$$Z = (c_H - [H])/c_L$$

where c_H and c_L are the total concentrations of H and L, [H] is the free equilibrium concentration of H and n_c is the number of species in solution.



Each curve $Z = f(\mathrm{pH})$ has n_s points and curves can be measured for n_B total concentrations to give finally an $n_s \times n_B$ matrix **Z**.
In matrix notation we can write:

$$\mathbf{Z} = \mathbf{EC}$$

where **Z** is $(n_s \times n_B)$, **E** is $(n_B \times n_C)$ and the concentration matrix **C** is $(n_C \times n_s)$.
The second moment matrix **M** is defined by

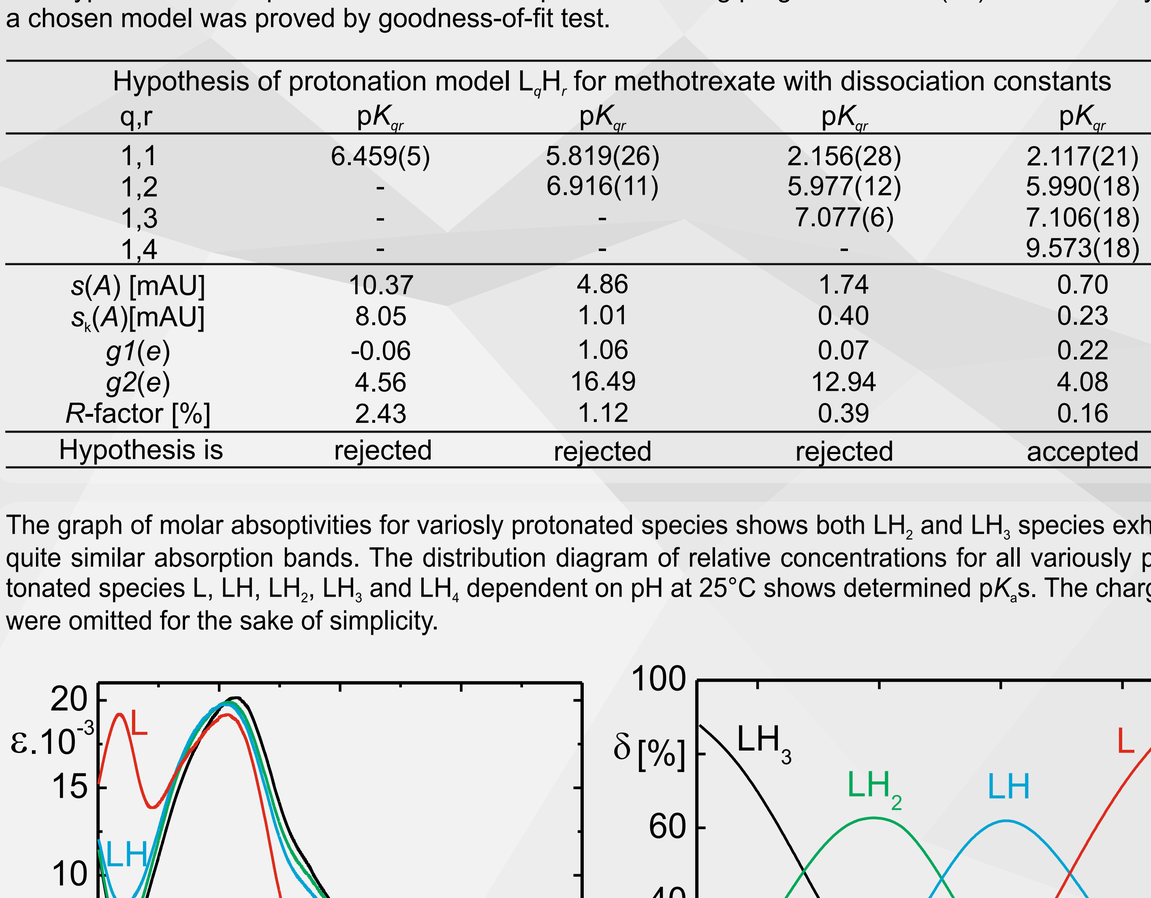
$$\mathbf{M} = (1/n_s)\cdot\mathbf{Z}\cdot\mathbf{Z}^T$$

where $\mathbf{Z}^T$ denotes the transpose of **Z**. The residual standard devition of **Z** is given by

$$s_k(\mathbf{Z}) = \sqrt{\frac{\mathrm{tr}(\mathbf{M}) - S_{i=1}^{k} r_i}{n_B - k}}$$

where tr(**M**)is the trace of **M** and k is the number of independent conponents in the equilibruim system.

The measured spectra were analyzed using INDICES algorithm to determine the number of light-absorbing species. The INDICES algorithm contains several methods of factor analysis including second and third derivation or ratio of derivations.



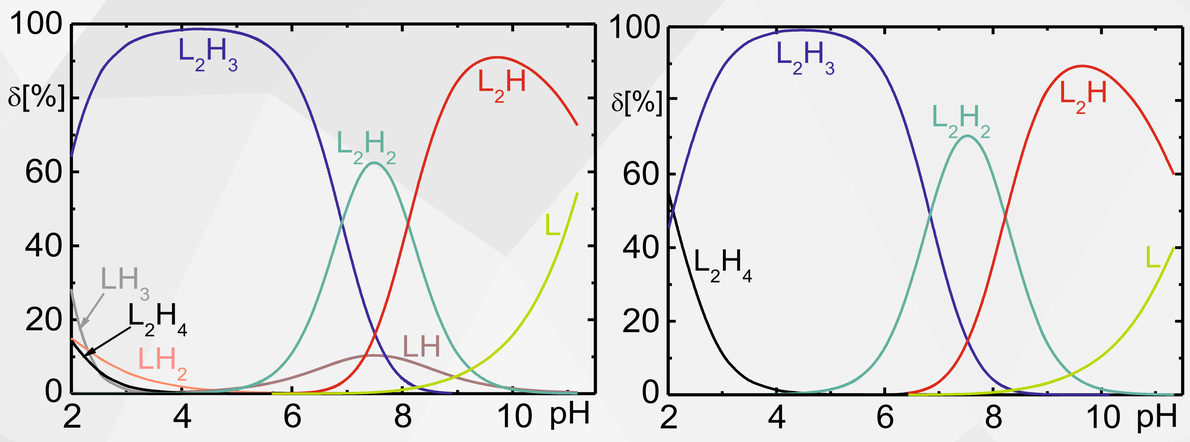
The hypothesis of the protonation model can be proven using program HYPERQUAD2008. The reliability of a chosen model was proved by goodness-of-fit test.

Hypothesis of protonation model L_qH_r for sitagliptin with protonation constants				
q,r	log β_{qr}	log β_{qr}	log β_{qr}	log β_{qr}
2,1	9.03(2)	10.10(4)	13.79(10)	13.78(1)
2,2	-	17.12(4)	21.94(10)	21.93(2)
2,3	-	-	28.69(10)	28.82(2)
2,4	-	-	-	30.90(2)
$\lvert\bar{e}\rvert$*100	16.8	23.1	17.4	1.3
s(e)*100	24.9	37.0	33.9	1.7
E(e)*100	-2.7	-13.6	-13.4	0.0
% c_L estimated	223.4	112.0	104.7	100.7
Sigma criterion	30.5	29.9	24.7	4.0
Hypothesis is	rejected	rejected	rejected	accepted

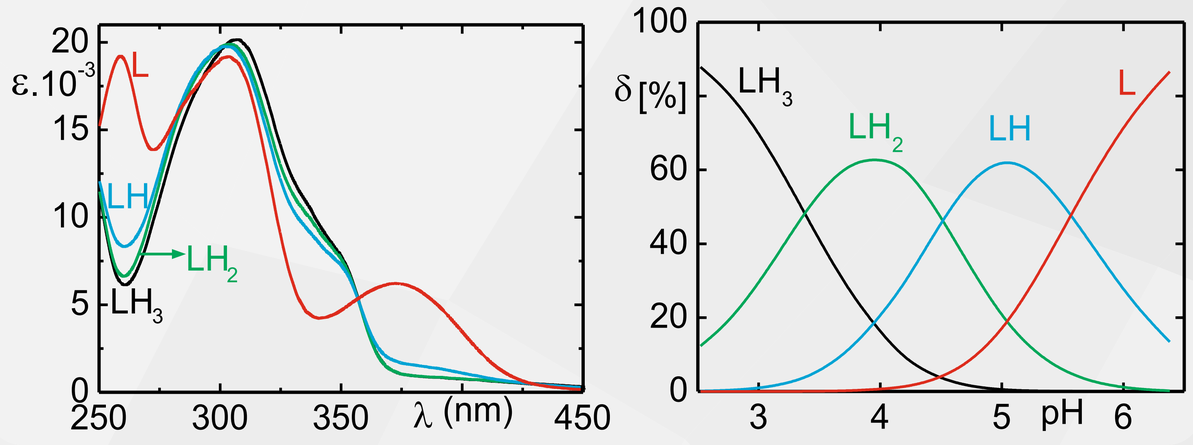
The hypothesis of the protonation model was performed using program SQUAD(84). The reliability of a chosen model was proved by goodness-of-fit test.

Hypothesis of protonation model L_qH_r for methotrexate with dissociation constants				
q,r	pK_{qr}	pK_{qr}	pK_{qr}	pK_{qr}
1,1	6.459(5)	5.819(26)	2.156(28)	2.117(21)
1,2	-	6.916(11)	5.977(12)	5.990(18)
1,3	-	-	7.077(6)	7.106(18)
1,4	-	-	-	9.573(18)
$s(A)$ [mAU]	10.37	4.86	1.74	0.70
$s_k(A)$[mAU]	8.05	1.01	0.40	0.23
$g1(e)$	-0.06	1.06	0.07	0.22
$g2(e)$	4.56	16.49	12.94	4.08
R-factor [%]	2.43	1.12	0.39	0.16
Hypothesis is	rejected	rejected	rejected	accepted

Two distribution diagrams of of relative concentrations of variously protonated species for two different concetrations. The first one is for concetration 13.5 mmol·dm^{-3} when monomers exist in minority together with dimers. The second one is for concetration 16.7 mmol·dm^{-3} when only dimers can be found. The charges were omitted for the sake of simplicity.



The graph of molar absoptivities for variosly protonated species shows both LH_2 and LH_3 species exhibit quite similar absorption bands. The distribution diagram of relative concentrations for all variously protonated species L, LH, LH_2, LH_3 and LH_4 dependent on pH at 25°C shows determined pK_as. The charges were omitted for the sake of simplicity.



Conclusion
Factor analysis and statistical analysis of proposed chemical models in case of sitagliptin and methotrexate can be used as an effective and powerful tool for determination of number of species in equilibrium mixture for spectrospocic and potentiometric titration data.

Literature
Meloun, M.; Syrový, T.; Vrána, A., Anal. Chim. Acta 2003, 489, 137-151
Havel, J.; Meloun, M., Talanta 1985, 32(3), 171-175
Meloun, M.; Havel, J.; Högfeldt, E., Ellis Horwood Publishing, Chichester 1988, pp. 297