Determination of Number of Species in Equilibrium Mixture

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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 parameters for predicting the extend of the ionization of the drug molecule in solution at different pHs. The methodology of determination consists of several steps. The first step is the prediction of pKₐ using MARVIN and SPARC programs based on quantum mechanical 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. Silagpilin 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 stoichiometries, 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 

\[ qL + pH \leftrightarrow \text{LH}_q \]

and the corresponding overall stability constants \( K_{q,r} = [LH_q]^q ([L]^m[H]^n) \).

Results and discussion
Silagpilin was measured using potentiometric titration. The existence of dimers was observed therefore several measurements at different concentrations of L and at different concentrations of KCl were made. The best chemical model was searched for each concentration 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.

The equation for potentiometric titration curve must be transformed into the form of normalized variables \( Z = f(pH) \) where the free equilibrium concentration of component H is usually measured potentiometrically and \( Z \) represents the average number of component H bound per H. The hypothesis of the protonation model can be proven using program HYPERQUAD08. The reliability of a chosen model was proved by goodness-of-fit test.

\[ Z = (c_0 - [H])c_1 \]

where \( c_0 \) and \( c_1 \) are the total concentrations of H and L. \([H]\) is the free equilibrium concentration of H and \( n_i \) is the number of species in solution.

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

The graph of molar absorbivities for variously protonated species shows both LH₂ and LH₃ species exhibit quite similar absorption bands. The distribution diagram of relative concentrations for all variously protonated species shows both LH₂ and LH₃ species exhibit quite similar absorption bands. The distribution diagram of relative concentrations for all variously protonated species shows both LH₂ and LH₃ species exhibit quite similar absorption bands.

## Literature
Havel, J.; Meloun, M., Talanta 1989, 32(3), 171-175

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