# UV-VIS SPECTROSCOPY IN THE DETERMINATION OF DISSOCIATION CONSTANTS OF SOME DRONATES

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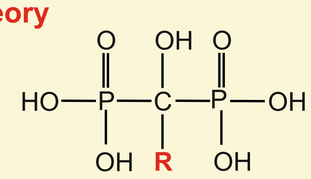
## Summary
Alendronate sodium and risedronate sodium belong to a group of drugs called nitrogen-containing bisphosphonates (N-BPs). The mixed dissociation constants at various ionic strengths I and at 25°C and 37°C have been determined with the use of the nonlinear regression analysis of the multiwavelength spectrophotometric pH-titration data.

## Experiment
The free hydrogen-ion concentration h was measured on an Hanna 3220 digital voltmeter using a THETA HC-103VFR glass electrode. The spectrophotometricmultiple-wavelength pH-titration was carried out as follows: an aqueous solution 20.0 $cm^3$ containing $10^{-5}$ mol/L drug, 1 mol/L hydrochloric acid and indifferent solution KCl for adjustment of constant value of an ionic strength was titrated with standard 1.0 mol/L KOH and absorption spectra were recorded. Titrations were performed in a water jacketed double-walled glass vessel of 100 mL,closed with a Teflon bung containing the electrodes, an argon inlet, a thermometer, a propeller stirrer and a capillary tip from a microburette. All pH measurements were carried out at 25.0°C and 37.0°C. The burettes used were syringe micro-burettes of 1250L capacity (META, Brno) with a 2.50 cm micrometer screw, [54]. The solution was pumped into the cuvette and spectrophotometric measurement was performed with the use of a Cintra 40 spectrophotometer (GBC,Australia).

## Theory


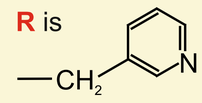

Alendronate

**R** is



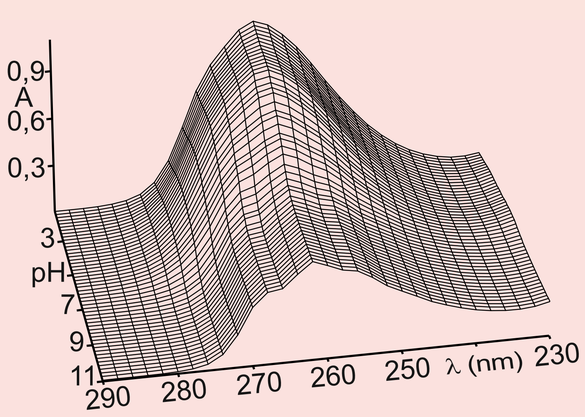

Risedronate

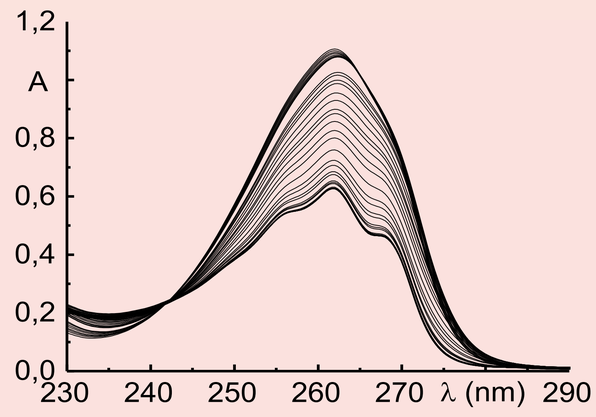
**R** is




The N-BPs have a six groups which can dissociate: five $H^+$ donors (four POH groups, one gemine $OH^-$ group) and one amino group as the $H^+$ acceptor. To determine dissociation constants for polyprotic system in which the dissociation steps are well separated ($\Delta pK_a = |pK_{a,i+1} - pK_{a,i}| > 3$) is possible; If the dissociation steps are close and overlapping, the $pK_{a,i}$ values describe the stoichiometry but not the site of protonationas there are intermediate species that have the same number of protons but at different protonation sites.The acid-base properties of two N-BPs in aqueous solution have been studied in pH range from 2 to 12 and can be described in terms of four dissociation steps: $pK_{a,2}$, $pK_{a,4}$, $pK_{a,5}$ related withdissociation of POH groups and $pK_{a,3}$ related with dissociation of $NH_3^+$. The mixed dissociation constants were determined at different ionic strengths and of 25°C and 37°C using SQUAD(84) and SPECFIT/32 for spectrophotometric data and ESAB2M and HYPERQUAD2008 for potentiometric data. The thermodynamic dissocaition constatns were estimated applying Debye-Hückel equation.

## Results and Discussion:
The mixed dissociation constants of Risedronate sodium were determined using multiwavelength spectrophotometric pH titration.The spectra were measured on spectrophotometr GBC Cintra 40 with an instumental error $s_{inst}(A)$= 0.35 mAU guaranteeing required accuracy for demanding regression analysis.

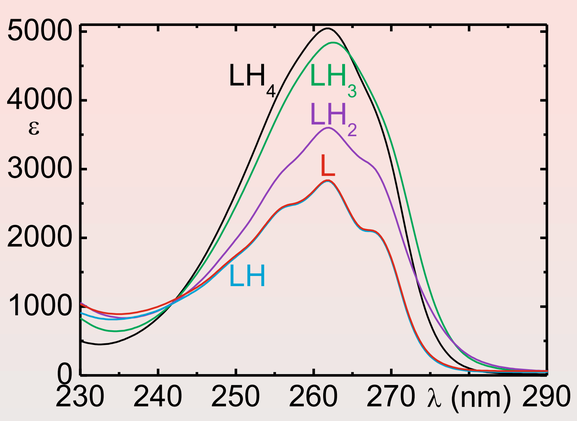


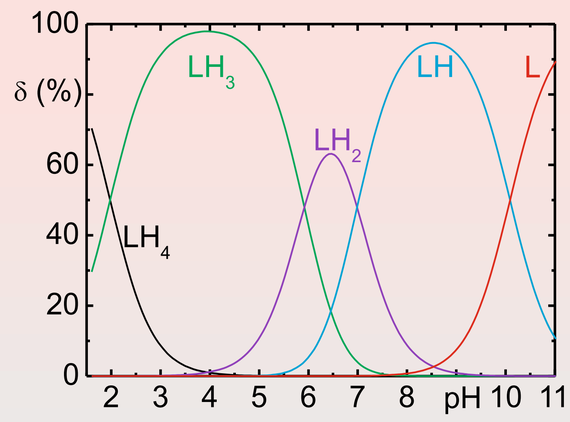




The hypothesis of the protonation model was performed using program SQUAD(84). The reliability of a chosen model was proved by goodness-of-fit test.

| Hypothesis of protonation model $L_qH_r$ with dissociation constants $pK_{qr}$ | | | | |
|---|---|---|---|---|
| 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 |

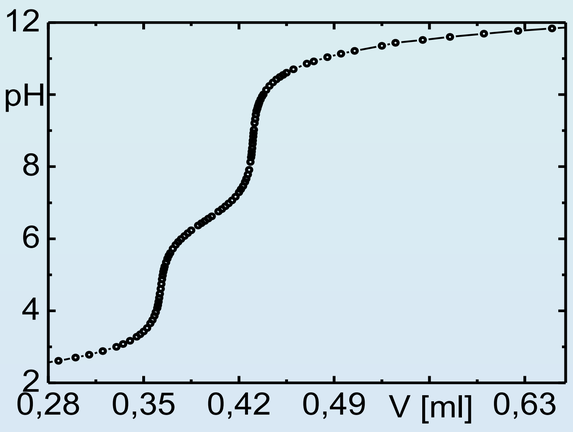
The graph of molar absorptivities for the variously protonated species shows both L and LH species exhibit quite similar absorption bands. The distribution diagram of relative concentrations for all variosly protonated species L, LH, $LH_2$, $LH_3$ and $LH_4$ dependent on pH at 25°C shows determined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

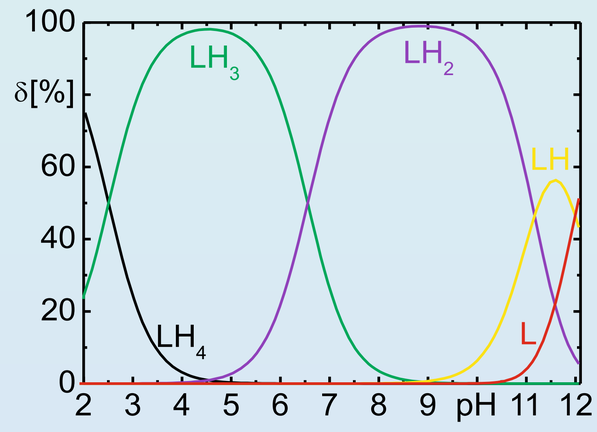


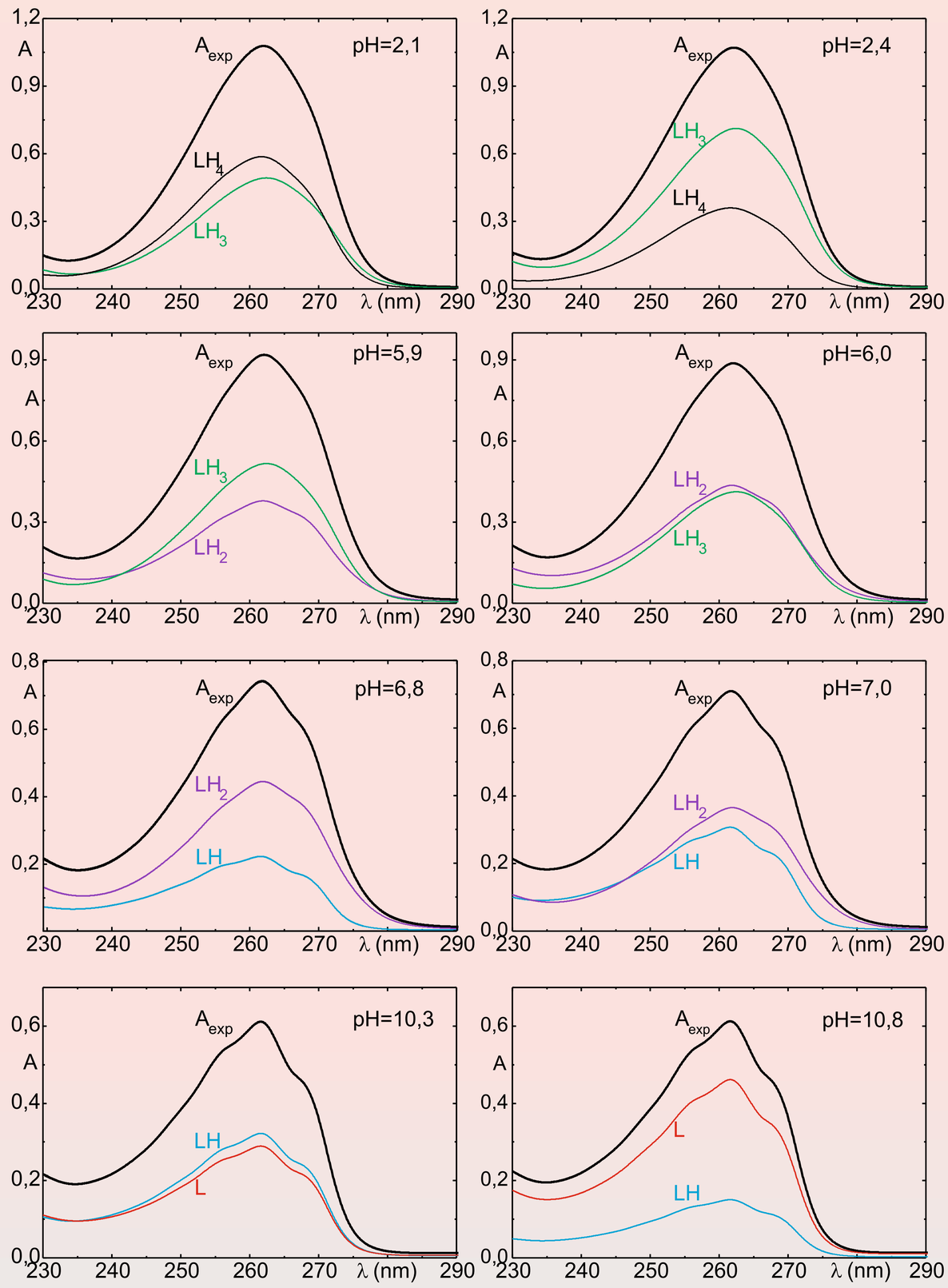

Risendronate and Alendronate are soluble enough to using a potentiometric pH-titration as a comparing method. We used the same electrode and pH meter as in case of spectrophotometric titration and the experiment conditions were the same.

The potentiometric titration curve of Alendronate shows three dissociations are possible. Using program ESAB and HYPERQUAD we determined four mixed dissociation constants. Because the constant $pK^T_{a4}$ and $pK^T_{a5}$ are very close we cannot distinguish them on the titration curve. Aplying the Debye-Hückel law we determined the thermodynamic dissociation constants for Alendronate: $pK^T_{a2}$= 2.60(0), $pK^T_{a3}$= 6.73(0), $pK^T_{a4}$= 11.51(2) and $pK^T_{a5}$= 12.44(3). Standard deviations of parameter estimated in last valid digits are in the brackets.




Deconvolution of the experimental absorption spectrum into a spectra of the individual variously protonated species for important values of pH show changes in spectra of each species in selected pH before and after dissociation.




## Conclusion:
The thermodynamic dissociation constants were estimated by nonlinear regression of ($pK_a$,$I$) data and a Debye-Hückel equation. Goodness-of-fit test based on regression diagnostics is measure of a reliability of parameters and prove that reliable estimates for alendronate $pK^T_{a2}$=2.13(9) and 2.35(3), $pK^T_{a3}$=6.68(1) and 6.41(5), $pK^T_{a4}$=8.92(12) and 9.22(4), $pK^T_{a5}$=11.47(3) and 11.38(3) at 25°C and 37°C, respectively, and for risedronate $pK^T_{a2}$=2.37(1) and 2.44(1), $pK^T_{a3}$=6.29(3) and 6.26(1), $pK^T_{a4}$=7.48(1) and 7.46(2), $pK^T_{a5}$=9.31(7) and 8.70(3) at 25°C and 37°C, respectively, were found.

## Acknowledgement:
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## Literature:
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