JOURNAL OF CHEMICAL & ENGINEERING DATA

ARTICLE

pubs.acs.org/jced

Thermodynamic Dissociation Constants of Alendronate and Ibandronate by Regression Analysis of Potentiometric Data

Milan Meloun,*[,†] Zuzana Ferenčíková,[†] Lucie Netolická,[†] and Tomáš Pekárek[‡]

[†]Department of Analytical Chemistry, University of Pardubice, 532 10 Pardubice, Czech Republic

[‡]Zentiva Group a.s., U Kabelovny 130, 102 37 Prague 10, Czech Republic

ABSTRACT: Mixed dissociation constant(s) of two nitrogen-containing bisphosphonate acids H_JL, *alendronate* and *ibandronate*, at various ionic strengths I in the range of (0.02 to 0.30) M at (298.15 and 310.15) K have been determined with the use of regression analysis of potentiometric titration data when common parameters $pK_{a,j}$, J = 1, ..., J, and group parameters L_0 and H_T are simultaneously refined. Ibandronate and alendronate are the antiresorptive drugs most widely used to treat osteoporosis owing to their particularly high potency at inhibiting osteoclast-mediated bone resorption. External calibration of the glass electrode cell in the activity scale a_{H+} was used. The estimate of ill-conditioned group parameters has a great influence on a systematic error in the estimated pK_a, and therefore it makes the computational strategy important. As more group parameters are refined and a better fit is achieved, a more reliable estimate of dissociation constants results. The thermodynamic dissociation constant pK_a^T was estimated by a nonlinear regression of $\{pK_a, I\}$ data and a Debye−Hückel equation. A goodness-of-fit test based on regression diagnostics is the measure of a reliability of parameters and proves that reliable estimates for alendronate pK_{a2}^T = 2.60(1) and 2.76(1), pK_{a3}^T = 6.73(1) and 6.77(1), pK_{a4}^T = 11.51(2) and 11.29(1), and pK_{a5}^T = 12.44(3) and 11.82(3) at (298.15 and 310.15) K, respectively, and for ibandronate pK_{a2}^T = 2.33(1) and 2.50(1), pK_{a3}^T = 6.31(1) and 6.37(1), and pK_{a4}^T = 10.74(1) and 10.65(1) at (298.15 and 310.15) K, respectively, were found.

INTRODUCTION

The bisphosphonates have been known to chemists since the middle of the 19th century, when the first synthesis occurred in 1865 in Germany and a historical survey of their mechanism of action brings a tutorial review by Fleisch.[1] Only in the past three decades have bisphosphonates been developed as drugs for use in various diseases of bone, tooth, and calcium metabolism. Bisphosphonates are compounds characterized by two C−P bonds. If the two bonds are located on the same carbon atom, the compounds are called germinal bisphosphonates, and they are analogues of pyrophosphates that contain an oxygen atom instead of a carbon atom.[2] Two nitrogen-containing bisphosphonates, for example, ibandronate and alendronate denoted as N-BPs (Figure 1), are the antiresorptive drugs[3] most widely used to treat osteoporosis owing to their particularly high potency at inhibiting osteoclast-mediated bone resorption.[4,5] They also inhibit growth of the amoeboid microorganism *Dictyostelium discoideum*.[6−8] These N-BPs are stable analogues of naturally occurring pyrophosphate compounds.[9,10] Bisphosphonate N-BPs reduce bone resorption and turnover through their interference with osteoclast activity.[1] Bisphosphonate N-BPs are now the major drugs used in the treatment of postmenopausal osteoporosis and represent the first-line therapy in the majority of patients.[11−16] The evolution of the chemical moieties on the R_1 and R_2 positions in the bisphosphonate structure in Figure 1 has resulted in a progressive increase in antiresorptive potency in binding affinities.[9,10,17−19] The R_1 moiety confers additional binding affinity;[9,17,20] for example, replacing a hydrogen atom with a hydroxyl group at R_1 increases the affinity for hydroxyapatite by about two-fold. The introduction of nitrogen components, such as primary and tertiary nitrogens or heterocyclic rings at the R_2 position, increased the antiresorptive potency of bisphosphonates by up to three orders of magnitude compared to that of non-nitrogen containing bisphosphonates.[1,19,21]

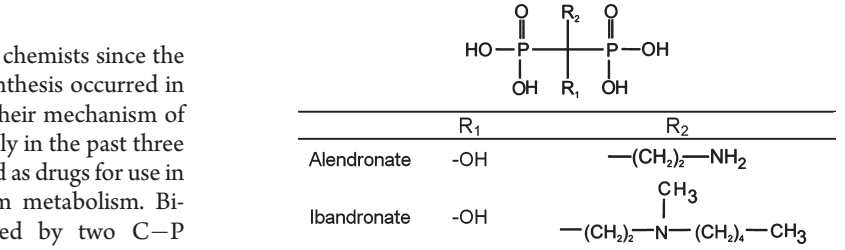

Figure 1. Basic structure of two nitrogen-containing bisphosphonates N-BPs, where R_1 means the group which assists binding to hydroxyapatite and R_2 means the side chain which determines potency dependent on mineral binding and biochemical action.

Boichenko et al.[22] has shown that the acid−base titrimetry is useful for routine analysis of sodium alendronate due to its accuracy, simplicity, and low cost. However, the theoretical basics of the determination of sodium alendronate by acid−base titration are complicated by very close third, fourth, and fifth dissociation constants of alendronic acid. Moreover, alendronic acid is very weak by the third dissociation step (Figure 2), and its titration should be problematic.[23,24] At ionic strength 0.1 M KCl and 298.15 K, dissociation constants were estimated as

Received: May 26, 2011
Accepted: August 20, 2011
Published: September 08, 2011

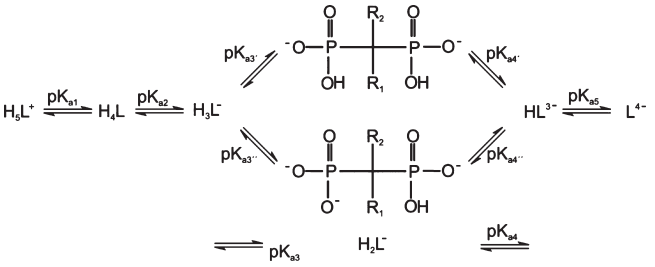

Figure 2. Schema of the dissociation of nitrogen-containing bisphosphonates (N-BPs) according to Fleisch.[1]

$pK_{a2} = 2.24 \pm 0.01$, $pK_{a3} = 6.38 \pm 0.03$, $pK_{a4} = 10.68 \pm 0.06$, and $pK_{a5} = 11.4 \pm 0.2$.

Hägele et al.[25] proved that the increasing distance between the bisphosphonate and the ammonium units (pamidronate → alendronate) leads to a significant increase in pK_{a1} to pK_{a4}; that is, pK_{a1} is 1.24 for pamidronate (P) and 1.33 for alendronate (A), pK_{a2} is 1.93 for P and 2.22 for A, pK_{a3} is 6.04 for P and 6.39 for A, pK_{a4} is 10.18 for P and 10.96 for A, and pK_{a5} is 12.14 for P and 11.82 for A at ionic strength 0.1 M and 298.15 K. The last acidity constant, pK_{a5}, assigned to the $NH_3^+ \rightarrow NH_2$ deprotonation exhibits, however, the opposite trend, indicating the increasing acidity-modifying interaction of bisphosphonate and amino sites with their decreasing covalent distance.

The reliability of dissociation constants obtained by regression analysis of potentiometric data is dependent upon (i) an algorithm used and (ii) the parameters selected for refinement[26,27] with ESAB[28−30] and with HYPERQUAD.[31] Both programs seem to be a powerful tool because they also permit the refinement of group parameters and the application of an internal calibration.

In this paper we decided to investigate the dissociation constants of these two drugs, alendronate and ibandronate, at various ionic strengths and at (298.15 and 310.15) K, and to prove their reliability and also to estimate their thermodynamic dissociation constant pK_a^T.

Procedure for the Determination of Mixed Dissociation Constants. The procedure for the determination of mixed dissociation constants with the use of two programs, ESAB and HYPERQUAD, was described previously.[46]

Procedure for the Determination of the Thermodynamic Dissociation Constant. Let us consider a dependence of the mixed dissociation constant $K_a = a_{H^+}[L^{z-1}]/[HL^z]$ on an ionic strength when both ions HL^z and L^{z-1} have roughly the same ion-size parameter $\mathring{a}$ in the dissociation equilibrium $HL^z \rightleftarrows L^{z-1} + H^+$ with the thermodynamic dissociation constant $K_a^T = a_{H^+} a_{L^-}/a_{HL}$ and that the overall specific interaction parameter is given as $C = C_{HL} - C_L$. This dependence is expressed by the extended Debye−Hückel equation

$$pK_a = pK_a^T - \frac{A(1-2z)\sqrt{I}}{(1 + B\mathring{a}\sqrt{I})} + CI$$

where $A = 0.5112\ mol^{-1/2}\cdot L^{1/2}\cdot K^{3/2}$ and $B = 0.3291\ mol^{-1/2}\cdot m^{-1}\cdot L^{1/2}\cdot K^{1/2}\cdot 10^{10}$ for aqueous solutions and 298.15 K. The mixed dissociation constant pK_a represents a dependent variable, while the ionic strength I stands for the independent variable. Three unknown parameters $\mathbf{b} = \{pK_a, \mathring{a}, C\}$ are to be estimated

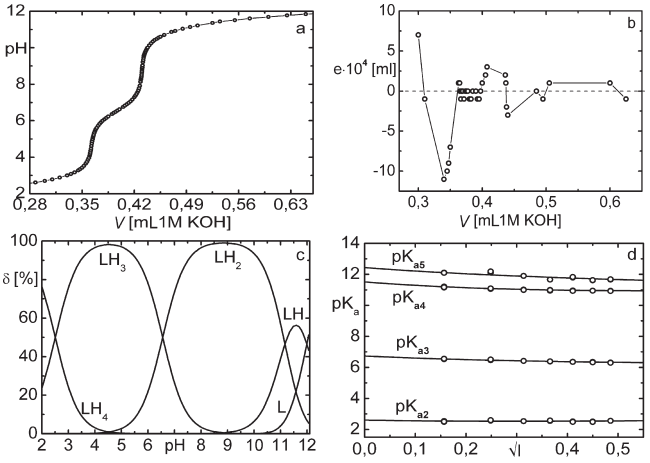

Figure 3. Protonation equilibria of alendronate analyzed with ESAB: (a) potentiometric titration curve of alendronate with KOH; $L_0 = 3.9035\cdot10^{-3}\ mol\cdot dm^{-3}$, $H_T = -0.8961\ mol\cdot dm^{-3}$, $V_0 = 15.3\ cm^3$, $I = 0.025$, $T = 298.15$ K; (b) plot of residuals; (c) distribution diagram of relative presentation of all species of protonation equilibrium; (d) dependence of the mixed dissociation constant pK_a of alendronate on the square root of an ionic strength, which leads to thermodynamic dissociation constants $pK_{a2} = 2.60(1)$, $pK_{a3} = 6.73(1)$, $pK_{a4} = 11.51(2)$, and $pK_{a5} = 12.44(3)$.

by a minimization of the sum of squared residuals

$$U(\mathbf{b}) = \sum_{i=1}^{n} w_i[pK_{a,exp,i} - pK_{a,calc,i}]^2$$

$$= \sum_{i=1}^{n} w_i[pK_{a,exp,i} - f(I; pK_a^T, \mathring{a}, C)]^2 = \text{minimum}$$

The nonlinear estimation problem is simply a problem of optimization in the parameter space in which the pK_a and I are known and given values, while the parameters pK_a^T, $\mathring{a}$, and C are unknown variables to be estimated.[32] However, for small values of an ionic strength the pK_a^T can be estimated only.

Reliability of the Estimated Dissociation Constants. The adequacy of a proposed regression model with experimental data and the reliability of found parameter estimates, b_j, $j = 1, ..., m$, may be examined by the goodness-of-fit test, also called the fitness test, which was described previously[46] and can be found on page 101 in ref 26.

EXPERIMENTAL SECTION

Chemicals and Solutions. Alendronate sodium (99.8 % in mass) and ibandronate sodium (100 % in mass) were donated by Zentiva Group, a.s. (Prague) with declared purity checked by a high-performance liquid chromatography (HPLC) method. These drugs have been weighed straight to a reaction vessel to reach a resulting concentration of about $0.005\ mol\cdot dm^{-3}$. Other chemicals and solutions were described previously.[46]

Apparatus. The free hydrogen-ion concentration h was measured on the digital voltmeter Hanna HI 3220 with a precision of ± 0.002 pH with the use of a combined glass electrode Theta HC 103-VFR. Apparatus, burets,[34] and titrations were described previously.[46]

Computation and Software Used. When the program ESAB estimated H_T and L_0 from an actual titration of a mixture of drug and hydrochloric acid with potassium hydroxide, some group parameters are given in the input data for ESAB[28,29] such as the

dx.doi.org/10.1021/je200512e |J. Chem. Eng. Data 2011, 56, 3848–3854

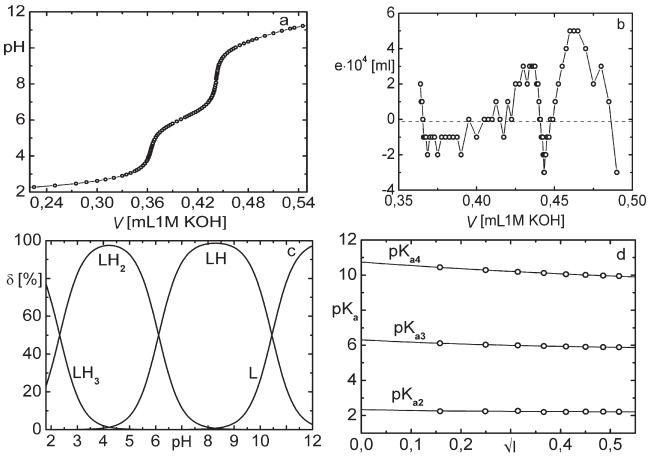

Figure 4. Protonation equilibria of ibandronate analyzed with ESAB: (a) potentiometric titration curve of ibandronate with KOH; L_0 = $4.8985 \cdot 10^{-3}$ mol·dm^{-3}, H_T = 0.9861 mol·dm^{-3}, V_0 = 15.25 cm^3, I = 0.025, T = 298.15 K; (b) plot of residuals; (c) distribution diagram of relative presentation of all species of protonation equilibrium; (d) dependence of the mixed dissociation constant pK_a of ibandronate on the square root of an ionic strength, which leads to thermodynamic dissociation constants pK_{a2} = 2.33(1) pK_{a3} = 6.31(1), and pK_{a4} = 10.74(1).

Nernstian slope and pK_w, which are both accessible from the literature.[35] Group parameters can be estimated by a regression analysis of both segments of a titration curve or from the acid segment only if the basic one might be affected by some carbonate as well as silicate in the alkali. With ESAB, two group parameters, L_0 and H_T, were refined to give the best fit, and the fitness may be examined by the goodness-of-fit criteria.

A computation relating to a determination of dissociation constants was performed by nonlinear regression analysis of titration curve using the ESAB program, version ESAB2M,[28,29] and the HYPERQUAD2008 program.[31] The thermodynamic dissociation constant pK_a^T, an ion-size parameter $\mathring{a}$, and salting-out coefficient C could be estimated with the nonlinear regression program MINOPT in the statistical system ADSTAT (TriloByte Statistical Software, Ltd. Pardubice).[36] PALLAS[37,38] and MARVIN[38,39] are programs for making predictions based on the structural formulas of drug compounds. Entering the compound topological structure descriptors graphically, pK_a values of organic compound are predicted using approximately hundreds of Hammett and Taft equations and quantum chemistry calculus.

The experimental and computational schemes for the determination of the protonation constants of the multicomponent system are taken from Meloun et al.,[26,27] and the details for the computer data treatment are collected in the webpage http://meloun.upce.cz and in the block DOWNLOAD and block DATA.

■ RESULTS AND DISCUSSION

Estimation of Dissociation Constants. In general, all two N-BPs are pentaprotic acids (Figure 2), but after dissolution, they may be treated as tetraprotic or triprotic acids. The titration of tetraprotic weak acid H_4L with a strong base (e.g., KOH) involves eight solution species H_3O^+, OH^-, H_4L, H_3L^-, H_2L^{2-}, HL^{3-}, L^{4-}, and the potassium cation K^+. Other variables are the initial weak acid concentration (L_0), the initial base concentration (H_T), volume of acid titrated (V_0), and the volume of base added (V). The algebraic description of a tetraprotic titration (neglecting activities) is completely specified by four ionization steps (K_{a1}, K_{a2}, K_{a3}, and K_{a4}), the equation for ionization of water (K_w), equations for the mass and charge balance, and in addition, two equations to account for dilution of the acid and base concentration during the titration. For adjusted value of ionic

Table 1. ESAB Refinement of Common and Group Parameters for a Titration of Alendronate with KOH[a]

i	titrant V/cm^3	residual ê	pa_H	protonation function
1	0.3000	0.0007	2.685	3.40
2	0.3100	−0.0001	2.768	3.26
3	0.3350	−0.0012	3.064	3.22
4	0.3400	−0.0011	3.156	3.18
5	0.3450	−0.0010	3.266	3.15
6	0.3475	−0.0009	3.334	3.13
7	0.3500	−0.0007	3.416	3.11
8	0.3630	0.0001	4.745	2.98
9	0.3635	0.0001	4.877	2.98
10	0.3640	0.0001	4.983	2.97
11	0.3645	0.0001	5.085	2.97
12	0.3650	0.0000	5.157	2.96
13	0.3655	0.0000	5.226	2.95
14	0.3665	−0.0001	5.347	2.94
15	0.3675	0.0000	5.448	2.93
16	0.3685	0.0000	5.531	2.91
17	0.3695	0.0000	5.606	2.90
18	0.3715	−0.0001	5.723	2.87
19	0.3735	0.0000	5.827	2.84
20	0.3755	0.0000	5.914	2.81
21	0.3775	0.0000	5.992	2.78
22	0.3800	−0.0001	6.076	2.75
23	0.3825	−0.0001	6.156	2.71
24	0.3850	0.0000	6.231	2.67
25	0.3900	0.0000	6.366	2.60
26	0.3925	−0.0001	6.428	0.257
27	0.3950	−0.0001	6.492	2.53
28	0.3975	0.0000	6.557	2.49
29	0.4000	0.0001	6.622	2.46
30	0.4050	0.0002	6.753	2.38
31	0.4075	0.0003	6.824	2.35
32	0.4360	0.0002	9.868	1.96
33	0.4370	0.0001	9.949	1.95
34	0.4380	−0.0002	10.007	1.94
35	0.4400	−0.0003	10.129	1.92
36	0.4850	0.0000	11.042	1.54
37	0.4950	−0.0001	11.134	1.47
38	0.5050	0.0001	11.216	1.41
39	0.6000	0.0001	11.691	0.97
40	0.6250	−0.0001	11.770	0.90

[a] Standard deviations of parameter estimates in last valid digits are in parentheses. Common parameters refined: pK_{a2} = 2.510(15), pK_{a3} = 6.546(2), pK_{a4} = 11.212(3), pK_{a5} = 12.113(4). Group parameters refined: L_0 = $3.9035 \cdot 10^{-3}$ mol·dm^{-3}, H_T = −0.8961 mol·dm^{-3} (negative sign means a base here, while the positive sign means the acid). Constants: H_0 = $1.992 \cdot 10^{-3}$ mol·dm^{-3}, T = 298.15 K, pK_w = 13.9799, V_0 =15.3 cm^3, $s(V)$ = 0.0001 cm^3, j_a = 0.0 mV, j_b = 0.0 mV, I_0 = 0.002 (in vessel), I_T = 0.8961 (in buret).

Table 2. Reliability of Parameter Estimates Proved by a Statistical Analysis of Residuals

bias, $E(\hat{e})$	-0.0000103 cm^3
lower and upper Hoaglin's limits	-0.0002 cm^3 and 0.0002 cm^3, no outliers
mean of absolute values of residuals, $E\|\hat{e}\|$	0.000213 cm^3
standard deviation, $s(\hat{e})$	0.000375 cm^3
skewness, $g_1(\hat{e})$	-1.49 (not differing from 0)
kurtosis, $g_2(\hat{e})$	5.44 (not differing from 3)
Jarque–Berra normality test of a residuals	normality accepted

Table 3. Mixed Dissociation pK_a Constant of Alendronate at 298.15 K and Various Values of an Ionic Strength I Estimated by Nonlinear Regression Programs ESAB and HYPERQUAD[a]

		ESAB		HYPERQUAD		
I	pK_a	pK_a	$\|\hat{e}\|/\mu$L	log β_{1q}	SIGMA	pK_a
0.025	pK_{a2}	2.506(27)	0.1	32.265(20)	2.08	2.532(20)
	pK_{a3}	6.546(2)		29.733(19)		6.553(19)
	pK_{a4}	11.212(3)		23.180(18)		11.229(18)
	pK_{a5}	12.113(4)		11.951(26)		11.951(26)
0.062	pK_{a2}	2.531(22)	0.2	32.166(47)	2.77	2.523(47)
	pK_{a3}	6.463(4)		29.643(46)		6.460(46)
	pK_{a4}	11.058(5)		23.183(46)		11.055(46)
	pK_{a5}	12.216(10)		12.128(50)		12.128(50)
0.099	pK_{a2}	2.533(13)	0.2	31.902(21)	1.59	2.535(21)
	pK_{a3}	6.416(4)		29.367(20)		6.416(20)
	pK_{a4}	11.041(7)		22.951(20)		10.992(20)
	pK_{a5}	11.850(10)		11.959(28)		11.959(28)
0.134	pK_{a2}	2.531(22)	0.2	31.661(52)	1.99	2.634(52)
	pK_{a3}	6.380(6)		29.027(43)		6.382(43)
	pK_{a4}	10.993(11)		22.645(39)		10.974(39)
	pK_{a5}	11.554(14)		11.671(53)		11.671(53)
0.169	pK_{a2}	2.511(18)	0.2	21.610(24)	1.76	2.486(24)
	pK_{a3}	6.362(6)		29.124(22)		6.362(22)
	pK_{a4}	10.956(6)		22.762(21)		10.942(21)
	pK_{a5}	11.792(10)		11.820(29)		11.820(29)
0.203	pK_{a2}	2.481(34)	0.4	31.404(29)	2.02	2.506(29)
	pK_{a3}	6.352(13)		28.898(25)		6.338(25)
	pK_{a4}	10.978(14)		22.560(24)		10.946(24)
	pK_{a5}	11.564(25)		11.614(24)		11.614(24)
0.236	pK_{a2}	2.547(55)	0.3	31.468(35)	2.3	2.556(35)
	pK_{a3}	6.310(8)		28.912(30)		6.308(30)
	pK_{a4}	10.938(11)		22.604(28)		10.925(28)
	pK_{a5}	11.663(15)		11.679(40)		11.679(40)

[a] Standard deviations of parameter estimates in the last valid digits are in parentheses.

strength the potentiometric titration of a mixture of HCl and N-BP drug acid with potassium hydroxide was carried out. The initial tentative value of the dissociation constant of the drug studied corresponding to the midpoint value in each plateau of the potentiometric titration curve data (Figures 3a and 4a) has been applied by the nonlinear regression ESAB and/or HYPERQUAD programs and gave the values of pK_{a3} and pK_{a4}. The pK_a values obtained by the deconvolution of the potentiometric titration curve indicated that this N-BP dissociates in a manner similar to that elucidated for alendronate.

Table 4. Mixed Dissociation pK_a Constant of Ibandronate at 298.15 K and Various Values of an Ionic Strength I Estimated by Nonlinear Regression Programs ESAB and HYPERQUAD[a]

		ESAB		HYPERQUAD		
I	pK_a	pK_a	$\|\hat{e}\|/\mu$L	log β_{1q}	SIGMA	pK_a
0.025	pK_{a2}	2.251(28)	0.1	18.813(6)	0.8596	2.261(6)
	pK_{a3}	6.110(1)		16.552(4)		6.108(4)
	pK_{a4}	10.450(2)		10.443(3)		10.443(3)
0.062	pK_{a2}	2.251(43)	0.1	18.580(8)	0.9452	2.253(8)
	pK_{a3}	6.036(2)		16.327(4)		6.036(4)
	pK_{a4}	10.291(2)		10.291(3)		10.291(3)
0.099	pK_{a2}	2.261(41)	0.2	18.435(8)	0.9972	2.259(8)
	pK_{a3}	5.997(3)		16.176(4)		5.992(4)
	pK_{a4}	10.183(3)		10.184(3)		10.184(3)
0.134	pK_{a2}	2.203(39)	0.1	18.254(7)	0.9270	2.198(7)
	pK_{a3}	5.949(2)		16.056(4)		5.950(4)
	pK_{a4}	10.106(2)		10.106(3)		10.106(3)
0.169	pK_{a2}	2.261(25)	0.2	18.232(10)	1.00	2.259(10)
	pK_{a3}	5.922(3)		15.973(6)		5.925(6)
	pK_{a4}	10.047(3)		10.048(4)		10.048(4)
0.203	pK_{a2}	2.221(16)	0.2	18.127(11)	1.41	2.219(11)
	pK_{a3}	5.906(3)		15.908(6)		5.902(6)
	pK_{a4}	10.009(3)		10.006(4)		10.006(4)
0.236	pK_{a2}	2.222(15)	0.2	18.081(8)	1.0561	2.225(8)
	pK_{a3}	5.893(3)		15.856(5)		5.895(5)
	pK_{a4}	9.963(3)		9.962(3)		9.962(3)
0.268	pK_{a2}	2.214(19)	0.2	18.055(7)	0.9929	2.214(7)
	pK_{a3}	5.888(3)		15.841(5)		5.892(5)
	pK_{a4}	9.948(3)		9.949(3)		9.949(3)

[a] Standard deviations of parameter estimates in last valid digits are in brackets.

Table 1 gives the results of the ESAB regression analysis of a part of a particular titration curve when a minimization process terminates. Besides the original data $\{V, pa_H\}$, also the residual $\hat{e}$ and the Bjerrum protonation function at each point are given. All common pK_{a2}, pK_{a3}, pK_{a4}, and pK_{a5} and group parameters L_0 and H_T are refined, and the best-fitting curve is proven by results of a statistical analysis of residuals in Table 2. The reliability of protonation constant may be classified according to a goodness-of-fit. As more group parameters are refined, a better fit is achieved, and therefore the more reliable estimate of protonation constants results. A quite sensitive criterion of reliability of protonation constant is the mean of absolute values of residuals $E|\hat{e}|$. Comparing residuals with the instrumental noise, $s_{inst}(y)$, represented here by either $s(V) = 0.0001$ cm^3 or $s(pa_H) = 0.01$, an excellent fit is considered because the mean $E|\hat{e}|$ and the residual standard deviation $s(\hat{e})$ are nearly the same magnitude and lower as a noise $s_{inst}(y)$. Here, $E|\hat{e}| = 0.0002$ cm^3 and $s(\hat{e}) = 0.00037$ cm^3 are lower than the instrumental error $s(V) = 0.0001$ cm^3. As the bias $E(\hat{e})$ is equal to $-1.0 \cdot 10^{-5}$, what may

be taken as zero, no systematic error in curve fitting can be expected. All residuals oscillate between lower and upper Hoaglińs inner bounds, and no residuals lay outside of these bounds. Residuals exhibit a normal distribution as confirmed by the Jarque-Berra normality test for combined sample skewness and kurtosis (cf. page 80 in ref 33) and also by parameters of distribution shape, the skewness $g_1(\hat{e}) = -1.49$ being not significantly different from zero and thus proving a symmetric distribution, and the kurtosis $g_2(\hat{e}) = 5.44$ being not significantly different from 3 and thus proving a normal distribution.

Figure 3 shows results of the graphical presentation of a regression analysis of (a) the *potentiometric titration curve* of a mixture of HCl and alendronate at 298.15 K; (b) the *overall diagram* of classical residuals, which gives an initial impression of residuals. The true model and reliable parameter estimates are proven because the residuals exhibit a normal distribution with a zero mean and also form a random pattern. No systematic departures from randomness indicate that the model proposed is true and estimates of the parameter are reliable; (c) the *distribution diagram* of a relative presentation of all variously protonated species, which seems to be more interesting than only a numerical value of a protonation constant. The intersection of both curves gives a value of the protonation constant on the pH axis; (d) dependence of the mixed dissociation constants pK_{a2}, pK_{a3}, and pK_{a4} of alendronate on the square root of the ionic strength, which leads to a parameter estimate of the thermodynamic dissociation constants, pK^T_{a2}, pK^T_{a3}, and pK^T_{a4}.

Table 3 shows the mixed dissociation constant of alendronate estimated by nonlinear regression programs ESAB and HYPERQUAD when residuals $e_i = (V_{exp,i} - V_{calc,i})$ are minimized. Low values of residuals in microliters prove an excellent fitness of the calculated regression curve through experimental points. For comparison of two programs ESAB and HYPERQUAD, three decimal places in estimation of pK_a were used in Tables 3 and 4.

Table 4 shows the estimate of dissociation constants of ibandronate determined at various values of ionic strength as results of regression analysis with two various mathematical approaches. The pK_a values obtained by the deconvolution of the potentiometric titration curve indicating that this N-BP dissociates in a manner similar to that for alendronate. The program ESAB minimizing residuals $e_i = (V_{exp,i} - V_{calc,i})$ reaches 0.1 μL and HYPERQUAD minimizing $e_i = (pa_{H,exp,i} - pa_{H,calc,i})$ and thus proving an excellent fit. When the statistical parameter SIGMA being of statistical weight nature is about 1 or less, it proves that an excellent goodness-of-fit is achieved and sufficient reliability of parameter estimates, while SIGMA greater than 1 indicates a poor goodness-of-fit.

Estimation of Thermodynamic Dissociation Constant. Applying an extended Debye−Hückel equation to the data of Tables 3 and 4 according to a regression criterion, from three unknown parameters pK^T_a, $\mathring{a}$, and C the pK^T_a could be estimated only as the range of an ionic strength contains small values and does not allow to estimate $\mathring{a}$ and C.

Table 5 shows the point estimate, calculated standard deviation of each parameter, and absolute and relative bias obtained when minimization process terminates. The linear parameter pK^T_a in the regression model are well-conditioned, and their estimation is sensitive. They have a strong influence on the residual-square sum function U. The well-conditioned parameter pK^T_a has a great influence on an elliptic hyperparaboloid shape, when the variable U is plotted against parameter pK^T_a, in a one-dimensional space. For well-conditioned parameters such a shape exhibits an obvious, sharp minimum, the pit point U_{min}.

Literature Comparison. The protonation of sodium salt of 4-amino-1-hydroxybutylidene-biphosphonic (alendronate) acid

Table 5. Survey of Estimated Thermodynamic Dissociation Constants for Two N-BPs Refined with ESAB

		298.15 K	310.15 K
alendronate	pK^T_{a2}	2.60(1)	2.76(1)
	pK^T_{a3}	6.73(1)	6.77(1)
	pK^T_{a4}	11.51(2)	11.29(1)
	pK^T_{a5}	12.44(3)	11.82(3)
ibandronate	pK^T_{a2}	2.33(1)	2.50(1)
	pK^T_{a3}	6.31(1)	6.37(1)
	pK^T_{a4}	10.74(1)	10.65(1)

Table 6. Dissociation Constants of Two N-BPs Found in the Literature and Estimated in This Work

ionic strength I, T/K	reference	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}	pK_{a5}
			Alendronate			
prediction with Pallas and Marvin		1.78	2.41	8.42	9.92	12.04
0.1 M KCl	24		2.72 ± 0.05	8.73 ± 0.05	10.5 ± 0.05	11.6 ± 0.10
0.2 M KCl	23		2.16	6.21	10.77	12.04
0.1 M KCl	25		2.22	6.39	10.96	11.82
0.1 M KCl	22		2.24 ± 0.01	6.38 ± 0.03	10.68 ± 0.06	11.4 ± 0.2
	3			6.17	≥ 10	
	44		2.35	6.55	10.09	
0.1 M KCl	45		2.66	6.01 ± 0.01	9.97 ± 0.02	10.74 ± 0.05
I = 0, 298.15 K, pK^T_a	this work		2.60(1)	6.73(1)	11.51(2)	12.44(3)
I = 0, 298.15 K, pK^T_a	this work		2.76(1)	6.77(1)	11.29(1)	11.82(3)
			Ibandronate			
prediction with Pallas and Marvin		1.60	2.39	6.71	9.94	11.89
	44		2.84	6.08	10.43	
I = 0, 298.15 K, pK^T_a	this work		2.33(1)	6.31(1)	10.74(1)	
I = 0, 298.15 K, pK^T_a	this work		2.50(1)	6.37(1)	10.65(1)	

dx.doi.org/10.1021/je200512e |J. Chem. Eng. Data 2011, 56, 3848–3854

and ibandronate have been studied at various temperatures and ionic strengths.[22,40,41] In only a few cases has the dependence of the dissociation constants on ionic strength been systematically investigated and the methods reviewed.[10,23,24] The dissociation constants of three nitrogen-containing bisphosphonic acids have been obtained[24] and are widely cited by other authors and referral databases.[42] However, it is interesting that other authors[23,25] have determined different dissociation constants and have not dissolved this discrepancy (Table 6).

Alendronate has six functional groups that could be ionized: five H^+ donors (four POH groups, one geminate OH group) and one amino group as a H^+ acceptor (Figure 1). The zwitterions are the most probable structures of the electroneutral forms of alendronate.[43] The dissociation of the geminal OH group of bisphosphonates in aqueous solutions was not observed up to pH 13, ref 22. The pK_{a1} values of alendronate that correspond to the dissociation of POH group in cationic acid H_5L^+ with the formation of zwitterions were obtained[25] by using NMR-controlled titrations. It is evident that the application of the standard titrimetric procedure for pK_{a1} determination does not allow for the calculation of pK_{a1} due to the minor presence of the H_5L^+ form in the equilibrium state of alendronate solution. Thus, the proteolytic properties of alendronates in pH ranging from 2 to 12 can be described in terms of four dissociation steps: pK_{a2}, pK_{a3}, pK_{a4} (related with dissociation of POH groups), and pK_{a5} related with dissociation of NH_3^+. Found estimates of two dissociation constants are in agreement with predicted values using both programs PALLAS and MARVIN; an elucidation may be found in ref 38.

CONCLUSIONS

The reliability of the dissociation constant of two N-BPs (alendronate and ibandronate) was proven even though two group parameters L_0 and H_T were ill-conditioned in a model. Their determination is uncertain and might lead to a false estimate of common parameters pK_a and therefore make the computational strategy important. These group parameters can have a great influence on a systematic error in estimated pK_a, and they should be refined together with common parameters pK_a. The external calibration pa_{H^+} of the glass electrode cell is performed. Comparing two computational approaches, ESAB and HYPERQUAD programs, ESAB led to better fitness of the potentiometric titration curve. The thermodynamic dissociation constant pK_a^T was estimated by a nonlinear regression of a dependence of the mixed dissociation constant pK_a on ionic strength I. The goodness-of-fit proved sufficient reliability of parameter estimates for two drugs at (298.15 and 310.15) K. Standard deviations of each parameter in the last digits are in brackets.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +420466037026. Fax: +420466037068. E-mail: milan.meloun@upce.cz.

Funding Sources

The financial support of the Grant Agency IGA MZ ČR (Grant No. NS9831-4/2008) and of the Czech Ministry of Education (Grant No. MSM0021627502) is gratefully acknowledged.

REFERENCES

(1) Fleisch, H. Bisphosphonates: Mechanisms of action. *Endocr. Rev.* **1998**, *19*, 80–100.

(2) Fleisch, H. Development of bisphosphonates. *Breast Cancer Res.* **2002**, *4*, 30–34.

(3) Hounslow, A. M.; Carran, J.; Brown, R. J.; Rejman, D.; Blackburn, G. M.; Watts, D. J. Determination of the microscopic equilibrium dissociation constants for risedronate and its analogues reveals two distinct roles for the nitrogen atom in nitrogen-containing bisphosphonate drugs. *J. Med. Chem.* **2008**, *51*, 4170–4178.

(4) Fleisch, H. *Bisphosphonates in bone disease: from the laboratory to the patient*; Parthenon Publishing Group: Nashville, TN, 1995.

(5) Russell, R. G. Bisphosphonates: from bench to bedside. *Ann. N.Y. Acad. Sci.* **2006**, *1068*, 367–401.

(6) Rogers, M. J.; Watts, D. J.; Russell, R. G.; Ji, X.; Xiong, X.; Blackburn, G. M.; Bayless, A. V.; Ebetino, F. H. Inhibitory effects of bisphosphonates on growth of amoebae of the cellular slime mold *Dictyostelium discoideum*. *J. Bone Miner. Res.* **1994**, *9*, 1029–39.

(7) Rogers, M. J.; Xiong, X.; Brown, R. J.; Watts, D. J.; Russell, R. G.; Bayless, A. V.; Ebetino, F. H. Structure-activity relationships of new heterocycle-containing bisphosphonates as inhibitors of bone resorption and as inhibitors of growth of Dictyostelium discoideum amoebae. *Mol. Pharmacol.* **1995**, *47*, 398–402.

(8) Grove, J. E.; Brown, R. J.; Watts, D. J. The intracellular target for the antiresorptive aminobisphosphonate drugs in *Dictyostelium discoideum* is the enzyme farnesyl diphosphate synthase. *J. Bone Miner. Res.* **2000**, *15*, 971–81.

(9) Russell, R. G. Ibandronate: pharmacology and preclinical studies. *Bone* **2006**, *38*, S7–12.

(10) Nancollas, G. H.; Tang, R.; Phipps, R. J.; Henneman, Z.; Gulde, S.; Wu, W.; Mangood, A.; Russell, R. G.; Ebetino, F. H. Novel insights into actions of bisphosphonates on bone: differences in interactions with hydroxyapatite. *Bone* **2006**, *38*, 617–27.

(11) Dunford, J. E.; Kwaasi, A. A.; Rogers, M. J.; Barnett, B. L.; Ebetino, F. H.; Russell, R. G.; Oppermann, U.; Kavanagh, K. L. Structure-activity relationships among the nitrogen containing bisphosphonates in clinical use and other analogues: time-dependent inhibition of human farnesyl pyrophosphate synthase. *J. Med. Chem.* **2008**, *51*, 2187–95.

(12) Hodgson, S. F.; Watts, N. B.; Bilezikian, J. P.; Clarke, B. L.; Gray, T. K.; Harris, D. W.; Johnston, C. C.; Kleerekoper, M.; Lindsay, R.; Luckey, M. M.; McClung, M. R.; Nankin, H. R.; Petak, S. M.; Recker, R. R. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the prevention and treatment of postmenopausal osteoporosis: 2001 edition, with selected updates for 2003. *Endocr. Pract.* **2003**, *9*, 544–64.

(13) Chesnut, C. H.; Skag, A.; Christiansen, C.; Recker, R.; Stakkestad, J. A.; Hoiseth, A.; Felsenberg, D.; Huss, H.; Gilbride, J.; Schimmer, R. C.; Delmas, P. D.; Vert, O. I. O. Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. *J. Bone Miner. Res.* **2004**, *19*, 1241–1249.

(14) Bone, H. G.; Hosking, D.; Devogelaer, J.; Tucci, J. R.; Emkey, R. D.; Tonino, R. P.; Rodriguez-Portales, J. A.; Downs, R. W.; Gupta, J.; Santora, A. C.; Liberman, U. A. Ten years' experience with alendronate for osteoporosis in postmenopausal women. *N. Eng. J. Med.* **2004**, *350*, 1189–1199.

(15) McClung, M. R.; Geusens, P.; Miller, P. D.; Zippel, H.; Bensen, W. G.; Roux, C.; Adami, S.; Fogelman, I.; Diamond, T.; Eastell, R.; Meunier, P. J.; Reginster, J. Y.; Wasnich, R. D.; Greenwald, M.; Kaufman, J.; Chestnut, C. H. Effect of risedronate on the risk of hip fracture in elderly women. *N. Engl. J. Med.* **2001**, *344*, 333–340.

(16) Bock, O.; Felsenberg, D. Bisphosphonates in the management of postmenopausal osteoporosis--optimizing efficacy in clinical practice. *Clin. Interv. Aging* **2008**, *3*, 279–97.

(17) Van Beek, E.; Lowik, C.; Que, I.; Papapoulos, S. Dissociation of binding and antiresorptive properties of hydroxybisphosphonates by substitution of the hydroxyl with an amino group. *J. Bone Miner. Res.* **1996**, *11*, 1492–7.

(18) Shinoda, H.; Adámek, G.; Felix, R.; Fleisch, H.; Schenk, R.; Hagan, P. Structure-activity relationships of various bisphosphonates. *Calcif. Tissue Int.* **1983**, *35*, 87–99.

(19) Geddes, A. D.; D'Souza, S. H.; Ebetino, F. H.; Ibbotson, K. J. Bisphosphonates: structure-activity relationships and therapeutic implications. In *Bone Mineral Research*; Heersche, J. N. M., Kanis, J. A., Eds.; Elsevier: Amsterdam, 1994; Vol. 8, pp 265−306.

(20) Lawson, M. A.; Triffitt, J. T.; Ebetino, F. H.; Barnett, B. L.; Phipps, R. J.; White, D. J.; Russell, R. G. G. Differences in the potential binding of phosphate containing compounds to bone mineral are revealed by the novel use of hydroxyapatite column chromatography. *J. Bone. Miner. Res.* **2005**, *20*, 1303–1303.

(21) Green, J. R.; Muller, K.; Jaeggi, K. A. Preclinical Pharmacology of Cgp 42′446, a New, Potent, Heterocyclic Bisphosphonate Compound. *J. Bone Miner. Res.* **1994**, *9*, 745–751.

(22) Boichenko, A. P.; Markov, V. V.; Kong, H. L.; Matveeva, A. G.; Loginova, L. P. Re-evaluated data of dissociation constants of alendronic, pamidronic and olpadronic acids. *Cent. Eur. J. Chem.* **2009**, *7*, 8–13.

(23) Dyba, M.; Jezowska-Bojczuk, M.; Kiss, E.; Kiss, T.; Kozlowski, H.; Leroux, Y.; El Manouni, D. 1-Hydroxyalkane-1,1-diyldiphosphonates as potent chelating agents for metal ions. Potentiometric and spectroscopic studies of copper(II) coordination. *J. Chem. Soc., Dalton. Trans.* **1996**, 1119–1123.

(24) Kabachnik, M. I.; Medved, T. Y.; Dyaglova, N. M.; Polikarpov, Y. M.; Shcherbakov, B. K.; Belskii, F. I. Synthesis and Acid-Base and Complexing Properties of Amino-Substituted "Alpha-Hydroxyalkylidenediphosphonic Acids". *Bull. Acad. Sci. USSR Div. Chem. Sci.* **1978**, *27*, 374–377.

(25) Hägele, G.; Szakacs, Z.; Ollig, J.; Hermens, S.; Pfaff, C. NMR-controlled titrations: Characterizing aminophosphonates and related structures. *Heteroatom Chem.* **2000**, *11*, 562–582.

(26) Meloun, M.; Havel, J.; Högfeldt, E. *Computation of solution equilibria: a guide to methods in potentiometry, extraction, and spectrophotometry*; Ellis Horwood: Chichester, U.K., 1988.

(27) Meloun, M.; Bartoš, M.; Högfeldt, E. Multiparametric curve fitting-XIII Reliability of formation constants determined by analysis of potentiometric titration data. *Talanta* **1988**, *35*, 981–91.

(28) Rigano, C.; Grasso, M.; Sammartano, S. Computer-Analysis of Equilibrium Data in Solution - a Compact Least-Squares Computer-Program for Acid-Base Titrations. *Ann. Chim.* **1984**, *74*, 537–532.

(29) De Stefano, C.; Princi, P.; Rigano, C.; Sammartano, S. Computer analysis of equilibrium data in solution ESAB2M: an improved version of the ESAB program. *Ann. Chim.* **1987**, *77*, 643–675.

(30) Capone, S.; Derobertis, A.; Destefano, C.; Sammartano, S.; Scarcella, R. Ionic-Strength Dependence of Formation-Constants 0.10. Proton Activity-Coefficients at Various Temperatures and Ionic Strengths and Their Use in the Study of Complex Equilibria. *Talanta* **1987**, *34*, 593–598.

(31) Gans, P.; Sabatini, A.; Vacca, A. Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs. *Talanta* **1996**, *43*, 1739–1753.

(32) Meloun, M.; Militký, J.; Forina, M. *Chemometrics for analytical chemistry*, Vol. 2: PC-aided regression and related methods; Ellis Horwood: Chichester, 1994; p 400.

(33) Meloun, M.; Militký, J.; Forina, M. *Chemometrics for analytical chemistry*, Vol. 1: PC-Aided Statistical Data Analysis; Ellis Horwood: Chichester, 1992; p 330.

(34) Meloun, M.; Říha, V.; Žáček, J. Piston Microburette for Dosing Aggressive Liquids. *Chem. Listy* **1988**, *82*, 765–767.

(35) May, P. M.; Williams, D. R.; Linder, P. W.; Torrington, R. G. The Use of Glass Electrodes for the Determination of Formation-Constants 0.1. A Definitive Method for Calibration. *Talanta* **1982**, *29*, 249–256.

(36) *ADSTAT 1.25, 2.0, 3.0* (Windows 95); TriloByte Statistical Software Ltd.: Pardubice, Czech Republic, 1995.

(37) Gulyás, Z.; Pöcze, G.; Petz, A.; Darvas, F. *PALLAS 3.0*; CompuDrug International Inc.: San Francisco, CA, 1994. http://www.compudrug.com.

(38) Meloun, M.; Bordovská, S.; Kupka, K. Outliers detection in the statistical accuracy test of a pK(a) prediction. *J. Math. Chem.* **2010**, *47*, 891–909.

(39) Szegezdi, J.; Csizmadia, F. *Prediction of dissociation constant using microconstants*; ChemAxon: Hungary, 1998−2011. http://www.chemaxon.com/marvin.

(40) Rondeau, J. M.; Bitsch, F.; Bourgier, E.; Geiser, M.; Hemmig, R.; Kroemer, M.; Lehmann, S.; Ramage, P.; Rieffel, S.; Strauss, A.; Green, J. R.; Jahnke, W. Structural basis for the exceptional in vivo efficacy of bisphosphonate drugs. *ChemMedChem* **2006**, *1*, 267–273.

(41) Tripathi, K. D. *Pharmacological Classification of Drugs*; Jaypee Brothers Medical Publishing: New Delhi, 2006.

(42) Index, T. M. *Merck Index*; Merck Co., Inc.: Whitehouse Station, NJ, 2010.

(43) Karamustafa, F.; Celebi, N. Bisphosphonates and Alendronate. *FABAD J. Pharm. Sci* **2006**, *31*, 31–42.

(44) Nancollas, G. H.; Tang, R.; Phipps, R. J.; Henneman, Z.; Gulde, S.; Wu, W.; Mangood, A.; Russell, R. G. G.; Ebetino, F. H. Novel insights into actions of bisphosphonates on bone: Differences in interactions with hydroxyapatite. *Bone* **2006**, *38*, 617–627.

(45) Cong, H. L.; Boichenko, A. P.; Levin, I. V.; Matveeva, A. G.; Loginova, L. P. Complexation of Ca^{2+} and Mg^{2+} with aminopropylidenebisphosphonic acids in aqueous and micellar solution of cetylpyridinium chloride. *J. Mol. Liq.* **2010**, *154*, 76–81. Boichenko, A. P.; Sidorenko, A. Yu.; Markov, V. V.; Cong, H. L.; Matveeva, A. G.; Loginova, L. P. Effect of micellar media Brij 35 on protolytic properties of series of aminodiphposphonic acids. *Kharkov Univ. Bull.* **2010**, *895*, 56–64.

(46) Meloun, M.; Ferenčíková, Z.; Netolická, L.; Vrána, A. The thermodynamic dissociation constant of naphazoline by the regression analysis of potentiometric data. *Cent. Eur. J. Chem.* **2011**, *9*, 66–74.