J. Chem. Eng. Data **2007,** *52,* 279−285

Thermodynamic Study for the Protonation of Branched Poly(ethylenimine) in NaCl(aq) and Its Dependence on Ionic Strength

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In this paper, we investigated the interactions between poly(ethylenimine) [BPEI] and the proton by using (H^+-glass electrode) potentiometry and titration calorimetry. The measurements were carried out in NaCl aqueous solutions, at $T = 298$ K in a wide range of ionic strength (up to $I \approx 5.0$ mol·L^{-1}). The protonation constants were calculated by following different approaches: (a) first, all the protonation constants were considered as true constants (considering BPEI as a simple low molecular weight tetramine); (b) second, the log K^{H}_{3} was considered as a linear function of $\bar{p}$ ($\bar{p}$ = protonation degree); and (c) the log K^{H}_{3} was considered as a nonlinear function of $\bar{p}$. The difference in the standard deviation on the fit between (a) and (b) calculations is quite significant, while no further improvement is observed by using the (c) model. The protonation enthalpies were calculated from the calorimetric titrations. In these calculations, all the protonation steps of BPEI were considered as independent of $\bar{p}$. The dependence of protonation constants and enthalpies on ionic strength was modeled using the Specific ion Interaction Theory (SIT).

Introduction

The amino compounds are, among the fundamental molecules of biological interest, widely present in natural and biological fluids, especially as amino acids, proteins, and enzymes. They are responsible for most of the complex functions that make life possible. The protonation behavior of low molecular weight polyamines is well-known, and several studies have been performed on this topic (refs 1−8 and references therein), although many of these refer to only one, generally low (0.1 mol·L^{-1}), value of ionic strength and often in KCl, KNO_3, or $NaClO_4$. Literature reports also a review[9] on the proton coordination in aqueous solution of many open-chain polyamines, macrocyclic polyamines, and polyazacycloalkanes unsubstituted and variably substituted. Some Finnish authors reported relevant data on the dependence on ionic strength for the protonation of amines (refs 10−14 and references therein). Our research group has previously studied the protonation of amines in different ionic media at different ionic strengths.[15−22] However, few studies have been reported on high molecular weight polyamine protonation,[23−29] although they have important applications in many industrial fields. In this work, we report protonation constants of branched poly(ethylenimine) (BPEI) MW 750 kDa. This high molecular weight polyamine has a monomeric unit that contains primary, secondary, and tertiary amino groups in the ratio 1:2:1, as shown in Figure 1. It is a polymer obtained from the aziridine through ring-opening cationic polymerization. BPEI has several applications in the biological, industrial, and pharmaceutical fields.[30−41] For example, it is used in bioprocesses to facilitate the transfer of nucleic acids through biological membranes for purification of DNA binding proteins, to transfer genes and oligonucleotides into living cells as a flocculating agent, and to immobilize enzymes and bacteria, or as a backbone of artificial enzymes. BPEI binds also heavy metal cations and therefore can be used in several environmental applications.[42] Moreover, the complexation behavior has been investigated toward some metal ions such as Fe(II), Co(II), Ni(II), and Cu(II).[43] Different methods have been used to explain the acid−base properties of BPEI: by applying the Ising model with short-range interactions,[24] by relating the BPEI protonation with ion condensation,[25] and by calculating the deprotonation quotient at different pH values.[26] However, few quantitative thermodynamic protonation data are present in literature. This work helps to clarify the acid−base proprieties of BPEI. For many years, our research group has been studying the acid−base proprieties of polyelectrolytes such as polyacrylates,[44,45] polymethacrylates,[46] and poly(allylamine).[29] In this paper, the protonation of

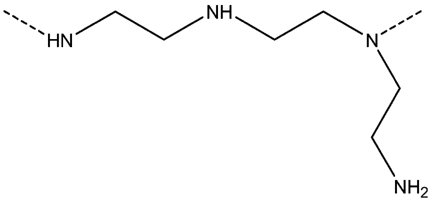

Figure 1. Representation of BPEI monomeric unit.

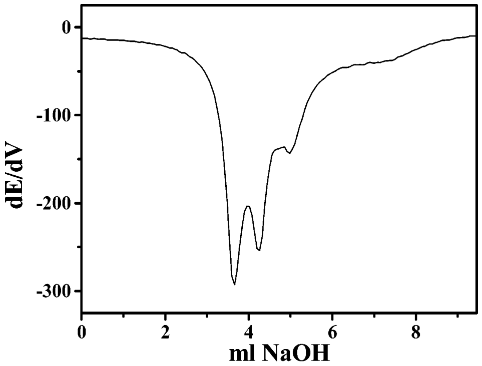

Figure 2. First derivative curve of BPEI titration at $T = 298$ K, C_{NaCl} = 4.5mol·L^{-1}, C_{BPEI} = 4.4 mmol·L^{-1}, and C_{H+} = 30 mmol·L^{-1}.

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10.1021/je060388z CCC: $37.00

Table 1. Protonation Constants of BPEI[a] in NaCl Aqueous Solutions at Different Ionic Strengths ($mol \cdot L^{-1}$)[a]

$I/mol \cdot L^{-1}$	$\log K^{H}_{1}$	$\log K^{H}_{2}$	$\log K^{H}_{3}$	$\log K^{H}_{4}$	σ^{c}
0.11	9.21 ± 0.07^{b}	8.18 ± 0.04^{b}	5.74 ± 0.05^{b}	2.67 ± 0.05^{b}	0.0268
	9.35 ± 0.03	8.22 ± 0.02	5.75 ± 0.04	2.43 ± 0.03	0.0187
	9.34 ± 0.03	8.22 ± 0.02	5.79 ± 0.03	2.46 ± 0.03	0.0403
	9.36 ± 0.03	8.24 ± 0.02	5.83 ± 0.03	2.51 ± 0.03	0.0395
	9.40 ± 0.07	8.32 ± 0.05	5.94 ± 0.07	2.75 ± 0.06	0.0173
	9.36 ± 0.04	8.27 ± 0.02	5.83 ± 0.03	2.50 ± 0.03	0.0093
mean value	9.34 ± 0.03	8.24 ± 0.02	5.81 ± 0.03	2.55 ± 0.05	
0.25	9.59 ± 0.04	8.52 ± 0.03	5.98 ± 0.04	2.46 ± 0.04	0.0220
	9.58 ± 0.04	8.52 ± 0.03	6.00 ± 0.04	2.51 ± 0.03	0.0209
	9.58 ± 0.04	8.52 ± 0.02	6.02 ± 0.04	2.50 ± 0.04	0.0473
	9.58 ± 0.04	8.51 ± 0.02	6.00 ± 0.04	2.48 ± 0.04	0.0478
	9.60 ± 0.05	8.58 ± 0.03	6.12 ± 0.04	2.69 ± 0.04	0.0124
	9.59 ± 0.05	8.51 ± 0.03	5.89 ± 0.05	2.28 ± 0.05	0.0156
mean value	9.59 ± 0.01	8.53 ± 0.01	6.00 ± 0.03	2.49 ± 0.05	
0.48	9.74 ± 0.04	8.75 ± 0.03	6.19 ± 0.04	2.55 ± 0.03	0.0227
	9.74 ± 0.04	8.75 ± 0.03	6.17 ± 0.04	2.52 ± 0.04	0.0230
	9.76 ± 0.04	8.77 ± 0.03	6.23 ± 0.04	2.58 ± 0.04	0.0503
	9.76 ± 0.04	8.78 ± 0.02	6.26 ± 0.04	2.63 ± 0.03	0.0491
	9.75 ± 0.04	8.79 ± 0.03	6.24 ± 0.04	2.60 ± 0.03	0.0113
	9.75 ± 0.05	8.77 ± 0.03	6.14 ± 0.05	2.46 ± 0.04	0.0138
mean value	9.75 ± 0.01	8.77 ± 0.01	6.21 ± 0.02	2.56 ± 0.02	
0.93	9.89 ± 0.05	8.96 ± 0.03	6.32 ± 0.05	2.55 ± 0.05	0.0233
	9.89 ± 0.05	8.97 ± 0.03	6.38 ± 0.05	2.61 ± 0.04	0.0513
	9.94 ± 0.04	9.01 ± 0.03	6.46 ± 0.04	2.66 ± 0.04	0.0513
	9.94 ± 0.04	9.01 ± 0.03	6.45 ± 0.03	2.64 ± 0.04	0.0533
	9.88 ± 0.05	9.00 ± 0.03	6.49 ± 0.05	2.78 ± 0.04	0.0145
	9.89 ± 0.06	8.99 ± 0.04	6.45 ± 0.07	2.81 ± 0.05	0.0174
mean value	9.91 ± 0.01	8.99 ± 0.01	6.43 ± 0.03	2.68 ± 0.04	
2.26	10.35 ± 0.05	9.48 ± 0.03	6.85 ± 0.05	2.77 ± 0.04	0.0270
	10.35 ± 0.05	9.48 ± 0.03	6.89 ± 0.05	2.86 ± 0.04	0.0260
	10.37 ± 0.05	9.52 ± 0.03	6.92 ± 0.05	2.82 ± 0.04	0.0570
	10.38 ± 0.05	9.53 ± 0.03	6.93 ± 0.04	2.88 ± 0.04	0.0575
	10.42 ± 0.10	9.41 ± 0.06	6.71 ± 0.09	2.99 ± 0.08	0.0259
mean value	10.37 ± 0.01	9.48 ± 0.02	6.86 ± 0.04	2.86 ± 0.04	
4.45	11.08 ± 0.10	10.05 ± 0.06	7.24 ± 0.09	3.05 ± 0.08	0.0518
	11.02 ± 0.07	10.10 ± 0.05	7.33 ± 0.07	3.11 ± 0.06	0.0388
	11.06 ± 0.05	10.19 ± 0.03	7.49 ± 0.05	3.01 ± 0.04	0.0670
	11.07 ± 0.06	10.20 ± 0.04	7.49 ± 0.06	3.04 ± 0.05	0.0680
mean value	11.06 ± 0.01	10.14 ± 0.04	7.39 ± 0.06	3.05 ± 0.02	

[a] BPEI concentration: $1.24 \leq C_{BPEI} \leq 4.39$ $mmol \cdot L^{-1}$ (calculated as a monomer). BPEI is considered as a simple (low molecular weight) tetramine, at $T = 298$ K. [b] ± standard deviation. [c] Standard deviation of the fit.

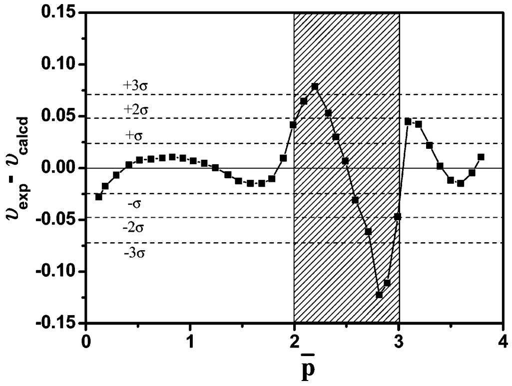

Figure 3. Error plot (ESAB2M calculation) at $I = 0.5$ $mol \cdot L^{-1}$.

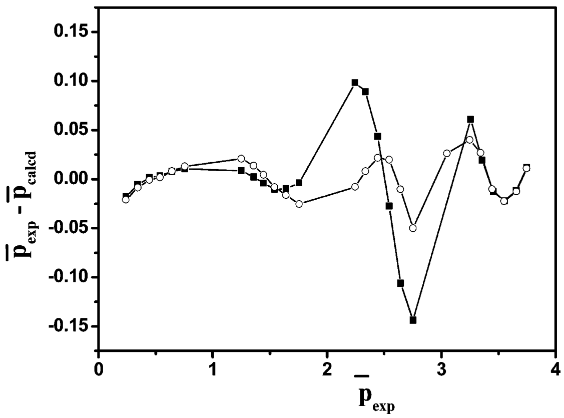

Figure 4. Error plot in the use of (a) and (b) calculation models at $I = 0.5$ $mol \cdot L^{-1}$. ■, model a; ○, model b.

BPEI acid−base properties are studied by pH potentiometry and by titration calorimetry in NaCl(aq) at different ionic strengths.

Experimental Section

Chemicals. All the reagents were Fluka analytical grade. A 50% aqueous solution of BPEI was used without further purification; its concentration was determined by potentiometric titrations, assuming the ligand as a monomer. Hydrochloric acid and sodium hydroxide working solutions were prepared by diluting concentrates and standardized against sodium carbonate or potassium biphthalate, respectively. NaCl solutions were prepared from the solids after drying under vacuum. All the solutions were prepared by using analytical grade water ($R \geq 18$ $M\Omega \cdot cm^{-1}$), and grade A glassware was employed.

Table 2. Protonation Constants of BPEI in NaCl Aqueous Solutions, at Different Ionic Strength (mol·L^{-1}), Calculated from Equation 5 Using Different Models at T = 298 K

I/mol·L^{-1}	model	log K^{H}_{1}	log K^{H}_{2}	log $K^{H}_{3(1)}$[a]	log $K^{H}_{3(0)}$[a]	log $K^{H}_{3(m)}$[a]	log $\bar{K}^{H}_{3}$	log K^{H}_{4}	σ[b]	σ^2/σ_0^2[c]
0.11	a	9.373 ± 0.003[d]	8.219 ± 0.001[d]				5.796 ± 0.006[d,e]	2.496 ± 0.001[d]	0.0237	1.492
	b	9.382 ± 0.003	8.169 ± 0.003	6.264 ± 0.009[d]	5.307 ± 0.012[d]		5.785	2.502 ± 0.001	0.0194	1.000
	c	9.364 ± 0.011	8.122 ± 0.011	6.343 ± 0.016	5.358 ± 0.020	5.706 ± 0.014[d]	5.802	2.499 ± 0.001	0.0194	1.000
0.25	a	9.597 ± 0.001	8.494 ± 0.002				5.998 ± 0.007	2.477 ± 0.001	0.0247	1.638
	b	9.607 ± 0.002	8.431 ± 0.003	6.542 ± 0.005	5.407 ± 0.009		5.975	2.483 ± 0.001	0.0196	1.031
	c	9.553 ± 0.002	8.372 ± 0.008	6.630 ± 0.012	5.461 ± 0.020	5.888 ± 0.012	5.993	2.480 ± 0.001	0.0193	1.000
0.48	a	9.765 ± 0.003	8.745 ± 0.004				6.203 ± 0.007	2.559 ± 0.002	0.0283	1.463
	b	9.775 ± 0.003	8.669 ± 0.008	6.792 ± 0.022	5.558 ± 0.024		6.175	2.564 ± 0.001	0.0237	1.026
	c	9.725 ± 0.028	8.617 ± 0.012	6.874 ± 0.020	5.583 ± 0.024	6.110 ± 0.017	6.189	2.563 ± 0.001	0.0234	1.000
0.93	a	9.920 ± 0.003	8.980 ± 0.003				6.427 ± 0.011	2.700 ± 0.003	0.0400	1.352
	b	9.926 ± 0.004	8.892 ± 0.007	7.042 ± 0.017	5.735 ± 0.024		6.389	2.706 ± 0.003	0.0344	1.000
	c	9.928 ± 0.003	8.897 ± 0.013	7.036 ± 0.020	5.722 ± 0.036	6.400 ± 0.026	6.386	2.707 ± 0.003	0.0344	1.000
2.26	a	10.378 ± 0.002	9.475 ± 0.002				6.868 ± 0.014	2.835 ± 0.003	0.0378	1.440
	b	10.371 ± 0.013	9.336 ± 0.024	7.622 ± 0.043	6.025 ± 0.045		6.823	2.843 ± 0.007	0.0317	1.013
	c	10.369 ± 0.014	9.334 ± 0.013	7.627 ± 0.020	6.024 ± 0.018	6.825 ± 0.015	6.825	2.840 ± 0.003	0.0315	1.000
4.45	a	11.054 ± 0.003	10.129 ± 0.001				7.382 ± 0.008	3.006 ± 0.004	0.0446	1.652
	b	11.038 ± 0.005	9.979 ± 0.016	8.211 ± 0.024	6.462 ± 0.028		7.336	3.009 ± 0.004	0.0359	1.070
	c	11.064 ± 0.004	10.086 ± 0.012	8.081 ± 0.041	6.277 ± 0.039	7.538 ± 0.033	7.299	3.031 ± 0.009	0.0347	1.000

[a] Values calculated from the Högfeldt three-parameter equation. [b] Standard deviation of the fit. [c] Variance ratio. [d] ± standard deviation. [e] log K^{H}_{3} values obtained from eq 10.

Table 3. Intrinsic Protonation Constants of BPEI in NaCl Aqueous Solutions at Different Ionic Strength (mol·L^{-1}) and T = 298 K According to Equations 6 and 7

	log K^{H}_{n1}	log K^{H}_{n2}			log K^{H}_{n4}
I/mol·L^{-1}	$n_1 = 1$	$n_2 = 1$	log K^{H}_{n3}	n_3	$n_4 = 1$
0.11	9.437 ± 0.005[a]	8.124 ± 0.004[a]	5.779 ± 0.005[a]	1.542 ± 0.019[a]	2.545 ± 0.003[a]
0.25	9.700 ± 0.005	8.405 ± 0.005	5.951 ± 0.005	1.672 ± 0.010	2.480 ± 0.002
0.48	9.857 ± 0.006	8.636 ± 0.003	6.154 ± 0.006	1.732 ± 0.018	2.551 ± 0.002
0.93	10.019 ± 0.006	8.859 ± 0.005	6.360 ± 0.006	1.802 ± 0.019	2.676 ± 0.003
2.26	10.478 ± 0.005	9.333 ± 0.005	6.798 ± 0.007	1.972 ± 0.018	2.848 ± 0.003
4.45	11.143 ± 0.005	9.986 ± 0.009	7.322 ± 0.009	2.174 ± 0.024	3.026 ± 0.004

[a] ± standard deviation.

Apparatus. Potentiometric measurements were carried out by using a potentiometer (Metrohm model E605) coupled with a combination ISE-H Orion Ross type glass electrode (model 8172) and with an automatic titrant dispenser (Metrohm Dosimat model 665). The potentiometer and the dispenser were connected to a PC, and suitable home-made software allows monitoring of the titrations. The program allows the equilibrium potentials to be read and the determination of the titrant volume to be added, based on the actual buffering properties of the solution under titration, so that the differences in successive readings were of ~0.1 as $-\log [H^+]$. The estimated precision of the system was ± 0.15 mV in the FEM and ± 0.003 mL in the titrant volume. The measurement cell was thermostatted at T = 25 ± 0.2 °C, and pure nitrogen presaturated with an ionic medium solution at the same ionic strength as the working solution was bubbled into the solution in order to avoid O_2 and CO_2 interference. The solutions were magnetically stirred. Calorimetric measurements were carried out by means of a Tronac (model 450) isoperibolic titration calorimeter coupled with a Keithley 196 digital multimeter. The titrant was delivered by a 2.5 mL capacity model 1002TLL Hamilton syringe. The acquisition of the calorimetric data was performed by a suitable computer program. Accuracy was checked by titrating a THAM [tris(hydroxymethyl)aminomethane] buffer with HCl. The enthalpy of dilution was measured before each experiment under the same experimental conditions as for the calorimetric measurements. The precision of the calorimetric apparatus was Q ± 0.008 J, and the precision of titrant volume was ± 0.001 cm^3. The enthalpy changes for the ionization of water used in the calculations were taken from De Stefano et al.[47]

Procedure. Potentiometric Measurements. 25.0 mL of solution containing $1.24 \leq C_{BPEI} \leq 4.39$ mmol·L^{-1} (calculated as a monomer), an excess of standard HCl ($C_{HCl} = 4 \times C_{BPEI} + 5$ mmol·L^{-1}), and suitable quantities of sodium chloride ($0.1 \leq C_{NaCl} \leq 5.0$ mol·L^{-1}) were titrated with standard NaOH up to pH ≈ 11. In Figure 2 the first derivative curve of BPEI obtained for C_{BPEI} = 4.4 mmol·L^{-1} and C_{NaCl} = 4.5 mol·L^{-1} is reported as an example. HCl is added to fully protonate the amino groups of BPEI. For each titration, a separate calculation of the formal potential (E^0) was carried out by means of a titration of 10 mmol·L^{-1} HCl at the same ionic strength as the system under study; this determination was carried out both before and after each experiment. Proton concentrations are given as pH = $-\log [H^+]$, i.e., the free hydrogen ion concentration was used. All the titrations were carried out in duplicate.

Calorimetric Measurements. 50.0 mL of a solution containing the supporting electrolyte ($0.25 \leq C_{NaCl} \leq 5.0$ mol·L^{-1}) and (0.981 and 4.904) mmol·L^{-1} BPEI was titrated with (0.5047 and 1.003) mol·L^{-1} HCl, respectively.

Calculations. The nonlinear least-squares computer program ESAB2M[48] was used to refine the parameters of the acid−base titrations (species concentrations, formal potential of electrode couple (E^0), water ionic product (K_w)) and the conditional protonation constants by minimizing the error squares sum:

$$U = \sum (V_{exp} - V_{calcd})^2 \qquad (1)$$

where V is the titrant volume.

The general nonlinear least-squares computer program LIANA[49] was used to analyze experimental data using different models (see later). For the analysis of the calorimetric titrations and the determination of enthalpy changes, the program ES5CM[50] was used.

Results and Discussion

Models for the Calculation of Protonation Constants. The protonation constants of a polyamine are related to the stepwise or overall reactions:

$$\mathrm{AH}_{i-1}^{(i-1)+} + \mathrm{H}^+ \rightleftarrows \mathrm{AH}_i^{i+} \qquad K^{\mathrm{H}}{}_i \tag{2}$$

$$\mathrm{A} + i\mathrm{H}^+ \rightleftarrows \mathrm{AH}_i^{i+} \qquad \beta^{\mathrm{H}}{}_i \tag{3}$$

where, in the present case, $i = 1$ to 4. The number of protons bound to the amine is given by

$$\bar{p} = \frac{[\mathrm{H}^+]_\mathrm{T} - [\mathrm{H}^+] + K_\mathrm{W}/[\mathrm{H}^+]}{[\mathrm{A}]_\mathrm{T}} \tag{4}$$

where $[\mathrm{A}]_\mathrm{T}$ = total concentration of amine or

$$\bar{p} = \frac{\sum i\beta^{\mathrm{H}}{}_i[\mathrm{H}]^i}{1 + \sum \beta^{\mathrm{H}}{}_i[\mathrm{H}]^i} \tag{5}$$

If the polyamine can be considered as a low molecular weight amine, one can calculate $K^{\mathrm{H}}{}_i$ or $\beta^{\mathrm{H}}{}_i$ from least-squares minimization using function 1. Direct calculation can be made using eq 5 also. For high molecular weight polyelectrolytes, often the protonation constants are dependent on the degree of protonation (or on the degree of ionization)[29,44−46] and therefore different calculation methods must be used. Katchalsky[51] proposed the modified Henderson−Hasselbalch equation:

$$\mathrm{pH} = \log K^{\mathrm{H}}{}_{ni} - n_i \log\,[(1 - i + \bar{p})/(i - \bar{p})] \tag{6}$$

where $K^{\mathrm{H}}{}_n$ is the so-called intrinsic protonation constant and n is an empirical parameter (for $n = 1$ we have the original equation for low molecular weight ligands). Equation 6 can be written, considering the protonation constant, K^{H}, at each pH value as

$$\log K^{\mathrm{H}}{}_i = \log K^{\mathrm{H}}{}_{ni} - (n_i - 1)\log[(1 - i + \bar{p})/(i - \bar{p})] \tag{7}$$

A slightly more sophisticated three-parameter equation was proposed by Högfeldt et al.:[52]

$$\log K^{\mathrm{H}}{}_i = (i - \bar{p})^2 \log K^{\mathrm{H}}{}_{i(1)} + (1 - i + \bar{p})^2 \log K^{\mathrm{H}}{}_{i(0)} + 2(i - \bar{p})(1 - i + \bar{p}) \log K^{\mathrm{H}}{}_{i(\mathrm{m})} \tag{8}$$

where $K^{\mathrm{H}}{}_{(1)}$ and $K^{\mathrm{H}}{}_{(0)}$ are the value at $\bar{p} \rightarrow i$ and $\bar{p} \rightarrow (i - 1)$, and $K^{\mathrm{H}}{}_{(\mathrm{m})}$ is an intermediate value that accounts for the nonlinearity of the function $\log K^{\mathrm{H}} = f(\bar{p})$. The thermodynamic protonation constant can be represented by a mean constant ($\bar{K}^{\mathrm{H}}$) obtained by the integral of the experimental curve: $\log K^{\mathrm{H}} = f(\bar{p})$ in the range $(i - \bar{p}) \le \bar{p} \le (1 - i + \bar{p})$, for example:

$$\log \bar{K}^{\mathrm{H}}{}_i = \int_{i-\bar{p}}^{1-i+\bar{p}} \log K^{\mathrm{H}}{}_i \, \mathrm{d}\bar{p} \tag{9}$$

or

$$\log \bar{K}^{\mathrm{H}}{}_i = (\log K^{\mathrm{H}}{}_{i(1)} + \log K^{\mathrm{H}}{}_{i(0)} + \log K^{\mathrm{H}}{}_{i(\mathrm{m})})/3 \tag{10}$$

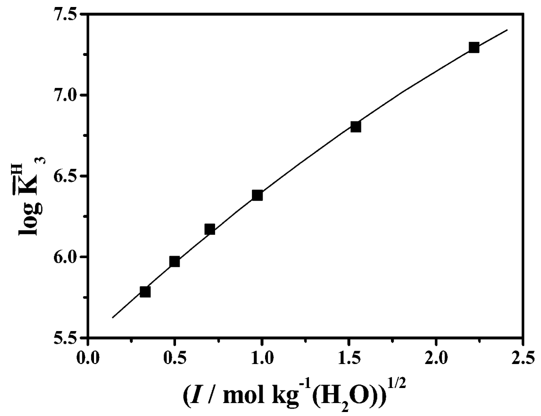

Figure 5. Ionic strength dependence of $\log \bar{K}^{\mathrm{H}}{}_3$ in NaCl(aq) at $T = 25$ °C.

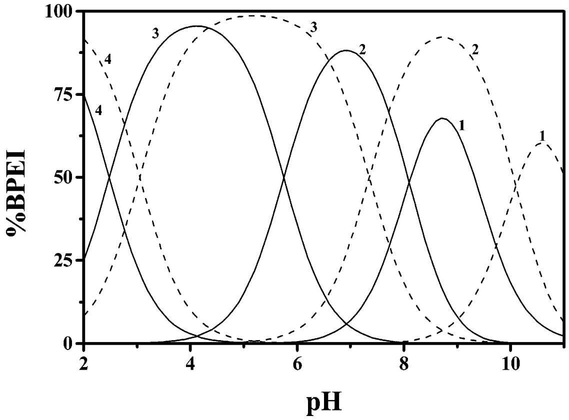

Figure 6. Speciation diagram for BPEI. Percentages of BPEI protonated species vs pH obtained by protonation constants reported in Table 1. Index $i = 1-4$ refer to BPEI-H$_i$ species. Analytical conditions: $C_{\mathrm{BPEI}} = 1$ mmol·L^{-1}, $T = 298$ K, and $I = 0.10$ mol·L^{-1} (—) and 4.50 mol·L^{-1} (– –) for NaCl.

The Högfeldt three-parameter eq 8 comes from the Guggenheim zeroth approximation.[53] When the function $\log K^{\mathrm{H}}{}_i$ versus $\bar{p}$ is linear, eq 8 is simplified to

$$\log K^{\mathrm{H}}{}_i = (i - \bar{p}) \log K^{\mathrm{H}}{}_{i(1)} + (1 - i + \bar{p}) \log K^{\mathrm{H}}{}_{i(0)} \tag{11}$$

and, therefore

$$\log \bar{K}^{\mathrm{H}}{}_i = \log K^{\mathrm{H}}{}_{i(\mathrm{m})} = (\log K^{\mathrm{H}}{}_{i(1)} + \log K^{\mathrm{H}}{}_{i(0)})/2 \tag{12}$$

Protonation Constants. First we considered, in the calculation, the BPEI polyelectrolyte as a simple low molecular weight tetramine and, by minimizing function (1) (ESAB2M computer program), we obtained the protonation constants reported in Table 1. Both standard deviations on the parameters and standard deviations on the fit are reasonable and indicate that, as a first approximation, this type of calculation can be used to obtain a simple picture of acid−base proprieties of BPEI. Nevertheless, by considering a typical error plot (Figure 3), one may observe that for the third protonation step, $2 < \bar{p} < 3$, there is a systematic trend and that $\Delta V = (V_{\mathrm{exp}} - V_{\mathrm{calcd}})$ exceeds the $\pm 3\sigma$ range. This behavior is confirmed by performing analogous calculations, using eq 5 and minimizing the error square sum:

$$U = \Sigma(\bar{p}_{\mathrm{exp}} - \bar{p}_{\mathrm{calcd}})^2 \tag{13}$$

(LIANA calculations) as shown in Figure 4. The trend observed in the error plots (see Figures 3 and 4) can be interpreted in terms of dependence of $\log K^{\mathrm{H}}{}_3$ on $\bar{p}$. By substituting the

Table 4. Protonation Constants of BPEI in NaCl Aqueous Solutions at Different Ionic Strength in the Molal Concentration Scale at $T = 298$ K

I/mol·kg^{-1}	log K^{H}_{1}	log K^{H}_{2}	log $K^{H}_{3(1)}$[a]	log $K^{H}_{3(0)}$[a]	log $\bar{K}^{H}_{3}$[b]	log K^{H}_{4}
0.11	9.380	8.166	6.262	5.305	5.784	2.500
0.25	9.604	8.427	6.539	5.404	5.972	2.480
0.49	9.770	8.664	6.787	5.553	6.170	2.559
0.95	9.917	8.884	7.034	5.726	6.380	2.697
2.37	10.350	9.316	7.601	6.004	6.803	2.822
4.92	10.994	9.936	8.168	6.419	7.294	2.966

[a] Values calculated from eq 11. [b] Values calculated from eq 12.

Table 5. Infinite Dilution Protonation Constants of BPEI at $T = 298$ K Together with $\Delta\epsilon_\infty$ and $\Delta\epsilon_0$ Parameters Referred to Molal Constants

		$\Delta\epsilon_\infty$	$\Delta\epsilon_0$	σ[a]
log K^{H0}_{1}	9.36 ± 0.06[b]	0.20 ± 0.02[b]	0.95 ± 0.15[b]	0.035
log K^{H0}_{2}	7.90 ± 0.05	0.16 ± 0.02	1.04 ± 0.12	0.032
log $\bar{K}^{H0}_{3}$[c]	5.29 ± 0.01	0.14 ± 0.01	0.48 ± 0.04	0.004
log K^{H0}_{4}	1.80 ± 0.06	0.05 ± 0.02	−0.71 ± 0.16	0.040

species	ϵ_∞[d]	ϵ_0[d]	σ[a]
ϵ(AH$^+$, Cl$^-$)	−0.068 ± 0.012[b]	−0.875 ± 0.048[b]	0.035
ϵ(AH$_2^{2+}$, Cl$^-$)	−0.089 ± 0.016	−1.829 ± 0.061	0.032
ϵ(AH$_3^{3+}$, Cl$^-$)	−0.093 ± 0.017	−2.208 ± 0.064	0.004
ϵ(AH$_4^{4+}$, Cl$^-$)	−0.003 ± 0.021	−1.417 ± 0.082	0.040

[a] Standard deviation of the fit. [b] ± standard deviation. [c] Value obtained from eq 10. [d] Parameters calculated using eq 16.

Table 6. ΔG, ΔH, and $T\Delta S$ Values for the Protonation of BPEI at $T = 298$ K at Different Ionic Strengths in NaCl Aqueous Solutions

I[a]	i[b]	$-\Delta G_i$[c]	ΔH_i[c]	$T\Delta S_i$[c]
0.25	1	54.6 ± 0.2 [d]	−47.4 ± 0.5[d]	7.2 ± 0.5[d]
	2	48.0 ± 0.2	−45.8 ± 0.5	2.2 ± 0.5
	3	34.1 ± 0.1	−31.4 ± 0.5	2.7 ± 0.5
	4	14.5 ± 0.2	−10.2 ± 1.0	4.2 ± 1.0
1.03	1	56.8 ± 0.1	−47.8 ± 0.1	9.0 ± 0.1
	2	50.9 ± 0.1	−46.1 ± 0.3	4.8 ± 0.3
	3	36.7 ± 0.1	−34.3 ± 0.5	2.4 ± 0.5
	4	15.4 ± 0.2	−10.5 ± 1.0	4.9 ± 0.2
3.21	1	60.4 ± 0.1	−50.2 ± 0.6	10.3 ± 0.6
	2	54.6 ± 0.1	−49.0 ± 0.5	5.6 ± 0.5
	3	39.8 ± 0.1	−38.5 ± 0.6	1.3 ± 0.6
	4	16.3 ± 0.1	−9.8 ± 1.7	6.5 ± 1.7
5.63	1	63.6 ± 0.2	−52.5 ± 0.7	11.1 ± 0.7
	2	57.5 ± 0.2	−51.0 ± 0.6	6.4 ± 0.6
	3	42.3 ± 0.1	−41.4 ± 0.7	0.9 ± 0.7
	4	17.2 ± 0.2	−15.8 ± 1.9	1.4 ± 1.9

[a] mol·kg^{-1}. [b] Step of BPEI protonation. [c] kJ·mol^{-1}. [d] Standard deviation.

Table 7. Infinite Dilution Values of ΔH^0_i (at $I = 0$ mol·kg^{-1}) of BPEI at $T = 298$ K Together with $\Delta\epsilon'$ Parameters (eq 17)

i[a]	ΔH^0_i[b]	$\Delta\epsilon'_i$	σ[c]
1	−47.1 ± 0.3	−1.0 ± 0.1	0.10
2	−46.4 ± 0.5	−1.1 ± 0.1	0.20
3	−34.0 ± 0.8	−1.9 ± 0.2	0.92
4	−12.4 ± 0.6	−1.3 ± 0.2	0.87

[a] Step of BPEI protonation. [b] kJ·mol^{-1}. [c] Standard deviation of the fit.

expression for log K^{H}_{3} from eqs 8 or 11 into eq 5, we may recalculate all the protonation constants with log $K^{H}_{3} = f(\bar{p})$. In Table 2, we report the results of these calculations: (a) all the protonation constants considered as true constants (first row); (b) log K^{H}_{3} considered as a linear function of $\bar{p}$, according to eq 11 (second row); and (c) log K^{H}_{3} considered as a nonlinear function of $\bar{p}$, according to eq 8 (third row). The difference in the standard deviation of the fit between (a) and (b) calculations is quite significant, while no further improvement is observed by using the (c) model. The difference between (a) and (b) calculations is also clearly evidenced in Figure 4. In Table 2, we report also the variance ratio σ^2/σ_0^2 that provides evidence of the statistical significance of the different models. In Figure 4, we show two error plots relative to the use of the (a) and (b) calculation models: also in this case the reduction of errors is quite evident. The proposed model for the protonation of BPEI was confirmed still further by using eqs 6 and 7 with $n_1 = n_2 = n_4 = 1$ and by refining n_3 with excellent results in terms of fitting. The protonation constants and n_3 values are reported in Table 3.

Dependence on Ionic Strength of Protonation Constants. The protonation constants of BPEI are strongly dependent on the ionic strength as shown in Figure 5, where as an example we report the trend observed for log $\bar{K}^{H}_{3}$. Moreover this dependence implies very different speciation profiles at low and high ionic strengths. In Figure 6, we plotted the formation percentage of each species versus pH at $I = 0.1$ mol·L^{-1} and $I = 4.5$ mol·L^{-1}; as can be seen the shift of the curves is noticeable (e.g., for the first protonation step we have a maximum shift of 1.9 pH units). The dependence on ionic strength of protonation constants can be modeled by using the SIT (Specific ion Interaction Theory) approach,[54−56] which can be expressed by the equation (at $T = 298$ K):

$$\log K^{H}_{i} = \log K^{H0}_{i} + 2(i-1)A\sqrt{I}/(1 + 1.5\sqrt{I}) + \Delta\epsilon_i I \tag{14}$$

where K^{H0} is the protonation constant at infinite dilution (molal concentration scale), A is the Debye-Hückel constant ($A = 0.51$ at $T = 298$ K), and $\Delta\epsilon$ is the difference between interaction coefficients. For example, when $i = 3$, we have

$$\Delta\epsilon_1 = \epsilon(\mathrm{H_2A^{2+}, Cl^-}) + \epsilon(\mathrm{H^+, Cl^-}) - \epsilon(\mathrm{H_3A^{3+}, Cl^-}) \tag{15}$$

By writing eq 15 also for the other steps, we have four equations with five unknown ϵ values, namely, ϵ(H$_i$A^{i+}, Cl$^-$), for $i = 1$ to 4, and ϵ (A^0, NaCl) ≡ K_m (Setschenow coefficient for the neutral species); ϵ(H$^+$, Cl$^-$) is known from the literature.[57] Initially we calculated simply the $\Delta\epsilon$ values that will allow single ϵ parameters to be obtained when one of them is available from independent measurements.

In the original SIT approach ϵ values are true constants, but in several cases, in particular for $I > 3.5$ mol·kg^{-1}, they are dependent on ionic strength. To take into account this dependence, the following equation was proposed:[57]

$$\epsilon = \epsilon_\infty + \frac{\epsilon_0 - \epsilon_\infty}{I + 1} \tag{16}$$

($\epsilon = \epsilon_\infty$ for $I \to \infty$; $\epsilon = \epsilon_0$ for $I \to 0$) that can be used also at very high ionic strengths. In this case we may calculate $\Delta\epsilon_\infty$ and $\Delta\epsilon_0$ for each protonation step. Note that, when dealing with the SIT method, the molal concentration scale must be used. In

Table 8. Comparison of Protonation Constants and Enthalpies at Infinite Dilution with Other Tetramines at $T = 298$ K

compound	$\log K^{H0}_1$	$\log K^{H0}_2$	$\log K^{H0}_3$	$\log K^{H0}_4$	ref
Trien[a]	9.67	8.87	6.11	2.38	19
TE1[b]	9.22	8.14	4.74	0.57	19
mean value	9.45	8.51	5.43	1.48	

compound	ΔH^0_1	ΔH^0_2	ΔH^0_3	ΔH^0_4	ref
Trien[a]	−42.6	−46.3	−42.3	−33.3	15
TE1[b]	−27.8	−29.9	−25.4	−15.0	20

[a] Triethylenetetramine (Trien). [b] 1,1,4,7,10,10-Hexamethyltriethylenetetraamine (TE1).

Table 4, we report the protonation constants at different ionic strengths converted to this scale. Values of $\Delta\epsilon_\infty$ and $\Delta\epsilon_0$ together with $\log K^{H0}_i$ values are given in Table 5. The values of $\Delta\epsilon_\infty$ and $\Delta\epsilon_0$ for each protonation step are quite different and indicate that the simple one-parameter equation cannot be used in a large ionic strength range. Both $\Delta\epsilon_\infty$ and $\Delta\epsilon_0$ are a decreasing function of the protonation step as expected owing to the interaction of Cl^- with the positive charges of protonated amino groups.[58] By assuming $\epsilon(A^0, NaCl) \equiv K_m = 0$ (as frequently done in the literature), we can calculate single $\epsilon_x (x = \infty, 0)$ parameters for H_iA^{i+} species, using for $\epsilon(H^+, Cl^-)$ the values $\epsilon_\infty(H^+, Cl^-) = 0.136$[57] and $\epsilon_0(H^+, Cl^-) = 0.0848$.[57] The interaction parameters for H_iA^{i+} species are reported in the second part of Table 5.

Protonation Enthalpies and Their Dependence on Ionic Strength. From titration calorimetric measurements, we were able to calculate ΔH values for the protonation of BPEI. In this case, since the precision of calorimetric data is lower than that of the potentiometric data, we considered also the third step as independent of $\bar{p}$. Some trial calculations showed that the loss of precision is comparable with the errors in the calorimetric data. Values of protonation ΔH are reported in Table 6 together with ΔG and $T\Delta S$. As for all the amines the protonation is enthalpically stabilized while the $T\Delta S$ contribution is quite small.

Protonation enthalpies are also dependent on ionic strength, and this dependence can be expressed by

$$\Delta H_i = \Delta H^0_i + A'2(i-1)\sqrt{I}/(1 + 1.5\sqrt{I}) + I\Delta\epsilon'_i \qquad (17)$$

Equation 17 is derived from eq 14; ΔH^0_i is the protonation enthalpy at infinite dilution referred to each protonation step, $A' = RT^2 \ln 10\,(dA/dT) = 1.5$, and

$$\Delta\epsilon' = RT^2 \ln 10 \frac{d\Delta\epsilon}{dT} \qquad (18)$$

As expected, the $\Delta\epsilon'$ values reported in Table 7 are fairly small, indicating a small dependence on temperature of the interaction parameters for the protonated species of polyamines. This is in accordance with the results of previous work on the dependence on ionic strength of protonation enthalpies of low molecular weight polyamines in NaCl(aq).[20]

Literature Comparison. Kim and Cho[43] studied the acid−base proprieties of BPEI at different ionic strengths, KCl(aq) (0.01 to 1) mol·L^{-1}, in the pH range (6 to 9). The reported values of $\log K^H$ were calculated by eqs 6 and 7, and protonated BPEI was considered as monoprotic. Their results can be compared with the data from present study since (a) $\log K^H$ ranges between (7.9 and 8.9) in the same ionic strength conditions; (b) the trend as a function of ionic strength is the same as that observed in this work. Thiele and Gronau[59] found at $I = 1$ mol·L^{-1} (KCl(aq)) $\log K^H = 8.9$ at a protonation degree of $\bar{p} \sim 1.3$. By considering the different approaches (both experimental and calculation) the agreement can be considered satisfactory.

Conclusions

Acid−base properties of BPEI, in terms of free energy, enthalpy, and entropy changes were well-defined over a wide range of ionic strengths in NaCl(aq). The protonation behavior of this high molecular weight polyamine is very similar to that of low molecular weight tetramines. The four protonation steps of BPEI can be described as for simple tetramines, and an improvement only for the third step is observed by considering $\log K^H_3$ as a function of protonation degree ($\bar{p}$). Moreover the values of both protonation constants and protonation enthalpies can be easily compared with that of some tetramines as shown in Table 8.

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Received for review September 5, 2006. Accepted November 6, 2006. This work was supported by FIRB (Fondo per gli Investimenti per la Ricerca di Base) RBAUO1HLFX.

JE060388Z