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The Proton Affinity Spectrum of Polyethylenimine

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Abstract. The characterization of polymers and surfaces with a large number of different sites is difficult, mainly due to problems in the numerical solution of the integral equation involved. New numerical tools developed which can be used on PCs allow to solve these problems today at the bench. This opens new possibilities to characterize equilibria in heterogeneous systems and polymer solutions. We encourage analytical chemists to use these new tools and to develop a feeling for the limits of the method in different applications. In this contribution, we report the interpretation of the titration curves of different types of polyethylenimines in aqueous solution as an example of this new approach.

Introduction

In the last few years, we have studied the protonation and the complex formation of water-soluble polymers [1–4]. Experimentally, a titration curve is measured by potentiometry or spectroscopy and the distribution of the proton or the metal ion, respectively, is determined as a function of the composition of the solution. There is no problem to calculate the binding isotherm from the measurement, if the number of binding sites per gram of polymer is known. The number of sites for H^+ is equal to the degree of polymerisation, if each repeating unit contains one basic position. The number of binding sites for metal ions depends of the number of coordination sites, but it is proportional to the mass of the polymer dissolved. The interpretation of the experimental binding isotherm for the polymer is, however, a difficult problem, which is discussed in the following.

Because the problems are identical for the interpretation of the binding isotherm of H^+ and of metal ions, with exception of the influence of the coordination number, we limit the discussion to the binding of protons. The binding of the proton is characterised by association constants, which is not general practice in acid-base reactions.

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Affinity Spectrum [5]

The interpretation of the binding isotherm in these systems is not possible by the same formalism as the interpretation of the binding isotherm of small molecules with a well defined limited number of basic sites. There are two main reasons why the conventional approach fails:

i) Polymers with one basic site in each repeating unit have a large number of binding sites corresponding to the degree of polymerisation. Hence, the same number of stepwise association constants K_i or cumulative association constants β_i are necessary to define the isotherm by the standard *Eqn. 1*.

$$x_{hb} = \frac{H_3O^+_{bound}}{L_{tot}} = \frac{1}{n} \cdot \frac{\sum_{i=0}^{n} i \cdot \beta_i \cdot [H_3O^+]^i}{\sum_{i=0}^{n} \beta_i \cdot [H_3O^+]^i}, \qquad \beta_i = \prod_{I=0}^{i} K_I \qquad (1)$$

x_{hb}: fraction of occupied sites
L_{tot}: number of binding sites in solution
$H_3O^+_{bound}$: number of protons bound
n: number of binding sites per macromolecule
β_i: cumulative association constant, $\beta_0 = 1$
K_i: stepwise association constant, $K_0 = 1$

Each of the n constants will be different even if the intrinsic affinity for the different sites is the same. K_i for the case of n identical non-interacting sites is related to the intrinsic constants k by *Eqn. 2*. This result is obtained, comparing the relative probabilities for association and dissociation.

$$K_i = \frac{n - i - 1}{i} \cdot k \qquad (2)$$

K_i: i-th stepwise association constant of a class of n sites with identical affinity
k: intrinsic association constant of the class

The negative logarithm of the stepwise association constant K_i is proportional to the change in free energy, ΔG^0 for the transfer of one proton from the solution to the polymer carrying already $i-1$ protons. The negative logarithm of the intrinsic association constant k is proportional to the change in free energy, ΔG^0 for the transfer of one proton from the solution to one site of the class, independent of the number of protons already bound.

ii) The degree of polymerisation of different macromolecules varies. Therefore, the number of coordination sites n is not the same for all the molecules in solution. It is defined by the molecular-weight distribution, which is characteristic for the polymer studied.

Under these conditions, the association to a polymer can be treated in the same way as the adsorption in heterogeneous systems. This is not surprising, as a diluted polymer solution is a heterogeneous system on the molecular level, even if the solution seems to be homogenous by eye. There is a high local concentration of binding sites within the different polymer coils. Inbetween, the polymer coils there are no binding sites at all. The isotherm of a heterogeneous system is given by *Eqn. 3*, if the association is in the order of increasing free energy, and if there is no cooperative interaction between different association steps.

$$x_{hb} = \frac{H_3O^+_{bound}}{L_{tot}} = \sum_{i=1}^{m} p_i \cdot \frac{k_i \cdot [H_3O^+]}{1 + k_i \cdot [H_3O^+]} \qquad (3)$$

x_{hb}: fraction of occupied sites
L_{tot}: number of binding sites in solution

$H_3O^+_{bound}$: number of protons bound
m: number different classes of binding site
k_i: association constant of class i
p_i: fraction of binding sites in class i

In this approach, the coordination sites are distributed in different classes, which are characterized by the same intrinsic association constants k_j, and the intrinsic free energy of association ΔG^0_j(intr), characterising the transfer of a proton from solution to one independent site of class j. The number of different classes depends on the resolution on the ΔG^0 scale. If the steps in ΔG^0 are infinitely small, the sum might be replaced by an integral, and the values p_i by a continuous function a of log k and ΔG^0.

$$x_{hb} = \int_{-\infty}^{+\infty} a(\log k) \cdot \frac{k \cdot [H_3O^+]}{1 + k \cdot [H_3O^+]} \, d\log k = \int_{-\infty}^{+\infty} a(\log k) \cdot R(k) \, d\log k \qquad (4)$$

$a(\log k)$: affinity spectrum, site-affinity density function, SADF
$R(k)$: response function

The function $a(\log k)$ is the affinity spectrum or the site affinity density function of the polymer in solution. The value $a(\log k_i)$ indicates the fraction of binding sites within the interval log k_i and log $k_i + \Delta$. The affinity spectrum can be interpreted as the free-energy spectrum of the polymer in solution as sensed by the H^+. The affinity spectrum is a conditional property, because it depends on activity of the species in solution and the composition of the solution.

Fredholm Volterra integral equations of the form *4* are general for any type of measurement. The characteristic property of the probe is convoluted by the response function characteristic for the experimental method. In a spectrometer, as an example, the response function is given by the slit width and the performance of the spectrometer. In our case, it is given by a *Langmuir* isotherm, characteristic for a large number of identical sites. The deconvolution of the experimental measurements to find the properties of the probe poses considerable numerical problems.

It is, in general, not possible to give a solution of *Eqn. 4*. One resorts, therefore, to the numerical solution of the *Eqn. 4*. If a grid of nx equidistant values log k_j is defined, *Eqn. 4* yields the system of linear *Eqn. 5*.

The solution of the linear system *5* without any further assumptions is cumbersome, because it is in general ill-posed [3]. The introduction of the additional condition $p_j > 0$ allows to find meaningful solutions of the system *5* using log k_i values in the range from 0.2 to 13.8 with the increment of 0.2 units. This increment in log k_j corresponds to a resolution of *ca.* 1.1 kJ/mol in ΔG^0 at room temperature. The limits of the window to probe the affinity spectrum of a polymer in aqueous solution are given by the intrinsic limits imposed by the pH in diluted solutions *ca.* 1–13.

The cumulative sum of p_i and the integral of a(log k) between the limits log k_a and log k_b gives the fraction of basic sites with an affinity in this interval. This information gives not only a characterization of a polymer in solution. It also allows to probe the qualitative (log k_i) and quantitative (p_i) composition of a mixture of acids in solution from the titration curve of the latter, even if no distinct inflection points and buffer regions are observed. The possibilities of this approach should be studied systematically.

Today, it is possible to transform the isotherm into an affinity spectrum on a PC using numerical 'tools' supplied by commercial subroutine packages [6] (see *Exper. Part*). In our laboratory, we use the subroutine *nnls* (nonlinear least square), which allows the solution of the linear system *5* with the condition of non negative values p_i. The interpretation of the isotherm experimentally obtained by a titration in a heterogeneous system is, therefore, possible without difficulties in the laboratory on a PC. We encourage workers in this field to make use of these possibilities to interpret. the isotherm without any assumption on the distribution of the site affinities.

Affinity Spectrum of a Linear Decaamine (N10)

To get some feeling of the possibilities of this approach, we simulated the titration curve (ml of base added *vs.* pH) of the linear decaamine **N10** (1,26-bis(methylamino)-3,6,9,12,15,18,21,24-octaazahexacosane) [7]. The ten stepwise protonation constants have been determined by *Aragó et al.* [7]. The calculated pH value have been rounded to 10^{-2} units, and a normal distributed error of 0.01 units was added. This corresponds to the precision of a pH measurement. The isotherm was calculated from the titration curve by *Eqn. 6* and the affinity spectrum obtained by *Eqn. 5* using an increment in log k of 0.02. The result is given in *Table 1*. The affinity spectrum shows a cluster of classes of binding sites with a log k_i between 9.8 and 7.8, which corresponds to 5 of the 10 binding sites. The position of the peaks does not correspond to the log K_i values in the literature. An inspection of the five log K_i values in this region shows that their ratio is close to the statistical ratio 5:2:1:0.5:0.2 for the stepwise constants of five identical independent sites. It is obvious that, for five identical sites, one would find only one peak in the affinity spectrum. The affinity of the next class of sites corresponding to a fraction of 10% is found at 5.2. This is much lower than log $K_6 = 6.68$. The 7th and 8th class are found at log $k_i = 4.5$ and log $k_i = 3.4$ corresponding to 4 and 13%. Their position corresponds to log K_7 and log K_8. The affinity spectrum is not able to differentiate between site log K_9 and log K_{10} at 2.71 and 1.46. Only one class of sites is found between log $k_i = 2.2$ and 2.0 corresponding to 17%. In the region where these sites are occupied, the H^+ concentration is much higher than the concentration of the polyamine. Therefore, only a minor part of the H^+ in solution are bound. Consequently, the x_{hb} values calculated by *Eqn. 6* depends strongly on the precision of the pH measurement. The standard deviation between the simulated isotherm and the recalculated isotherm using the affinity spectrum is $6.5 \cdot 10^{-3}$.

$$\vec{x_{hb}}(\vec{nb}) = \mathbf{A}(nb \cdot nx) \cdot \vec{p}(nx)$$
$$A_{i,j} = \frac{k_j \cdot [H_3O^+]_i}{1 + k_j \cdot [H_3O^+]_i} = \frac{10^{\log k_j - pH_i}}{1 + 10^{\log k_j - pH_i}} \qquad i = 1...nb,\ j = 1...nx \qquad (5)$$

Affinity Spectrum of 3,6,9,12-Tetraazatetradecane-1,14-diamine (N6)

A solution of the linear hexaamine in acid solution of known concentration was titrated with NaOH. From the titration curve, we calculated the isotherm for the protons. The six stepwise protonation constants have been obtained by a conventional least-square fit of the isotherm. In addition, we calculated for comparison the affinity spectrum. The results of the two treatments are given in *Table 2* together with the log K values of the linear hexaamine 1,14-bis(methylamino)-3,6,9,12-tetraazatetradecane for comparison. The least-square calculation yields three log K values at 9.91, 9.54, and 8.87, indicating that *ca.* 50% of the basic sites are protonated in the pH region form 11 to 8.

Table 1. *Affinity Spectrum Calculated from a Simulated Titration Curve of* **N10**

$\log k_i$	p_i	$\log k_i$ Σp_i 14	$\log K$ [7]
9.8	0.13	0.13	10.27
9.6	0.03	0.16	9.72
9.4	0.03	0.19	9.27
9.0	0.02	0.21	
8.8	0.19	0.49	8.72
8.0	0.01	0.41	8.24
7.8	0.09	0.50	
5.4	0.01	0.51	6.58
5.2	0.10	0.60	
4.4	0.02	0.62	4.54
4.2	0.02	0.64	
3.4	0.12	0.76	3.50
3.2	0.01	0.77	
2.2	0.06	0.83	2.71
2.0	0.15	0.98	1.46

Table. 2. *Affinity Spectrum of 3,6,9,12-Tetraazatetradecane-1,14-diamine*

$\log k_i$	p_i	$\log k_i$ Σp_i 14	$\log K^{a)}$	$\log K^{b)}$ [7]
9.6	0.22	0.22	9.91	10.28
9.4	0.27	0.49	9.64	9.52
8.2	0.01	0.50		
8.0	0.02	0.52		
7.2	0.11	0.63	6.94	6.54
6.8	0.01	0.64		
6.6	0.03	0.67		
5.2	0.04	0.71	4.60	3.80
5.0	0.08	0.79		
3.8	0.18	0.97		
3.6	0.03	1.00	3.21	2.52

[a)] Result of least square fit.

[b)] 1,14-Bis(methylamino)-3,6,9,12-tetraazatetradecane.

Table.3. *Affinity Spectrum of BPEI 600 and Polymin P*

BPEI 600 0.01M N, 0.02M H^+ pH 2.24–10.85			Polymin P, 0.01M N, 0.02M H^+ pH 2.42–11.22		
$\log k_i$	p_i	$\log k_i$ Σp_i 14	$\log k_i$	p_i	$\log k_i$ Σp_i 14
9.8	0.18	0.18	9.6	0.06	0.06
9.6	0.25	0.43	9.4	0.32	0.38
7.8	0.06	0.49	7.8	0.05	0.43
7.6	0.04	0.53	7.6	0.04	0.47
6.6	0.10	0.63	6.6	0.08	0.55
6.4	0.02	0.65	6.4	0.04	0.59
5.4	0.02	0.67	5.0	0.05	0.64
5.2	0.02	0.69	4.8	0.05	0.69
4.4	0.07	0.76			
4.2	0.01	0.78			
3.0	0.06	0.83			

The ratio of these three log K values is close to the statistical ratio 3:1:0.33 for three identical sites. The affinity spectrum shows two important values at log k = 9.6 and 9.4, corresponding to 49% of the binding sites and some minor features at log k = 8.2 and 8.0 corresponding to only 3% of the binding sites. As in the case of **N10**, the affinity spectrum is not able to distinguish the three most basic sites, which have nearly the same intrinsic affinity. The deviation from the pK values found for three identical sites is to small. There is 11% sites with an log k of 7.2 and 4% of the sites with a log k of 6.8 and 6.6. The fraction corresponds to *ca.* one proton in a hexaamine. The log K_4 is found to be 6.94 by the least square fit. Of the two most acid sites with log K 4.60 and 3.21, each corresponds to the two neighboring peaks in the affinity spectrum at 5.2 (4%), 5.0 (8%) and 3.8 (18%), 3.6 (3%). The standard deviation between the experimental and the recalculated isotherm is $1.2 \cdot 10^{-2}$ for the least square fit and $0.8 \cdot 10^{-2}$ for the affinity spectrum.

Affinity Spectrum of BPEI(Branched Polyethylenimine)

BPEI is an industrial product. The structure of the polymer is not well defined. Polymers contain primary, secondary, and tertiary amines in the ratio of *ca.* 1:2:1 [8]. We studied three different types of BPEI, with the molecular weight 600, 1800, and 600 000–1 000 000 according to the specification of the producer. In a standard titration (*Fig. 1*), a solution containing N and H^+ in the ratio of 1:2 is titrated with base. The proton isotherm (*Fig. 2*) of the polymer is calculated from the titration curve. The maximum of the isotherms is 0.83 at pH 2.23 the beginning of the titration for the BPEI 600 and 1800. This indicates that, at this pH, not all of the N-atoms are protonated. The isotherm of the BPEI with the high molecular weight starts at 0.69 at pH 1.90. The number of basic sites available to protons in a solution of pH 1.9 is smaller. This might indicate that the affinity of N buried in the large polymer is smaller than the affinity of amines at the surface. The affinity spectra of BPEI 600 and Polymin P are given in *Table 3* (*Fig. 3*). 40–45% of the sites are characterized by a log k between 9.8 and 9.2 independent of the type of polymer. This corresponds to the affinity of an unperturbed amine. Up to this point in the isotherm there is no or only little interaction between the protonation at different sites. Further protonation of the polymer is more difficult. In BPEI 600, five different types of protonation sites are distinguished in

addition, each one corresponding to a fraction somewhat less than 10%. *Ca.* 10% of the sites have log k between 7.8 and 7.6, 12% between 6.6 and 6.4, 4% between 5.4 and 5.2, 8% between 4.4 and 4.2, and 6% at 3.0. Three classes of sites of similar affinity are found for Polymin P. 10% of the sites are at log k between 8.2 and 8.0 *ca.* 13% at log k between 6.8 and 6.6, and 9% log k between 4.8 and 5.0. This analysis shows clearly, that the sites not available for protonation in the high-molecular-weight BPEI are the ones with low affinity. They are probably, as indicated above, buried in the polymer coil. Comparing BPEI with analogous molecular amines shows that their acid base properties are similar. It is not possible to protonate all the N-atoms in these compounds. In tris(2-aminoethyl)amine, only the three primary N-atoms are protonated, in *N,N,N',N'*-tetrakis(2-aminoethyl)ethylenediamine, only the four primary nitrogens [9]. By comparison with these values, one would guess that in BPEI *ca.* 40% of the sites are terminal N-atoms. In *N*-methyl-*N,N',N'*-tris(2-aminoethyl)ethylenediamine, one finds in addition to the log K values of the three primary N-atoms between 8.5 and 10.5 a fourth log K value at 5.3, which must be attributed to the tertiary N-atoms, which has only one substituent with a terminal amino group [9].

Affinity Spectra of LPEI (Linear Polyethylenimine)

If an acid solution of LPEI is neutralized with base, the polymer precipitates out of solution between pH 7 and 8. This precipitation is accompanied by a sudden release of protons. The concept of affinity spectra can, therefore, only be applied up to pH 7. The titration curve shows, that, at this pH, the degree of protonation is *ca.* 0.5. The proton isotherm is, therefore, only known between 0.91 at pH 2.2 and 0.5 at pH 7. It is not possible to protonate the polymer completely within the limits of diluted solutions. The affinity of *ca.* the 10% of the N-atoms is to low to be observed at pH values down to two. The protonation of these sites is hindered by the large positive charge of the linear polyammonium ion. The affinity spectrum (*Fig. 4*) was calculated from the isotherm between pH 2 and 6. It is given for two different concentrations in *Table 4*. In this pH region, one observes three classes of binding sites, with log k values of 5.8 (*ca.* 10%), 4.2–4.6 (*ca.* 10%), and 2.8–3.0 (*ca.* 25%). These values correspond to those values found for well defined linear polyamines. The examples of **N10** and **N6** discussed above show that in

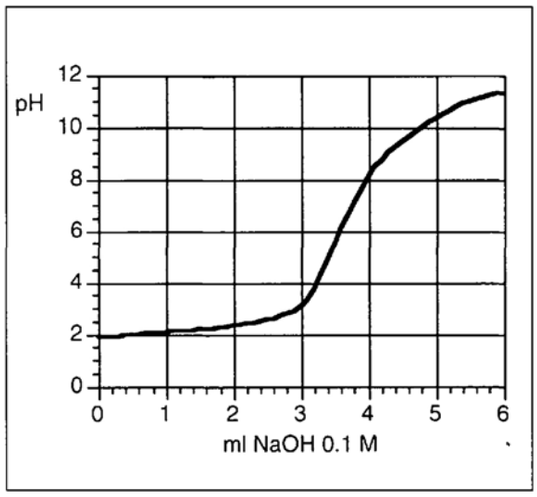

Fig. 1. *Titration curve of 25-ml solution, Polymin P N 10^{-2} M, $2 \cdot 10^{-2}$ M HCl, 0.5M NaCl with 10^{-1} M NaOH*

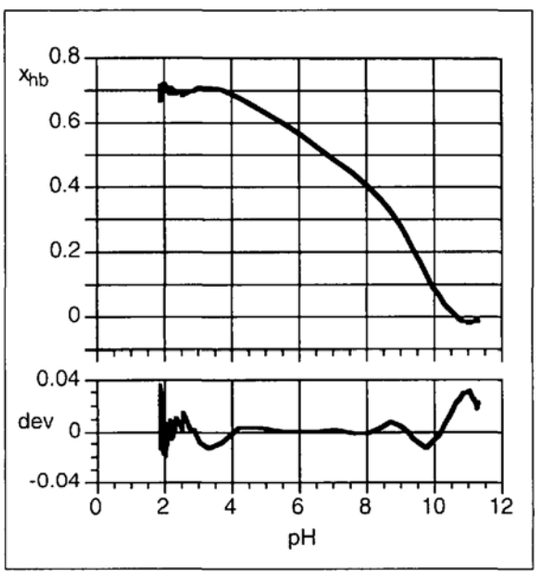

Fig. 2. *Proton isotherm for Polymin P calculated from the titration curve in* Fig. 1. dev: difference between experimental isotherm and the isotherm recalculated with the affinity spectrum in *Fig. 3*.

Table. 4. *Affinity Spectrum of LPEI at Different Concentrations*

LPEI 0.01M N, 0.02M H^+ pH = 2.24–6.70			LPEI, 0.1M N, 0.2M H^+ pH = 2.07 –6.87		
log k_i	p_i	log k_i $\sum_{14}^{} p_i$	log k_i	p_i	log k_i $\sum_{14}^{} p_i$
		0.43[a])			0.50[b])
5.6	0.12	0.55	5.8	0.07	0.57
4.4	0.03	0.58	4.6	0.04	0.61
4.2	0.09	0.67	4.4	0.06	0.67
			3.6	0.10	0.77
3.2	0.11	0.78	3.0	0.10	0.87
3.0	0.13	0.91	2.8	0.04	0.91

[a]) $\sum_{14}^{6.70} p_i$ at the precipitation of LPEI.

[b]) $\sum_{14}^{6.78} p_i$ at the precipitation of LPEI.

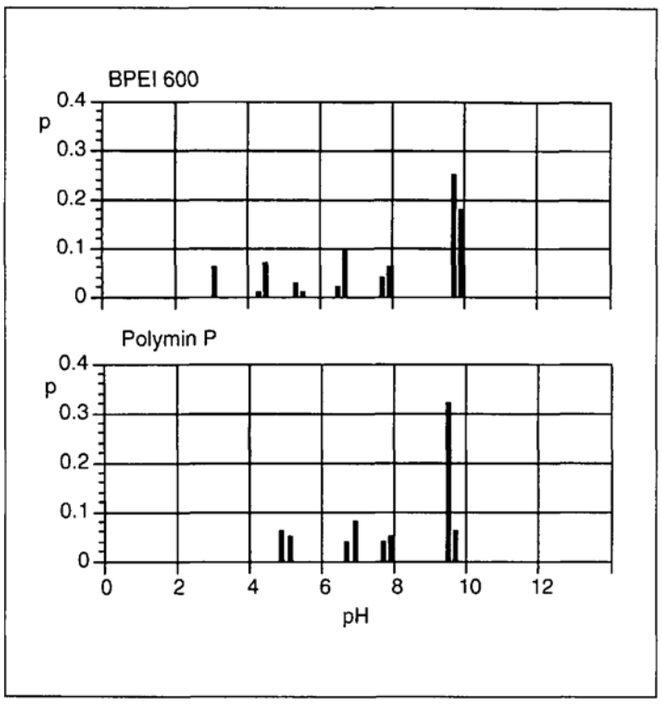

Fig. 3. *Affinity spectrum of BPEI 600 and Polymin P*

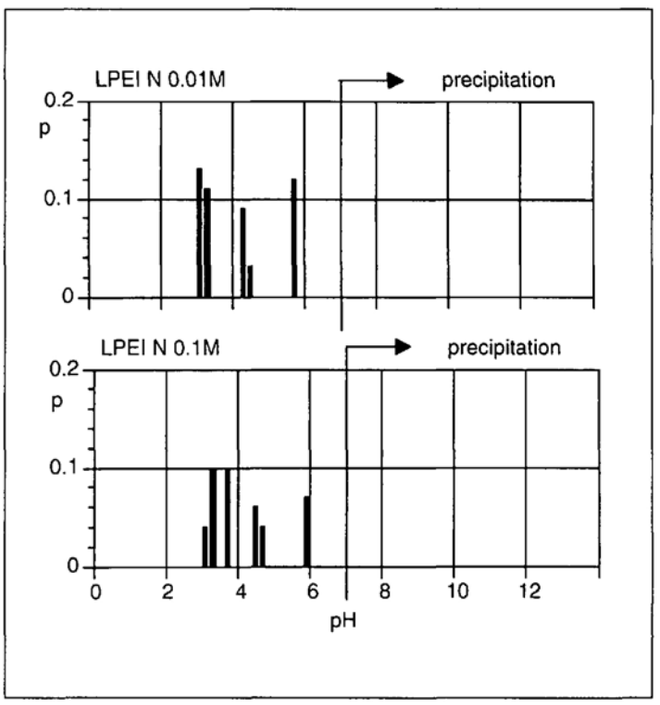

Fig. 4. *Affinity spectrum of LPEI, calculated from two solutions of different concentration*

general one half of the H^+ bind between pH 7.5 and 10. This correspond to the pH region where the LPEI precipitates. The protonation of the second half of the sites is more difficult and extends to low log K values [7][9]. It is, therefore, tempting to postulate, that each proton interacts with two N-atoms as long as the degree of protonation is lower as 0.5. If more protons are bound, the interaction with a second N-atom has to be broken, making the protonation more difficult.

Experimental

Polymin P, mol. wt. 500000–1000000, was obtained as 50% aq. soln. from *Fluka AG*. BPEI 800 and 1800 were obtained as powder from *Polyscience INC*. LPEI was prepared by polymerisation of ethyloxazoline and acid hydrolyses of the polyoxazoline [10][11]. 3,6,9,12-Tetraazatetradecane-1,14-diamine was prepared according to [12].

In a typical titration experiment, a soln., with a ratio N/H^+ of 1:2, prepared from the corresponding mass of polymer and HCl in NaCl 0.5M, was neutralized with NaOH. The pH was measured with a pH-meter *Methrom 632* or a *Mettler DL 21* titrator controlled by a *Olivetti M 290 S*. The glass electrode was calibrated in concentration of H^+ by titration of a mixture of $HClO_4$ and AcOH of known concentration in the same inert electrolyte.

c_a: concentration of strong acid (HCl)
V_a: volume of strong acid added
c_b: concentration of strong base (NaOH)
V_b: volume of strong base added
V_{tot}: total volume of the titration solution
m: mass of polymer dissolved
mol. wt.: molecular weight of repeating unit

The isotherm was calculated from the titration curve by *Eqn. 6* and transferred to MACIIci. The affinity spectrum was obtained using the subroutine *nnls* (non negative least square) from the Matlab OPTIM toolbox [13].

The results reported here are a part of the general research project on the acid-base and complex-formation equilibria of polymers in solution. I acknowledge with pleasure the doctorands, which have contributed with great commitment and enthusiasm during the last years to this project at the borderline of conventional coordination and polymer chemistry. Those that have completed their theses are: *Karin Weiss*, *Vincent Dudler*, *Dominique Sallin*, *Manfred Schweizer*, *Lydia Barbosa*, *Jean Bernard Ballif*, *Claude Lerf*, and *Vincent Ruffieux*. Presently, two doctorands are working in this field, *Urs Meier* and *Manfred Kurt*. The good and efficient infrastructure supplied by the University and the Canton of Fribourg created an environment, which was favourable for our work. The project was supported by the *Swiss National Science Foundation*. Finally, I like to thank my colleagues for their stimulating friendship, which contributes a lot to the success of the scientific work.

$$x_{hb} = \frac{c_a \bullet V_a - c_b \bullet V_b + \left(10^{pH-pKw} - 10^{-pH}\right) \bullet V_{tot}}{\frac{m}{MGW}} \qquad (6)$$

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[1] A. v. Zelewsky, L. Barbosa, C.W. Schläpfer, *Coord. Chem. Rev.* **1993**, *123*, 229.
[2] C.W. Schläpfer, A. v. Zelewsky, *Comments Inorg. Chem.* **1990**, *9*, 181.
[3] J.B. Ballif, Thesis No. 1005, University of Fribourg, 1991.
[4] C. Lerf, Thesis No. 1054, University of Fribourg, 1993.
[5] J. Buffle, 'Complexation Reactions in Aquatic Systems, an Analytical Approach', Ellis Horwood, Series in analytical chemistry, 1988.
[6] MATHLAB™, High Performance Computational Software, Mathworks Inc.
[7] J. Aragó, A. Bencini, A. Bianchi, E. García-España, M. Micheloni, P. Paoletti, J. Ramirez. P. Paoli, *Inorg. Chem.* **1991**, *30*, 1843.
[8] D. Horn, in 'Polymeric Amines and Ammonium Salts', Ed. E.D. Goethals, Pergamon, Oxford, 1980, p. 333.
[9] R.M. Smith, A.E. Martell, 'Critical Stability Constants', Plenum Press, 1975.
[10] D.A. Tomalia, D.P. Sheetz, *J. Polym. Sci., Part A-1* **1966**, *4*, 2253.
[11] R. Tanaka, I. Ueoka, Y. Takaki, K. Kazuya, S. Saito, *Macromolecules* **1983**, *16*, 849.
[12] I.W. Stapelton, *Aust. J. Chem.* **1985**, *38*, 633.
[13] A. Grace, 'Optimization Toolbox for use with MATHLAB', Mathworks Inc., 1992.