The Proton Affinity Spectrum of Polyethylenimine

Jean Bernard Ballif, Claude Lerf, and Carl Willhelm Schläfper*

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 can be used on PCs 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 polyethyleneimines 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 potentiometrically or spectroscopically 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 on 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.

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 number of coordination sites per gram of polymer studied.

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.

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.

\[ K_i = n - i^{-1} \cdot k \]  

\[ K_i: i\text{-th stepwise association constant of a class of } n \text{ sites with identical affinity} \]

$K$: intrinsic association constant of the class

\[ \beta_i: \text{cumulative association constant} \]

\[ K_0: \text{stepwise association constant} \]

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

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\[ \Delta H^\circ = -R \cdot \ln \beta_i \]  

\[ \Delta S^\circ = R \cdot \delta \ln \beta_i \]

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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 \( p_i > 0 \) 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 \( \log k_i \) corresponds to a resolution of \( 1.1 \) kJ/mol in \( \Delta G^0 \) 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 9.1-13.

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 (\( \log k \)) 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 nls (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-octazaahexacosane) [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 distribution 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 \( \log k \) 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 \( \log k \) values in the literature. An inspection of the five \( \log k \) 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 \( \log k = 6.68 \). The 7th and 8th class are found at \( \log k = 4.5 \) and \( \log k = 3.4 \) 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 \( \log k_7 \) and \( \log k_{10} \) at 2.71 and 1.46. Only one class of sites is found between \( \log k = 2.2 \) 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 polynfamide. Therefore, only a minor part of the H+ in solution are bound. Consequently, the \( \Delta G^0 \) 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 \times 10^{-2}.

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

A solution of the linear hexamine 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 hexamine 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 from 11 to 8.
Table 1. Affinity Spectrum Calculated from a Simulated Titration Curve of N10

| log $k_i$ | $p_i$ | $\log k$ | $\log K$ |
|-----------|-------|-----------|-----------|
| 9.8       | 0.13  | 10.27     |           |
| 9.6       | 0.03  | 9.72      |           |
| 9.4       | 0.03  | 9.27      |           |
| 9.0       | 0.02  | 8.72      |           |
| 8.8       | 0.19  | 8.24      |           |
| 8.0       | 0.01  | 8.07      |           |
| 7.8       | 0.14  | 7.65      |           |
| 5.4       | 0.01  | 5.10      |           |
| 5.2       | 0.10  | 5.68      |           |
| 4.4       | 0.02  | 4.54      |           |
| 4.2       | 0.02  | 4.66      |           |
| 3.4       | 0.01  | 3.50      |           |
| 3.2       | 0.01  | 3.80      |           |
| 2.2       | 0.06  | 2.71      |           |
| 2.0       | 0.15  | 1.46      |           |

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

| log $k_i$ | $p_i$ | $\log k$ | $\log K$ |
|-----------|-------|-----------|-----------|
| 9.6       | 0.22  | 9.91      | 10.28     |
| 9.4       | 0.27  | 9.64      | 9.52      |
| 8.2       | 0.01  | 6.94      | 6.54      |
| 8.0       | 0.02  | 6.71      | 6.67      |
| 7.2       | 0.11  | 7.65      |           |
| 6.8       | 0.01  | 7.40      |           |
| 6.6       | 0.03  | 7.21      |           |
| 5.2       | 0.04  | 6.40      | 3.80      |
| 5.0       | 0.08  | 0.80      |           |
| 3.6       | 0.18  | 0.97      |           |
| 3.0       | 0.03  | 1.00      | 2.52      |

*Result of least square fit.

Table 3. Affinity Spectrum of BPEI 600 and Polymin P

| BPEI 600 0.01m N, 0.02m H⁺ | Polymin P, 0.01m N, 0.02m H⁺ |
|---------------------------|-------------------------------|
| pH 2.24–10.85             | pH 2.42–11.22                |
| log $k_i$ | $p_i$ | log $k_i$ | $p_i$ | log $k_i$ | $p_i$ |
| 9.8       | 0.18  | 9.6       | 0.06  | 9.4       | 0.06  |
| 9.6       | 0.25  | 9.4       | 0.32  | 9.3       | 0.38  |
| 7.6       | 0.06  | 7.8       | 0.05  | 7.6       | 0.43  |
| 6.6       | 0.04  | 6.6       | 0.08  | 6.4       | 0.55  |
| 5.4       | 0.02  | 5.0       | 0.05  | 4.8       | 0.64  |
| 4.4       | 0.07  | 4.4       | 0.76  |           |       |
| 4.2       | 0.01  | 4.2       | 0.78  |           |       |
| 3.0       | 0.06  | 3.0       | 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 $K_i$ 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 · 10⁻² for the least square fit and 0.8 · 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 600000–1000000 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 $N,N',N'$-tetraakis(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 isomer 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 $N_{10}$ and $N_{6}$ discussed above show that in

![Fig. 1. Titrations curve of 25-nl solution, Polymin P N $10^{-2}$ m, $2 \times 10^{-2}$ m HCl, 0.5x NaCl with $10^{-4}$ m NaOH](image)

![Fig. 2. Protioisotherm 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.](image)

Table 4. Affinity Spectrum of LPEI at Different Concentrations

| Concentration | pH | $\log k$ | $\rho_1$ | $\sum \rho_1$ | $\log k$ | $\rho_1$ | $\sum \rho_1$ |
|---------------|----|----------|----------|--------------|----------|----------|--------------|
| LPEI 0.01 m N, 0.02 m $H^+$ | 2.24–6.70 | | | | | | | |
| LPEI 0.1 m N, 0.2 m $H^+$ | 2.07–6.87 | | | | | | | |

\[ a) \sum_{14}^n \rho_i \text{ at the precipitation of LPEI.} \]

\[ b) \sum_{14}^n \rho_i \text{ at the precipitation of LPEI.} \]
The glass electrode was calibrated in concentration of ethyloxazoline and acid hydrolyses electrolyte. DL 21 was neutralized with NaOH. The pH was measured with a pH-meter responding mass of polymer and HC\textsubscript{2} in NaCl. According to \[12\].

In a typical titration experiment, a soln., with a ratio Na\textsubscript{OH} of 1:2, prepared from the corresponding mass of polymer and HCl in NaCl 0.5\textdegree, was neutralized with NaOH. The pH was measured with a pH-meter Methrom 632 or a Mettler DL 27 titrator controlled by a Olivetti M 290 S. The glass electrode was calibrated in concentration of H\textsuperscript{+} by titration of a mixture of HCl 0.1\textdegree and AcOH of known concentration in the same inert electrolyte.

\[ x_{hb} = \frac{c_a \cdot V_a - c_b \cdot V_b + (10^{pH} - pK_w - 10^{pH}) \cdot V_{tot}}{m \cdot MGW} \]  

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[1] A. v. Zelewsky, L. Barbosa, C.W. Schläfner, Coord. Chem. Rev. 1993, 133, 229.
[2] C.W. Schläfner, 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] MATLAB\textsuperscript{TM}, 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. Uesaka, Y. Takaki, K. Kazuya, S. Saito, Macromolecules 1983, 16, 849.
[12] I.W. Stapleton, Aust. J. Chem. 1985, 38, 633.
[13] A. Grace, 'Optimization Toolbox for use with MATLAB\textsuperscript{TM}, Mathworks Inc., 1992.