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Dissociation of polyvalent electrolytes

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Abstract

Understanding of the dissociation mechanism for polyvalent electrolytes remains a matter of critical importance. Different theoretical approaches could result in different values for the microscopic parameters of the system under analysis, as well as for its integral characteristics (such as conductivity and buffering power). A unified classification of dissociation schemes is proposed here. Some examples of hybrid type schemes are considered and the expressions relating the macroscopic and microscopic constants are given. The possibility of applying the equation of Linderstrøm–Lang to multi-dissociating systems with non-constant total concentration is considered. The problem of proton binding curves (so-called titration curves) modeling is discussed. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Any chemically reacting system may be described with a set of all possible reactions taking place between all of the particles, the knowledge of the kinetic constants for each reaction (direct and inverse, as well) allowing us, in principle, to see the evolution of such a system. The information about equilibrium constants only gives an opportunity of obtaining the values of each microconcentration at steady state and no more, that is we have no information about the life time of microstates. On the other hand, for the purpose of describing some properties of such systems, we do not really need even the precise scheme of the general reaction. For example, for any polyvalent substance, the complete information about proton binding reactions taking

place in solution and their constants (equilibrium) give us an opportunity of obtaining a comprehensive description of the entire system (average microstate concentrations) but, for obtaining the pH–electric charge relationship (and the buffering capacity as well) there is no necessity of knowing the complete set of microconstant values. Also, when the other task of interpreting the experimental protein binding isotherm or “titration curve” is considered, only limited information about the so-called intrinsic dissociation constants may be derived from the appropriate data. This fact was pointed out as early as in 1925 by Adair [1], who analyzed the system with a set of parallel reactions and concluded that only “apparent” or “macroscopic” constants could be experimentally measured (the term of Adair constant is also used). A general treatment of acid–base equilibria can be found in the book of Cohn and Edsall [2].

Except for the case of very simple compounds, there is no possibility of obtaining a precise in-

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formation about the intrinsic dissociation constants, so for titration curves modeling, which is a matter of great importance in some separation technologies (electrophoresis, ion-exchange chromatography), usually we need to adopt a simplified approach. This may be either an assumption of independent dissociation or some other way of postulating all the necessary microscopic constant values, but the result will depend on the reaction scheme which is selected for description. We analyzed recently the properties of parallel and sequential dissociation schemes [3,4] and we propose now a unified classification. In the present report some general properties of possible dissociation schemes and some problems of interpretation of experimental titration curves will be analyzed. We will also discuss which scheme should be used in order to have a correct description of the entire system and of some of its particular properties as well.

2. Theory

2.1. Acid–base equilibria, general considerations

A system containing a set of groups (N), capable of parallel dissociation of protons, results in a rather high number of microscopic states, the latter growing rapidly with the number of dissociating groups. For such a system, a number of possible reactions, in turn, increases more rapidly. Consider the most simple case of only two ($N=2$) ionogenic groups (Fig. 1A): here we have four possible microstates and the same number of chemical reactions. Here we are not specifying the nature of dissociating group (acid or base); any reaction corresponds to a proton loss. The symbols ‘P’ and ‘U’ are used to designate the fully protonated and fully unprotonated states, respectively; the other intermediate states are numbered with small letters and the same horizontal level implies the same charge value. If we analyze a process with a pure type of parallel dissociation, for $N=3$ the number of microstates becomes equal to eight with twelve different reactions among them (see Fig. 1B). Obviously, for calculating all the relative microconcentrations, we do not need all of the microconstant values, e.g., for the system with

only two ionogenic groups (Fig.1A) there is one additional relation connecting microconstant values:

$$k_{Pa}k_{aU} = k_{Pb}k_{bU} \quad (1)$$

and thus any three microconstants provide all the information about the relative concentration of each microstate.

For the system in Fig. 1B one can easily obtain five independent relations, connecting the dissociation constant values, for example we may take them in the following form:

$$k_{ad}k_{dU} = k_{af}k_{fU} \quad (2)$$

$$k_{Pa}k_{ad}k_{dU} = k_{Pb}k_{bd}k_{dU} \quad (3)$$

$$k_{Pa}k_{ad}k_{dU} = k_{Pb}k_{bc}k_{cU} \quad (4)$$

$$k_{Pa}k_{ad}k_{dU} = k_{Pc}k_{ce}k_{eU} \quad (5)$$

$$k_{Pa}k_{ad}k_{dU} = k_{Pc}k_{cf}k_{fU} \quad (6)$$

and, starting from only seven ($7=12-5$) independent equations¹ of dissociation we arrive at a complete description of our system. As we see, in order to know the concentrations of each microstate, we need only seven microconstants. Note that the general form of a titration curve does not depend on whether acidic or basic groups are involved (provided the set of microconstants remains the same), their balance influences only the isoelectric point value. Further, if we want to obtain the proton binding isotherm only, we do not need the precise distribution between all the possible microstates in our system, it is sufficient to know only the ‘total’ concentrations of microstates with the same charge values. The pH–charge relationship $Q((H^+))$ for the system shown on Fig. 1A may be described with

$$Q((H^+)) = \frac{1 + K_1/(H^+)}{1 + K_1/(H^+) + K_1K_2/(H^+)^2} \quad (7)$$

$$K_1 = k_{Pa} + k_{Pb} \quad (8)$$

¹These equations may be selected, for example, by following the simple rule, namely:

“All of the possible reaction paths, obtained by specification of microreactions, do not form closed trajectories”.

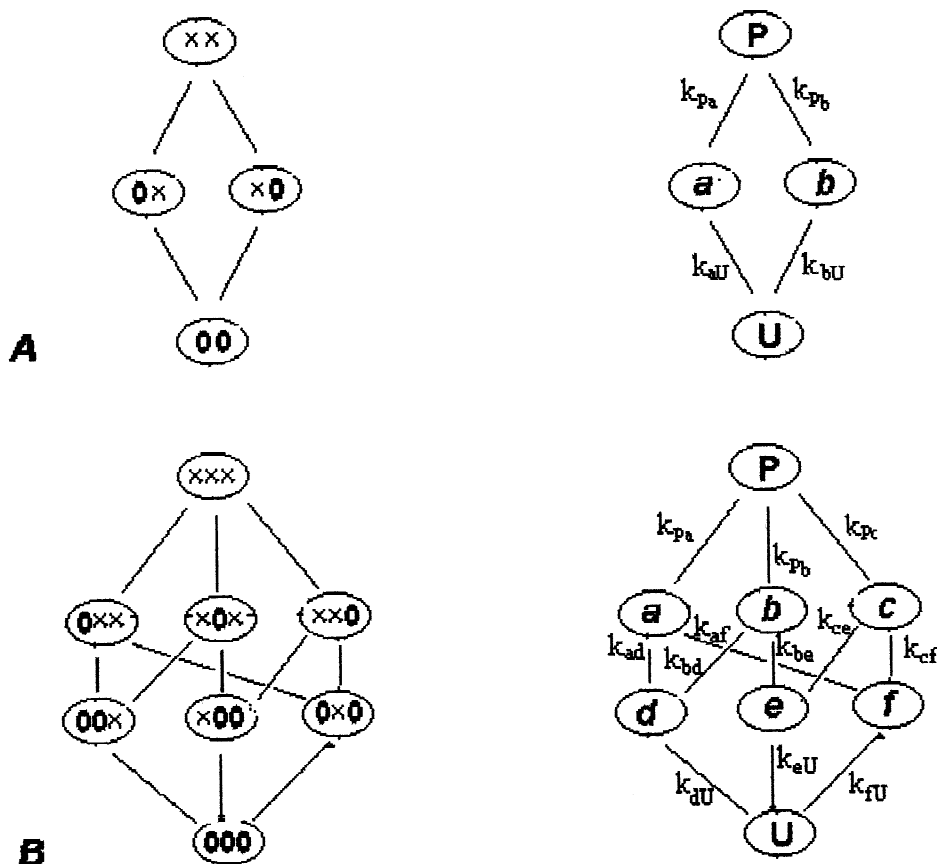


Fig. 1. Reaction schemes of parallel dissociation for the cases of two (A) and three (B) ionogenic groups. The nature of the dissociating group (acid or base) is not specified, since any reaction corresponds to a proton loss. X's and zeroes are used to designate the presence or the absence of a proton (left panel). The two extremes 'P' and 'U' correspond to the fully protonated and fully unprotonated states, respectively; the other intermediate states are numbered with small letters. The double subindexes of the microscopic constants reflect the two microstates involved in the microreaction (right panel). The appropriate relation connecting macroscopic and microscopic constants are given in the text.

$$K_2 = \frac{k_{aU}k_{bU}}{k_{Pa} + k_{Pb}} \quad (9)$$

$$K_3 = \frac{k_{Pa}k_{ad}k_{dU}}{k_{Pa}k_{ad} + k_{Pb}k_{be} + k_{Pc}k_{cf}} \quad (12)$$

the help of two macroscopic constants only².

A three dissociating group system (Fig. 1B) is characterized by three macroconstants:

$$K_1 = K_{Pa} + K_{Pb} + K_{Pc} \quad (10)$$

$$K_2 = \frac{k_{Pa}k_{ad} + k_{Pb}k_{be} + k_{Pc}k_{cf}}{k_{Pa} + k_{Pb} + k_{Pc}} \quad (11)$$

And the expression for the electric charge is given by

$$Q((H^+)) = \frac{1 + K_1/(H^+) + K_1K_2/(H^+)^2}{1 + k_1/(H^+) + K_1K_2/(H^+)^2 + K_1K_2K_3/(H^+)^3} \quad (13)$$

²In order to write the expression for the electric charge we need to specify the nature of ionogenic groups. In this and in the following examples all of them are taken as acid ones.

In the case of a pure parallel dissociation process of *N* groups we are dealing with 2^{*N*} microstates (this

result obtained by Wyman, see ref. in [5]). The number of possible microreactions (M) increases very rapidly with the number of ionizable groups:

$$M = \sum_{k=0}^{N-1} N_k = N! \sum_{k=0}^{N-1} \frac{1}{k!(N-k-1)!} \quad (14)$$

see Appendix A. These data on the numbers of possible microstates and microreactions are summarised in Table 1 (from one to eight steps of dissociation). Fortunately, in order to find all the equilibrium concentrations we do not need to know all of the microconstant values, it sufficient to have only $2^N - 1$ independent constants.

Moreover when someone is interested in proton binding curves only, a further reduction of necessary parameters is possible. For an arbitrary number N of ionogenic groups, acting parallelly, it is sufficient to operate with N macroscopic constants. Indeed, since we are interested in only the total concentration of microstates with the same charge value, in order to know the concentration distribution between the $N + 1$ possible ‘‘macro-states’’ (which are connected with the normalization equation), we need only N macroscopic constants.

2.2. Dissociation schemes of hybrid type

This conclusion can be easily generalized to an arbitrary closed process, no matter what general scheme of dissociation is taking place, but obviously, the expressions for macroconstants must differ from the case of pure parallel dissociation. As an example let us take a scheme of ‘‘hybrid type’’, which is shown on Fig. 2A. In this system two groups

dissociate consecutively and the third one may dissociate irrespective of which states the two first groups are in. There are six microstates and seven chemical reactions in such a system, with the relative concentrations of each microstate being shown in Fig. 2B. The appropriate expressions for the three macroconstants will be:

$$K_1 = k_{p_a} + k_{p_b} \quad (15)$$

$$K_2 = \frac{k_{p_b}k_{b_d} + k_{p_a}k_{a_c}}{k_{p_a} + k_{p_b}} \quad (16)$$

$$K_3 = \frac{k_{p_a}k_{a_c}k_{c_u}}{k_{p_b}k_{b_d} + k_{p_a}k_{a_c}} \quad (17)$$

A system composed of two sets (each one representing a true two-phase stepwise process) is a particular case of reaction with four ‘‘macrostages’’, see Fig. 3. This system includes nine microstates and the number of chemical reaction is twelve. The appropriate expressions for the four macroconstants are:

$$K_1 = k_{p_a} + k_{p_b} \quad (18)$$

$$K_2 = \frac{k_{p_a}k_{a_c} + k_{p_a}k_{a_d} + k_{p_b}k_{b_c}}{k_{p_a} + k_{p_b}} \quad (19)$$

$$K_3 = \frac{k_{p_a}k_{a_c}k_{c_f} + k_{p_b}k_{b_e}k_{e_g}}{k_{p_a}k_{a_c} + k_{p_a}k_{a_d} + k_{p_b}k_{b_e}} \quad (20)$$

$$K_4 = \frac{k_{p_a}k_{a_c}k_{c_f}k_{f_u}}{k_{p_a}k_{a_c}k_{c_f} + k_{p_b}k_{b_e}k_{e_g}} \quad (21)$$

Should we be interested in the concentration of each microstate for this scheme, obviously, we need to specify eight independent microconstants.

Other examples of four-microstages of the hybrid

Table 1
Number of possible microreactions and their increase with the number of ionizable groups (N)

N	Number of microstates	Number of reactions	Number of independent microconstants
1	2	1	1
2	4	4	3
3	8	12	7
4	16	32	15
5	32	80	31
6	64	182	63
7	128	348	127
8	256	744	255

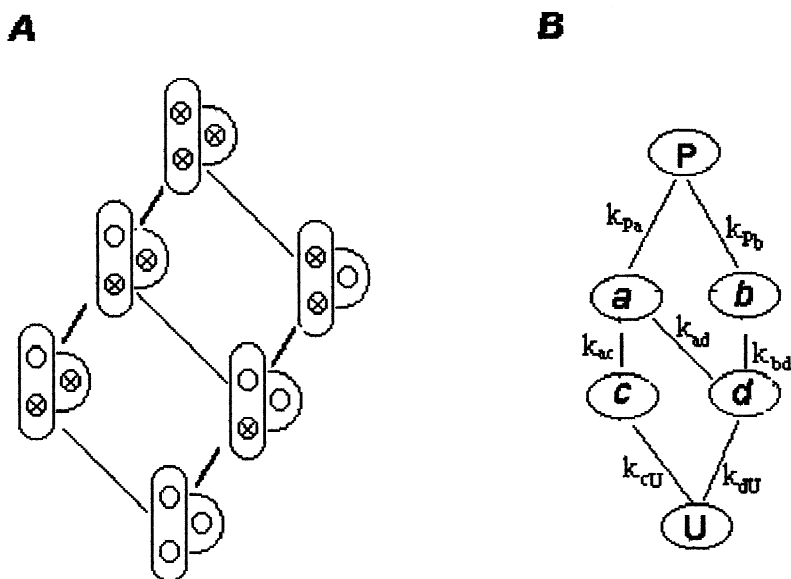


Fig. 2. Dissociation scheme of the “hybrid type”, where two groups dissociate consecutively and the third one may dissociate either in a parallel or consecutive fashion, regardless of which states the two first groups are in. Prolonged ovals symbolize a subsystem of two groups dissociating stepwise, while a semicircle corresponds to one independently dissociating group. (B) shows the appropriate designations for microstates and microconstants. The expressions for macroconstants are given in the text.

type are given in Fig. 4A, where two groups dissociate sequentially and the other two may act in their own way. Here we have twelve microstates and twenty reactions. In contrast with the pure parallel process (Fig. 4B) this scheme not only contains a different number of microstates, but it is also a non-symmetrical one.

2.3. Schemes with independent dissociation

The assumption of independent dissociation essentially simplifies the treatment; in this case the set of apparent constants uniquely defines all of the intrinsic constants. For a process of parallel dissociation with an arbitrary number M of ionogenic groups, acting independently, as shown by Simms [6], the $M-1$ macroscopic constants (K_i) are connected with the microscopic ones (k_i) as follows:

$$K_1 = k_1 + k_2 + \dots + k_M = \sum_i k_i$$

$$K_1 K_2 = k_1 k_2 + k_1 k_3 + \dots + k_2 k_3 + \dots + k_{N-1} k_N \\ = \sum_{ij} k_i k_j$$

$$K_1 K_2 K_3 = k_1 k_2 k_3 + k_1 k_2 k_4 + \dots = \sum_{ijl} k_i k_j k_l$$

$$K_1 K_2 K_3 \dots K_N = k_1 k_2 k_3 \dots k_N \quad (22)$$

For any “hybrid” scheme the appropriate relations are different from (22), see for example a transformation of the set (10–12) to (15–17). Regardless of a concrete dissociation scheme, the assumption about independent dissociation for each ionogenic group leads to a “one-to-one” correspondence between the sets of intrinsic (microscopic) and apparent (macroscopic) constants.

2.4. Linderstrøm–Lang equation and its universalization

The idea of connecting such different matters, at a first glance, as standard charge deviation of multi-dissociating systems with the form of the proton binding curve belongs to Linderstrøm–Lang (see Edsall [5], p. 462). The treatment given there is connected with the consideration of a parallel process, but may be generalized to any other, which is

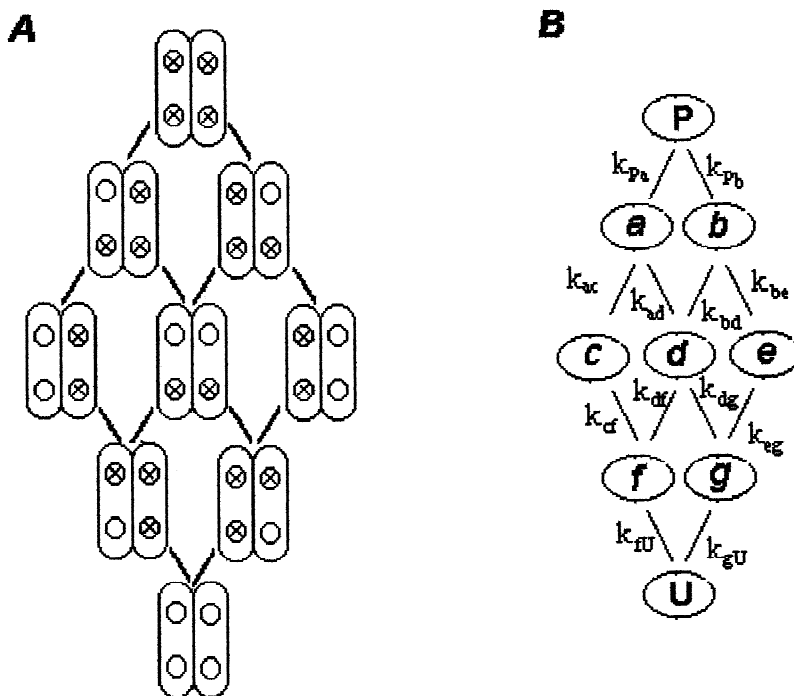


Fig. 3. Dissociation scheme of “hybrid type”, describing the case of two subsystems, in which two groups dissociate consecutively, but these subsystems, in turn, may act parallelly (two stepwise plus two stepwise). The symbols used are the same as in Figs. 1 and 2. The expressions for macroconstants are given in the text.

formally reducible to a stepwise scheme, by an appropriate selection of the macroscopic constant values. Moreover, this equation

$$\frac{\partial \bar{Q}}{\partial \text{pH}} = 2.303[\bar{Q}^2 - (\bar{Q})^2] \quad (23)$$

remains valid also for non-closed processes (non constant total concentration). Obviously, due to a lack of normalization equation, we will need an additional independent microconstant value for describing such systems.

As any formal stepwise process is defined by a limited number of parameters, it is clear that an essential part of information about such a complex system is lost. Thus, with the value of standard deviation one can say nothing about charges distribution within each “macrostate”. This leaves us an opportunity of modeling, for example, such an important characteristic as dipole moment. Let us come back to a system that is described with the scheme of Fig. 1A. As we know, such a system may

be described completely by the set of three microscopic constants. Let us suppose that we have such constants:

$$k_{Pa} = k^1; \quad k_{Pb} = k^2; \quad k_{aU} = k^3.$$

They give us the values of two macroscopic constants – K_1 and K_2 [see (7–9)], which are quite sufficient to derive a pH-charge relationship. But one can easily find that any other set of three new microconstants designed according to the following rule:

$$k_{Pa} = k^1 \cdot \theta; \quad k_{Pb} = k^2 - k^1 \cdot (\theta - 1); \quad k_{aU} = k^3 / \theta,$$

where θ is an arbitrary parameter, will result in the same macroscopic constants. Coming back to the dipole moment one can easily write the appropriate expression, provided one knows all of the independent microconstant values and the dissociating center coordinates. Usually, it is more important to know this value in the vicinity of the isoelectric point where the total net electric charge approaches zero.

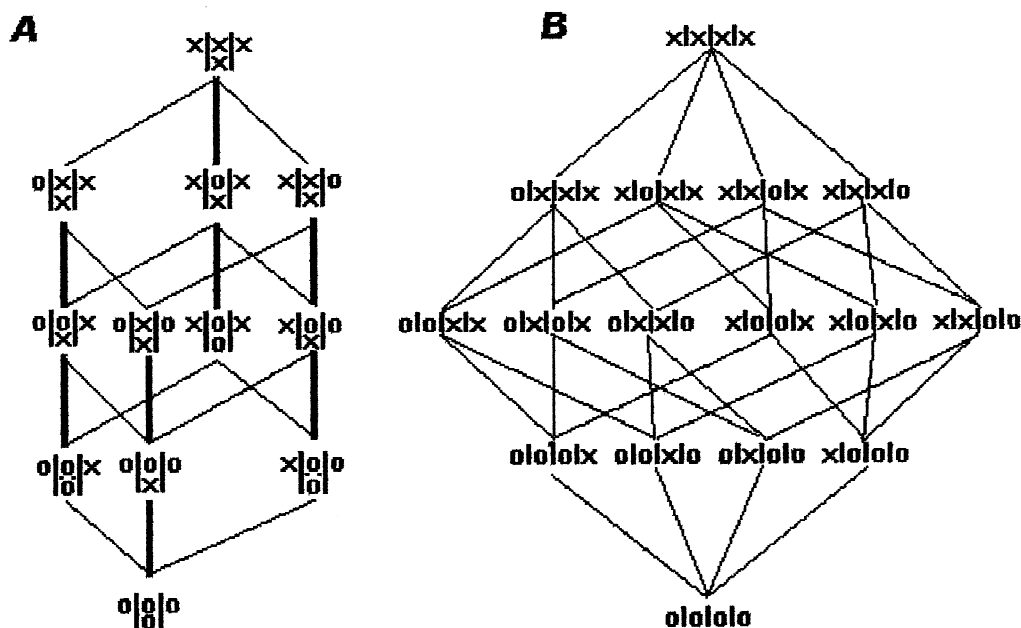


Fig. 4. Dissociation scheme of “hybrid type”, where two groups dissociate consecutively and the other two act in parallel (two stepwise plus two parallel), (A). Two symbols, one above the other, between vertical lines correspond to “stepwise” subsystems. The four-step scheme with pure parallel dissociation (all the four ionogenic groups act in parallel) is shown on (B). The expressions for macroconstants are not given.

At the isoelectric point for the case of two independently dissociating centers, the value of dipole moment (d) will be directly proportional to the relative concentration of the zwitterionic state (in our symbols b or C_b , see Fig. 1):

$$\vec{d} = C_b \vec{l} \tag{24}$$

where \vec{l} is the vector connecting the centers of positive and negative charge.

For the zwitterionic state concentration we have the following expression:

$$C_b((H^+)) = \frac{(H^+)_0 k^1}{(H^+)_0^2 + (H^+)_0(k^1 + k^2) + k^1 k^3} \tag{25}$$

where

$$(H^+)_0 = (K_1 K_2)^{1/2} \tag{26}$$

The dipole of a new system $d(\theta)$ is connected with its old value $d_0 = d(\theta = 1)$ as

$$\vec{d}(\theta) / \vec{d}_0 = 1 + (1 - \theta) k_1 / k_2 \tag{27}$$

3. Discussion

3.1. On the modeling of titration curves

Although the titration curve of any polyelectrolyte may be described in terms of a limited number of macroconstant values, the use of macroconstants is not a very suitable instrument for titration curves modeling, based on the information about chemical composition. For this we may operate only with intrinsic constants, since the macroscopic (apparent) constants are not invariable³. As it was already mentioned in the introduction, an experimental binding isotherm offers an opportunity of obtaining the macroscopic constant (by means of the best interpolation using the theoretical proton binding equation), so even in the case of simple substances we need

³For example, a single change (or an addition) of one intrinsic constant may *completely* change the initial set of apparent constants. Certainly, when the necessary intrinsic dissociation constant values are found as tabulated data or evaluated by some other way, the value of macro- or “apparent” constant are easily calculated, also.

other independent measurements in order to have microconstant values.

Complete prediction of the protein binding curve may be achieved only if we know all of the intrinsic constant values. The assumption of independent dissociation enormously simplifies the theoretical treatment, but unfortunately this approach is not always legitimate, and usually an evaluation of structural formulas is sufficient to understand if it is possible to use this approach or not. For example, for the description of polycarboxylic acids ionization the model of independent dissociation is incorrect, since ionogenic groups are located rather close to each other (note that the difference between “apparent” pK values has no relations to the possibility of using the model of independent dissociation; it only allows us to treat the true “stepwise” process as a “parallel” one and no more). The same for amino acids, where we may not neglect the interaction between α -amino and α -carboxyl groups. Just opposite is the situation in the case of polypeptide chains, where the amino acid side chains, capable of ionization, may be treated as acting independently.

When a truly parallel dissociation process is treated as a stepwise one, but the dissociation constant values are taken as intrinsic, essential errors in calculating the isoelectric point and also an unusual form of the final titration curves (some portions possessing a very high derivative) may be obtained. Only in the case of small compounds with few ionizable groups a deviation may be non-essential, and the smaller is the difference in “reduced microconstants”⁴, the more evident is the deviation. This is clearly visible in the case of several pK values with the same or rather close values, when one obtains completely different pattern. An important question still remains – how to determine the intrinsic constants for side chain groups. When using titration data for small compounds (few groups capable of ionization) in some simple cases we may approximate an equivalence between micro and

macro-constants. Should we not neglect the interaction, a refinement is possible by using tabulated data of the same ionogenic groups in other compounds (see, for example [7]), or with the help of alternative techniques.

One should also pay attention when interpreting some maxima on the titration curve slope (buffering capacity maximum). The latter does not necessarily correspond to the presence of ionogenic groups with appropriate pK values. That result is valid only for pK values which are rather widely separated in the case of pure parallel dissociation (with the assumption of an independent mechanism). It is easy to evaluate the minimal Δ pK distance, by which it is possible to detect two separated maxima on the buffering capacity curve, see Appendix B.

Often we do not specify any difference between the true “titration curve”, i.e. the quantity of strong titrant versus pH and the “dissociation curve” (Q ver. pH). That is correct only if we operate “not very far from neutrality” [8] (the question of bulk water contribution for the system containing one amphoteric substance is treated in detail in [9]).

4. Conclusions

With respect to pH-charge relationship, the latter is completely described with the help of macroconstants (we need N constants, N being the number of dissociating groups). This statement is generalized to a system with an arbitrary dissociation scheme (hybrid type). The set of macroconstants thus gives an opportunity to write the number of relations connecting microconstants (correct choice of dissociation scheme determines the concrete look). In the case of independent dissociation, the set of macroconstants completely defines all of the microconstants. While for any individual substance, its macroconstant data set is no doubt a very useful information, these data may not be applicable directly to another one with similar composition. For molecules with many dissociating groups, one simple way of predicting its pH-dependent properties is to accept the assumption of an independent dissociation model and to operate in terms of intrinsic (micro-) constants. The appropriate dissociation model should be used, and the structural formulae will help to

⁴For an arbitrary system with known apparent constants, the proton binding curve may be obtained with the help of a set of “reduced” microconstants, formally calculated according to (22). In case one cannot neglect an interaction between neighboring ionogenic groups, the latter constants will have no direct relation to intrinsic constants of “monomeric” units.

write the scheme of the reaction. Another important question is “how to use the tabulated data of dissociation constant values”, and one should pay attention to its correct interpretation.

5. Symbols

$K_1, K_2 \dots K_N$	macroscopic constants, the subindexes denoting the appropriate “step” of dissociation process.
P	«initial» microscopic state (fully protonated).
U	«final» microscopic state (unprotonated).
<i>a, b, c</i>	are used to designate the intermediate microstates for any dissociation scheme (in each case the correspondence is given with the appropriate figure), the same letters in italic are used for concentration.
k_{Pa}, k_{Pb}, k_{Pc}	microscopic constants, the double subindexes reflecting the two microstates involved in microreaction, e.g. $k_{Pa} = a(H^+)/P$.
$k_1, k_2, k_3 \dots k_N$	microscopic (intrinsic) constants for a true parallel process with independent dissociation.

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Appendix A. The total number of reactions in a multi-dissociating system with parallel reactions

For any true parallel dissociation process with N groups capable of dissociating a proton consider the general scheme as a “ N -stage” process. Each “stage” includes all of microreactions of one proton loss, so if we denote by k the number of deprotonated groups the total number of microstates (n_k) for given k is obtained by the binomial coefficient

for given k is obtained by the binomial coefficient

$$n_k = C_k^N = N!/(k!(N-k)!)$$

Any of these microstates can loose a proton in a number of ways which can be expressed by another binomial coefficient:

$$n_{k-1}^k = C_1^{N-k} = (N-k)!/(1!(N-k-1)!)$$

Thus each stage (k) includes N_k reactions, where:

$$N_k = n_k \cdot n_{k-1}^k = C_k^N \cdot C_1^{N-k}$$

And the number of all possible reactions (M) is given by the sum (14).

Appendix B. Condition for the existence of two maxima in the buffer power curve of a bi-valent electrolyte

An assumption of independent dissociation leads to relations:

$$k_{Pa} = k_{bU}; \quad k_{Pb} = k_{aU}$$

and the buffering power is described as a function of (H^+), by the equation:

$$\beta = 2.303(H^+) \left\{ \frac{Ck_1}{[(H^+) + k_1]^2} + \frac{Ck_2}{[(H^+) + k_2]^2} \right\}$$

where, for simplicity, we used $k_1 = k_{Pa}$ and $k_2 = k_{Pb}$.

In order to find the conditions for the existence of two maxima, we need to take a second derivative:

$$\frac{d}{d(\text{pH})} \beta = 2.303^2(H^+)C \left\{ \frac{d}{d(H^+)} \left\{ \frac{Ck_1}{[(H^+) + k_1]^2} + \frac{Ck_2}{[(H^+) + k_2]^2} \right\} \right\}$$

and, by supposing its value equal to zero, we may easily derive the appropriate limitation on the pK difference. Bringing to a common denominator we obtain an equation of the fourth degree relative to

(H^+), which easily reduces to a quadratic equation.
By analyzing its discriminant we come to:

$$k_1/k_2 = (7 + 4\sqrt{3}) \approx 13.93$$

The latter gives

$$\Delta pK \approx 1.144$$

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