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NOTE

On the Influence of pH and Salt Composition on the Partition of Polyelectrolytes in Aqueous Polymer Two-Phase Systems

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Abstract

The drawbacks of the usual explanation of the influence of pH and salt composition on partition of polyelectrolytes in aqueous polymer two-phase systems are outlined. An explanation is presented that avoids the introduction of thermodynamic data on single ions and of single potential differences. It leads to an equation that describes the relation between the ratio of the partition coefficients of a polyelectrolyte in the presence of either of two salts on one hand, and its charge and the partition coefficients of the involved salts on the other. Examples of the application of this equation to literature data are given.

Partition in aqueous polymer two-phase systems is an extremely versatile separation method for macromolecules and cellular particles that has been developed by Albertsson (*1*). It is well known that the partition of polyelectrolytes (e.g., proteins) between the two phases is strongly dependent on the pH and the type of salt that is present in the system. The usual explanation (*2–4*) is as follows: different ions have different affinities for the two phases. Thus, when a salt is added to the system, an electrical potential difference between the two phases is created that exactly compensates the different affinities of the cation and the anion of the salt. The value of the potential difference depends on the type of salt (not on its concentration). If a small amount of a polyelectrolyte is partitioned in an aqueous polymer two-phase

system containing a salt, the value of its partition coefficient depends on its charge and on the potential difference between the two phases, i.e., on the pH and on the type of salt, present in the system. This argument leads to the equation (*4*)

$$\ln K_P = \ln K_P^0 + \frac{zF}{RT}\Psi \qquad (1)$$

where K_P is the partition coefficient of the polyelectrolyte, and K_P^0 is the value of this coefficient when the potential difference Ψ between the phases (generated by the excess of salt) is zero.

This reasoning is intuitively appealing, but it suffers from a number of drawbacks.

(a) It leads to an equation with a thermodynamic constant *of a single ion* K_P^0 and a *single potential difference* Ψ. Data of this kind cannot be determined, on principle, and have, strictly spoken, no physical meaning (*5*). The difficulties involved in obtaining even rough estimates are well documented (*6–9*).

(b) Of course, consistent results can be obtained from Eq. (1) by assigning arbitrarily some value to *one* of the above-mentioned data, e.g., by assuming Ψ to be zero in the presence of NaCl. However, Eq. (1) has been used to estimate Ψ from a plot of $RT/F \ln K_P$ vs z, assuming that $\ln K_P^0$ does not depend on z. This involves not one but a whole series of arbitrary assumptions:

$$\ln K_{P,z^+}^0 = \ln K_{P,(z-1)^+}^0 = \ldots = \ln K_{P,(z-n)^+}^0 \qquad (2)$$

which may lead to inconsistent results. Moreover, if one starts from the assumption that different ions have different affinities for the two phases of the system, it is illogical to assume subsequently that this does not hold for polyelectrolyte ions. In short, a plot of $RT/F \ln K_P$ vs z needs not to be linear [as has been observed (*3*)], and even if it is linear, one cannot estimate Ψ from it.

(c) The argument does not lead to a quantitative relationship between the $\log K_S$ values of different salts on one hand and the $\log K_P$ values of a polyelectrolyte in the presence of either of these salts on the other hand. In the field of partition of biopolymers, no one seems to be aware of the existence of this relationship.

An argument that avoids these drawbacks is given below. It is based on the fact that only electroneutral combinations of ions can be transferred from one phase to another, and that consequently the partition coefficient of a polyelectrolyte ion depends on the properties of the counterion. It must be stressed that there is no fundamental difference between the partition of

inorganic (or organic drug) ions in, e.g., a water–chloroform system and the partition of charged biopolymers in an aqueous polymer two-phase system.

Consider the partition equilibrium of a polyelectrolyte with charge z, P^z, in the presence of a salt MX. In the equation an electroneutral combination of ions must occur:

$$P_1^z + zX_1^- \rightleftharpoons P_2^z + zX_2^- \tag{3}$$

The charge z may be either positive or negative, and may be caused by ionization of acidic or basic groups, or by the adsorption of ions other than M^+ and X^- (e.g., buffer ions). The two phases are denoted by *1* and *2*. The equilibrium condition is

$$RT \ln \frac{[P^z]^1}{[P^z]^2} + zRT \ln \frac{[X^-]^1}{[X^-]^2} = \mu_2^{0,P} - \mu_1^{0,P} + z(\mu_2^{0,X} - \mu_1^{0,X}) \tag{4}$$

where the brackets denote concentrations and μ^0 denotes the standard chemical potential. Equation (4) can be simplified to

$$RT \ln K_P + zRT \ln K_{MX} = -\Delta\mu^{0,P} - z\Delta\mu^{0,X} \tag{5}$$

For partition of the polyelectrolyte P^z in the presence of the salts MX and NY, respectively, it holds that

$$RT \ln \frac{K_P(MX)}{K_P(NY)} + zRT \ln \frac{K_{MX}}{K_{NY}} = -z(\Delta\mu^{0,X} - \Delta\mu^{0,Y}) \tag{6}$$

For partition of the salts NX and NY it holds that

$$2RT \ln K_{NX} = -\Delta\mu^{0,N} - \Delta\mu^{0,X} \tag{7}$$

$$2RT \ln K_{NY} = -\Delta\mu^{0,N} - \Delta\mu^{0,Y} \tag{8}$$

from which it follows that

$$2RT \ln \frac{K_{NX}}{K_{NY}} = -\Delta\mu^{0,X} + \Delta\mu^{0,Y} \tag{9}$$

Combination of Eqs. (6) and (9) yields

$$RT \ln \frac{K_P(MX)}{K_P(NY)} + zRT \ln \frac{K_{MX}K_{NY}}{(K_{NX})^2} = 0 \tag{10}$$

Equation (10) may be compared with Eq. (1). It appears that a linear relationship can be expected between $\log K_P(MX)/K_P(NY)$ and z, but not between $\log K_P(MX)$ and z. This is illustrated in Fig. 1.

Figure 1(A) demonstrates that $\log K_P$ and the net proton charge z_H are not linearly related in either of the salts investigated. On the contrary, a linear

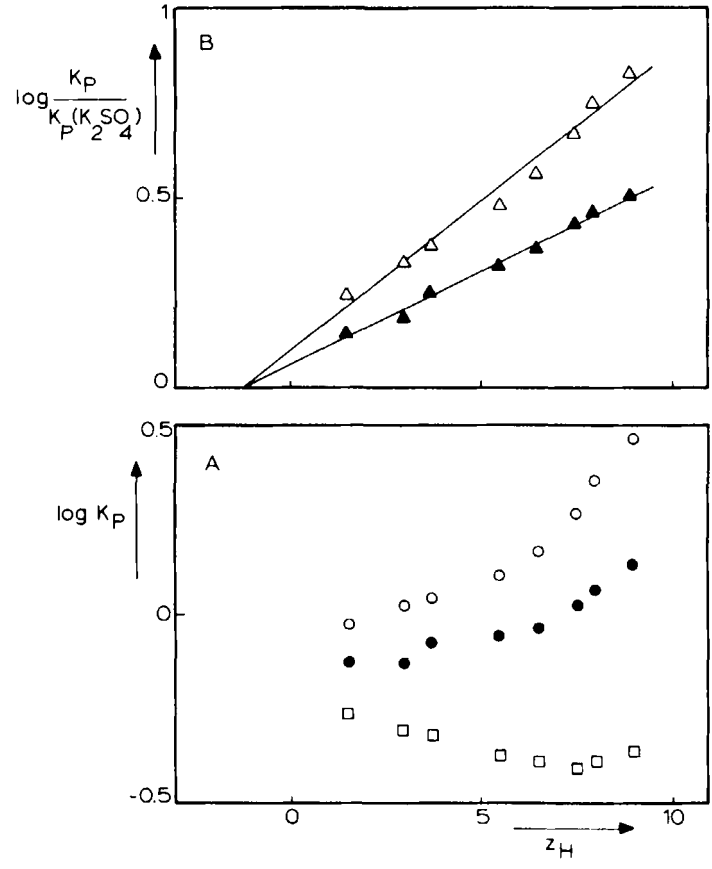

FIG. 1. (A) The variation of log K_P of ribonuclease with its net proton charge z_H when partitioned in a 9.80% dextran–7% polyethylene glycol system containing 0.1 *M* KSCN (○) 0.1 *M* KCl (●), or 0.05 *M* K_2SO_4 (□). The data are from Ref. *3*, Fig. 2d. (B) The variation of log K_P/K_P (K_2SO_4) with z_H, in 0.1 *M* KSCN (△), or in 0.1 *M* KCl (▲).

relationship does exist between log $K_P/K_P(K_2SO_4)$ and z_H (Fig. 1B), but it is puzzling that the graphs do not intersect the axis at $z_H = 0$, but at $z_H = -1.2$.

Johansson (*10*) has published data that enable us to check the accuracy of Eq. (10), *viz.*, data on the partition coefficients of the alkali halides and on the partition coefficients of ovalbumin in the presence of these salts at two pH values, for partition in a two-phase polymer system. From these data and Eq. (10), values of z can be calculated and compared with the values of the net

TABLE I

Data on the Partition of Alkali Halides, and on the Partition of Ovalbumin in the Presence of These Halides at Two pH Values, between the Two Phases of a 7% Dextran-7% Polyethylene Glycol System (*10*). The Concentration of the Alkali Halides is 0.1 *M*, the pH is Maintained by a 0.01 *M* Sodium Acetate Buffer

		At pH = 3.70			At pH = 5.59		
Salt	log K_S	log K_P/K_P(KBr)	z	z_H	log K_P/K_P(KBr)	z	z_H
LiCl	0.0212	−0.17	4	20	0.20	−5	−6
LiBr	0.0294	−0.12	4		0.18	−6	
LiI	0.0453	−0.11	10		0.06	−5	
NaCl	−0.0044	−0.07	5		0.05	−4	
NaBr	0.0043	−0.01	2		0.02	−5	
NaI	0.0212	0.06	5		−0.04	−3	
KCl	−0.0088	−0.06	7		0.03	−3	
KBr	0.0000						
KI	0.0170	0.10	6		−0.07	−4	

proton charge (*11*) z_H at the same pH values. Table 1 gives the results of the calculations.

It appears from Table 1 that at pH 5.59 [i.e., above the isoionic point (*11*) pI = 4.88] the values of z are in good accord with the value of z_H. However, at pH 3.70, z is much smaller than z_H, and we have no good explanation for this difference. One might suggest three causes for the discrepancy: neglect of activity coefficients in Eq. (10), adsorption of acetate buffer anions to the positively charged ovalbumin, or a dependence (*11*) of z_H on the electrolyte composition of the solution at low pH (in which case Eq. 10 cannot be applied to partition coefficients of a polyelectrolyte in the presence of two different salts at the same pH), but neither suggestion offers a satisfactory explanation. It is not clear why the neglect of activity coefficients should have a much larger effect at pH 3.70 than at pH 5.59. We could not detect any adsorption of acetate ions by the method of Hummel and Dreyer (*12*). While it is conceivable that z_H of a protein below its isoionic point depends on the anions present in the solution (*11, 13*), it is difficult to envisage that z_H depends on which alkali metal cation is present. So it is not clear why Eq. (10), when applied to the partition of ovalbumin in LiBr, NaBr, or KBr, should yield values of z that differ from those of z_H.

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