Journal of Chromatography, 367 (1986) 160–162
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 875

Note

On the stoichiometric model of electrostatic interaction chromatography for biopolymers

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(Received June 20th, 1986)

Recent advances in high-performance liquid chromatography (HPLC) have demonstrated the potential of the technique in the separation of biopolymers. The pioneering work of Regnier and co-workers[1,2] has shown that the use of porous siliceous bonded stationary phases with a hydrophilic coating and fixed ionogenic functions is a particularly promising approach. Such stationary phases can be used to retain selectively charged biopolymers by electrostatic interactions; by analogy with the other interactive chromatographic methods, such as hydrophobic interaction chromatography, that are commonly used for biopolymer separation, we will use the term electrostatic interaction chromatography rather than ion-exchange chromatography to describe this separation technique.

It is well known that the logarithmic retention factor of an eluite in electrostatic interaction chromatography decreases linearly with the logarithm of the eluent salt concentration. This observation motivated Boardman and Partridge[3,4] in 1955 to formulate a stoichiometric model for the retention of proteins on ion-exchange columns. Recently, Regnier and co-workers[5,6] expanded the model and verified it experimentally for monovalent salts. However, there is considerable ambiguity in the literature about the corresponding result for multivalent salts, and the purpose of this note is to present a rigorous derivation of this result.

The binding process in the absence of specific salt binding effects is expressed by the equation

$$\mathrm{PC}_{N_c} + N_s\bar{\mathrm{S}} \rightleftharpoons \bar{\mathrm{P}} + N_s\mathrm{S} + N_c\mathrm{C} \qquad (1)$$

where P is the protein in the mobile phase, C is the co-ion to the protein which is assumed to be monovalent, and S is the salt counterion. Overbars indicate that the species are bound to the stationary phase.

The characteristic charge on the protein, Z_p, under the prevailing conditions such as pH is taken to be the number of bound monovalent counterions that would be freed when one molecule of the protein is bound. If the valency of the salt counterion is Z_s, stoichiometric exchange requires that the number of its counterions, N_s, expelled upon binding one molecule of protein equal Z_p/Z_s that need not be an integer. Similarly, the number of co-ions, N_c, associated with one molecule of the protein in solution equals Z_p.

The appropriate binding constant K_b is given by

0021-9673/86/$03.50

$$K_b = \frac{[\bar{P}]\,[S]^{N_s}\,[C]^{N_c}}{[PC_{N_c}]\,[\bar{S}]^{N_s}} \tag{2}$$

where square brackets represent concentrations.

If A represents the total number of active sites, the following mass balance is valid at all times:

$$Z_p\,[\bar{P}] + Z_s\,[\bar{S}] = [A] \tag{3}$$

which can be written as

$$Z_p \frac{[\bar{P}]}{[A]} + Z_s \frac{[\bar{S}]}{[A]} = 1 \tag{4}$$

Limiting ourselves to the linear part of the protein binding isotherm is equivalent to requiring that the first term on the left hand side of eqn. 4 be negligible in relation to the right hand side. Thus,

$$Z_s \frac{[\bar{S}]}{[A]} \approx 1 \tag{5}$$

Defining the distribution constant K by

$$K = \frac{[\bar{P}]}{[PC_{N_c}]} \tag{6}$$

and substituting eqns. 5 and 6 into eqn. 2 yields

$$K = \left(\frac{[A]}{Z_s}\right)^{N_s} \frac{K_b}{[S]^{N_s}\,[C]^{N_c}} \tag{7}$$

Now, if the binding process is a stoichiometric exchange of one counterion for another, the co-ion only serves to maintain electroneutrality, and its concentration in the mobile phase remains effectively constant. We can therefore rewrite eqn. 7 as

$$K = \frac{Q}{[S]^{N_s}} \tag{8}$$

where Q is a constant given by

$$Q = \left(\frac{[A]}{Z_s}\right)^{N_s} \frac{K_b}{[C]^{N_c}} \tag{9}$$

The chromatographic retention factor k' is given by

$$k' = \varphi K \tag{10}$$

where φ is the phase ratio. From eqns. 8 and 10, we obtain the dependence of the logarithmic retention factor on the salt concentration as

$$\log k' = \log (\varphi Q) - \frac{Z_p}{Z_s} \log [S] \tag{11}$$

A plot of log k' against log [S] should therefore be a straight line with slope $-Z_p/Z_s$ (which is equal to $-N_s$), and multiplying the experimentally obtained slope by the valency of the salt counterion will yield the protein's characteristic charge. For algebraic convenience eqn. 1 was written for monovalent co-ions; however, the result presented in eqn. 11 is unaffected by this constraint, and can be shown to be rigorously valid for co-ions of arbitrary valence.

The result stated in eqn. 11 has experimental support, particularly in the ion-exchange of small species; it has also arisen in the context of certain non-stoichiometric theories. Manning[7] in his theory of counterion condensation has derived a similar result for the case where one counterion species' concentration is vastly in excess of the other's, which is precisely the condition for linear chromatography. Counterion condensation theory has been employed recently in our laboratory[8] to analyze the effect of salt on the HPLC of proteins under the conditions of electrostatic interaction chromatography.

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