AN APPROACH TO THE ION EXCHANGE CHROMATOGRAPHY OF POLYELECTROLYTES

I. A MODEL BASED ON THE LAW OF MASS ACTION

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(Received August 31st, 1964)

INTRODUCTION

It is recognized that at the present state of development of the theory of polyelectrolyte solutions no entirely satisfactory theory for the ion exchange of polyelectrolytes can be worked out. However, it would be convenient to have a relatively simple theoretical tool, if it would help to explain most, even if not all, of the peculiarities of the ion exchange chromatography of homologous polyelectrolytes. Such an approach is presented below. It accounts reasonably well for the experimental results which have been obtained with polylysines on carboxymethylcellulose[1], though it only takes into consideration ion exchange as interaction of electrostatic forces, while purposefully ignoring for the moment several other factors which undoubtedly do play a role in the chromatography of polyelectrolytes on ion exchangers.

However, we feel justified in publishing this approach, because its rather close agreement with the experimental data[1] indicates that it might have practical value in suggesting the optimal conditions for chromatography of polyelectrolytes on ion exchangers, and that this treatment may be taken as a basis for future developments, in which other factors will also be considered.

THE MODEL

Only the case where those groups of the polyelectrolyte that take part in the exchange and the groups on the exchanger's surface have the same affinity for each other is considered. This means that in the present model: (1) the charges of the groups of the polyelectrolyte, which take part in the exchange, are equal; (2) the charges on the groups of the exchanger are also equal; (3) no mutual interaction exists among the exchanger's groups, likewise among the polyelectrolyte's groups; (4) only negligible conformational changes (if any) accompany the ion exchange. That is, the steric fit between the charges of the exchanger and the charges of the polyelectrolyte only involves conformational changes corresponding to changes in free energy which are negligible in comparison to those of the exchange proper.

It is obvious that this model corresponds to a highly idealized case. Conditions

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(1), (3) and probably (4) do not hold in the case of proteins, for example. Condition (3) certainly does not hold with highly substituted synthetic ion exchangers, where a large pH- and ionic strength-dependent swelling obviously shows some interaction of the charged groups. Even in the case of ion exchange of polylysines on low-substituted carboxymethyl-cellulose[1] some interactions among the polylysine groups and among the exchanger's groups certainly occur. It is clear, however, that the ion exchange of polylysines on carboxymethyl-cellulose is closer to the idealized case than any ion exchange chromatography of proteins.

It is compatible with the proposed model that some groups of the polyelectrolyte are in an unfavourable steric position. Thus, n, the number of charges which take part in the exchange, may be smaller than the total number of charges of the polyelectrolyte and is not necessarily equal to the degree of polymerization of, say, polylysines or polyglutamic acids. However, except for the lower mers, it is reasonable to assume that n is proportional to the degree of polymerization. For the same reason the "monovalent ion" M (see next section) does not necessarily correspond to the monomer of the series considered.

It is assumed that a monolayer is formed on the exchanger's surface. This is likely to be the case with polyelectrolytes carrying only one type of charge, but it is probably not so with proteins.

As it has been pointed out in the introduction we are not attempting here to treat the effect of changes of the ionic strength on activity coefficients, on counter ion distribution, on conformation, on solvation etc. A change in counter ion distribution as well as a change in tertiary structure will, for example, affect n (the number of charged groups taking part in the exchange), and therefore also K_P (see later). Finally, we assume that the ion exchange is reversible.

SYMBOLS

(P) = concentration of the polyelectrolyte P.
(E) = concentration of the eluent E, monovalent.
(M) = concentration of the monovalent ion, M, having a mol. wt. equal to W_P/n.
Its only charged group is identical with one of the charged groups of P, which take part in the exchange.
n = number of the charges on P's surface which take part in the exchange. They are all identical (see above).
Z = total number of charges on the surface of 1 g exchanger (in equivalents).
W_P = molecular weight of the polyelectrolyte P.
θ = fraction of the groups on the surface of the exchanger, which are bound to the polyelectrolyte, and to the eluent: $\theta_P + \theta_E = 1$.
α = sorption velocity constant.
f = activity coefficient.
K_P = $\alpha_P n/\alpha_E$.
K_M = α_M/α_E.
q = g of polyelectrolyte (q_P), and of monovalent ion (q_M), respectively, which are fixed by 1 g of exchanger.
Other symbols are used with their usual meanings.

THE ISOTHERM

A Langmuir treatment has already been applied to ion exchange of ions of equal charge[2]. Within the limits pointed out in the section on the model we will try to apply it to ion exchange of polyelectrolytes.

Let us consider the exchange reaction:

$$P^{n\pm} + AE_n \rightleftharpoons AP + n\,E^{1\pm} \tag{1}$$

The probability that a group of the exchanger is bound to E and is thus available for exchange with one group of the polyelectrolyte, is equal to θ_E. The probability that n groups of the exchanger should occur in sterically favourable positions, so that the corresponding groups of the polyelectrolyte could bind to them is, of course, $\theta_E{}^n$. The velocity of sorption of the polyelectrolyte is thus:

$$\alpha_P \theta_E^n f_P(P) \tag{2}$$

Similarly, since n E ions will take the place of one P ion on the exchanger's surface (*cf.* above), the velocity of sorption of E will be:

$$\alpha_E \theta_P f_E^n (E)^n \tag{3}$$

At equilibrium:

$$\alpha_P \theta_E^n f_P(P) = \frac{1}{n} \alpha_E \theta_P f_E^n (E)^n \tag{4}$$

By introducing symbols already defined and by rearranging, one obtains the sorption (ion exchange) coefficient:

$$\frac{q_P}{(P)} = \frac{K_P Z W_P (1 - \theta_P)^n f_P}{n (E)^n f_E^n} \tag{5}$$

This equation, which is an obvious consequence of what has been said previously, agrees well with what is found experimentally. The very high dependence of R_F values of polyelectrolytes on (E) is a common observation and is shown by the fact that (E) enters into the formulation of the sorption coefficient (Eqn. 5) at the nth power, n being by definition higher (and probably much higher) than 1. TISELIUS[3] and BOARDMAN AND PARTRIDGE[4] and others have already pointed out that every equation for the sorption coefficient of polyelectrolytes, if based on the law of mass action, should indicate this. The simple derivation given above is essentially an application of the law of mass action to surface phenomena.

It is also well known that this "all or nothing" chromatographic behaviour of polyelectrolytes becomes less and less evident as their groups are titrated—and this is actually one of the reasons why pH gradients in the isoelectric range are extensively used in protein chromatography[5,6]. This fact is also shown in Eqn. (5): a decrease of

n will decrease this strong dependence of the sorption coefficient on (E). (K_P also will be affected, see later).

Equation 5 also indicates that the isotherms of a polyelectrolyte increase in curvature as n increases. In fact, the higher n, the more sensitive the sorption coefficient towards small changes of θ_P. It is known that the chromatographic behaviour of proteins on ion exchangers corresponds to that of substances having strongly curved isotherms (see *e.g.* ref. 7): where tailings or mutual displacement phenomena are very frequent. This fact is also very clearly indicated by the chromatographic behaviour of polylysines on carboxymethyl-cellulose (ref. 1, Fig. 2). At constant eluent concentration the zones become more skew as n increases. Since equilibrium conditions were approached, this observation has to be referred to an increased curvature of the isotherms[8–11].

BOMAN[12] has reported that the displacement of proteins depends not only on the concentration of the displacer[8], but also on that of the small "eluting" ions. A likely explanation is provided by Eqn. (5) (and probably by other formulations[13] as well). At low (E) the isotherm of the displacer D is too high for a displacement to take place at the chosen (D) (Fig. 1a). An increase in (E) brings about a flattening of the isotherms, which is more evident for D than for P (Fig. 1b). Thus at this (E), a displacement can take place. At still higher (E), both isotherms become essentially linear and therefore displacement is no longer possible.

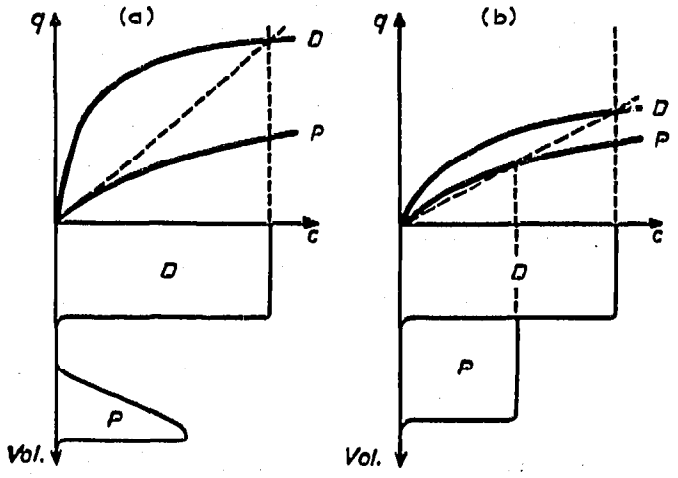

Fig. 1. Effect of (E) on the ion exchange isotherms of a polyelectrolyte P and of its displacer D. (a) at low (E); (b) at higher (E). For explanations of this suggested mechanism, see the section THE ISOTHERM.

Equation (5) is therefore in good agreement with the experimental observations available. It is unfortunate that not more than a qualitative check of this equation is possible at present for the limited amounts of polylysines available[1]. A quantitative check has been carried out, however, for the dependence of the chromatographic behaviour of polylysines on their polymerization number[1]. Since the equations tested (see later) are based on the same assumptions (see the Section MODEL), the fact that they fit the experimental data well is also indirect evidence that Eqn. (5) is essentially correct.

THE DEPENDENCE OF K_P ON n

In the reaction (1) the chemical potentials at the equilibrium are given by:

$$\frac{\mu^\circ_{AE_n} + \mu^\circ_P - \mu^\circ_{AP} - n\mu^\circ_E}{RT} = \ln \frac{\theta_P(E)^n f_E^n}{\theta_E^n f_P(P)} = \ln K_P \tag{6}$$

Similarly, for the exchange reaction:

$$M^{1\pm} + AE \rightleftharpoons AM + E^{1\pm} \tag{7}$$

the chemical potentials are given by:

$$\frac{\mu^\circ_{AE} + \mu^\circ_M - \mu^\circ_{AM} - \mu^\circ_E}{RT} = \ln \frac{\theta_M f_E(E)}{\theta_E f_M(M)} = \ln K_M \tag{8}$$

From the assumptions made in the section MODEL

$$\mu^\circ_{AE_n} = n\mu^\circ_{AE} \qquad \mu^\circ_{AP} = n\mu^\circ_{AM} \qquad \mu^\circ_P = n\mu^\circ_M$$

Thus,

$$n \ln K_M = \ln K_P \tag{9}$$

At constant (E), for $(E) \gg (P)$ and for $(E) \gg (M)$ (*i.e.*, at the initial part of the isotherm, where θ_P and θ_M can be neglected), by assuming that:

$$\frac{f_P}{f_M f_E^{n-1}} = 1 \tag{10}$$

one obtains, by comparison between the sorption coefficients of P and M at the same (E), that they are related as follows:

$$\ln \frac{q_P}{(P)} = \ln \frac{q_M}{(M)} + (n-1) \ln \frac{K_M}{(E)} \tag{11}$$

Within a homologous series of polymers $\ln[q_M/(M)]$ is, of course, constant. Since the retention volumes Δ are directly proportional to the sorption coefficients[9–11], one obtains:

$$\ln \Delta = a + (n-1) \ln \frac{K_M}{(E)} \tag{12}$$

where a is a constant, which depends on the type of polymer series, on the exchanger, on the column and on the initial conditions.

Equation (11) has been checked in batchwise isotherms and equation (12) in column experiments with elution at constant (E) (ref. 1, Fig. 1). In each set of ex-

periments the agreement with the theory was excellent. Moreover, similar values of K_M were found in both cases, in spite of the somewhat different experimental conditions.

These equations also provide an explanation for the common observation that in ion exchange chromatography of polyelectrolytes (*e.g.*, proteins, nucleic acids, etc.) it is usually not possible to elute more than a few components with a single concentration of the eluent. It is apparent, in fact (Eqn. 12), that at high values of n the retention volumes reach unworkable values.

Elution of polyelectrolytes is often accomplished by increasing (E) gradually. The individual polyelectrolytes travel more slowly than the gradient until they are reached by the lowest (E) in which they have an R_F of 1. From this moment on all polyelectrolytes have the same sorption coefficient and travel at the same speed as the gradient. Thus, if the column is long enough for the gradient used, a simple correlation exists between n and the (E) in which the individual polyelectrolytes emerge from the column. At $(E) \gg (P)$ and $(E) \gg (M)$, from the formulations of the adsorption coefficients of P (Eqn. 9) and of M (from Eqn. 9), and assuming the validity of Eqn. (10), it is easy to show that such a relation is:

$$\frac{K_M{}^n}{(E_P)^n} = \frac{K_M}{(E_M)}, \text{ i.e. } \ln (E_P) = \ln K_M - \frac{1}{n} \ln \frac{K_M}{(E_M)} \qquad (13)$$

where (E_P) and (E_M) are the lowest (E) at which P and M, respectively, have an R_F of 1.

This equation also has been tested with polylysines on carboxymethyl-cellulose (ref. 1, Fig. 3): it fits well the experimental data, except for the higher mers, which require a higher (E) than predicted. This is probably due to salting out effects, which are likely to be more evident with higher mers than with low ones. It is interesting to note that the value of K_M obtained from Eqn. (13) agrees well with those obtained from Eqns. (11) and (12), in spite of the different experimental approach.

CONCLUSIONS

The present equations should not be expected to hold quantitatively in ion exchange of proteins. In fact, in the latter case, some of the stated or understated assumptions of the present treatment are not likely to hold: (1) The charged groups on the protein molecule are of different types and often show evident interactions; (2) proteins, being polyfunctional, may well form multiple layers on the exchanger surface; and (3) it is doubtful whether equilibrium conditions are ever attained in the chromatography of very large molecules; etc.

The combined effects of these and other factors are that the sorption isotherm is more curved, and it may not reach a saturation level, the "elution isotherms" do not necessarily coincide with the sorption isotherms, etc. In the case of proteins, the present treatment can therefore only be regarded as semiquantitative. However, the equations obtained can be used as a guide to establish the conditions for protein chromatography, as they point out some of the main parameters affecting the sorption coefficient; in changing the conditions care should of course be taken that no opposing effects influence the sorption coefficient.

ACKNOWLEDGEMENTS

The author is greatly indebted for fruitful discussions to Prof. F. LEUTHARDT, Zürich, to Prof. A. TISELIUS, Uppsala, to Prof. E. SCHUMACHER, Zürich, as well as to Docent H. G. BOMAN and Dr. W. BJÖRK, Uppsala. The author is very grateful to Prof. M. A. STAHMANN and Dr. M. A. SMITH, Madison, Wisc., who have communicated to him many data on the chromatographic behaviour of polylysines on carboxymethyl-cellulose.

The financial support of the Schweiz. Nationalfonds für wissenschaftliche Forschung and of the Stiftung zur Förderung der wissenschaftliche Forschung an der Universität Zürich is gratefully acknowledged.

SUMMARY

Some considerations on the ion exchange of polyelectrolytes are presented. The approach is limited by the several assumptions. Nevertheless, the equations presented describe reasonably well the ion exchange of polylysines on carboxymethyl-cellulose (described in the following paper) and they agree, also, at first approximation, with the chromatographic behaviour of proteins on ion exchangers.

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