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Studies on the adsorption capacities of proteins with a tentacle-type ion exchanger and their relationship to the stoichiometric retention parameter Z_c [1]

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Abstract

The adsorption capacities of five proteins and an amino acid derivative with the so-called "tentacle-type" ion exchanger LiChrospher 1000 SO_3^- were studied as a function of the concentration of the displacing salt NaCl in batch equilibrium experiments, thus allowing the displacement slope parameter, B, to be calculated. In general, the slopes (B) of the plots of adsorption capacity versus displacing ion concentration were negative under the experimental conditions used, consistent with the adsorption mechanism having a dominant ion-exchange contribution. However, the results for lysozyme and insulin demonstrated that their adsorption capacities increased with the concentration of displacing salt in the solution, indicative of a significant contribution of hydrophobic interaction to the binding process of these two biosolutes. The relationship between the adsorption capacity at zero concentration of displacing ion ($q_{m,i\ 0}$), the displacement slope parameter (B) and the experimentally derived stoichiometric displacement parameter (Z_c), calculated from the ion-exchange gradient elution chromatographic results of these test solutes were compared under the same buffer, temperature and displacing salt conditions. These investigations have, in particular, confirmed that a reciprocal relationship exists between the value of the displacement slope parameter B measured in batch equilibrium experiments and the value of the parameter Z_c calculated from the plot of the logarithm of the capacity factor ($\log k'$) versus the logarithm of the reciprocal of the ionic strength at elution ($\log 1/C$). It can thus be concluded that B is directly related to the reciprocal of the effective (or average) number of binding sites on a protein molecule involved in the interaction with the ion-exchange adsorbent.

1. Introduction

Since the advent of the so-called tentacle-type silica-based ion exchangers several years ago, a number of research publications detailing the isothermal and retention features of this class of adsorbent have appeared in the scientific literature (see for example Refs. [1]–[5]). One characteristic property of these tentacle-type ion-exchange chromatographic materials is their high

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affinity for various classes of proteins with the chromatographic behaviour of these biomolecules described mainly in terms of an ON–OFF mechanism [4]. The adsorption isotherm for this type of interaction between a protein and this class of ion exchanger can theoretically assume a rectangular form in the absence of displacing ions. It has been found experimentally [5] that this rectangular form of the adsorption isotherm is observed over particular concentrations of displacing ions. However, with such adsorption systems, the maximum value of the plateau of the isotherm (i.e. the maximum capacity after adsorption saturation has occurred) has been found to be inversely proportional to the concentration of the displacing ion present in the feedstock. The characteristics of this dependency have been described in our earlier publication [5] in terms of the displacement slope parameter, B. Moreover, our earlier investigations have indicated that the magnitude of the displacement slope parameter B of a protein under such saturation conditions can also be affected by the conformational status of the protein, implying that the magnitude of the B value may possibly be related to the number of effective binding sites established between the protein molecule and the ion-exchange adsorbent in the presence of a particular concentration of the displacing ion. Based on these considerations, the conclusion was reached that the interaction between a protein molecule and an ion-exchange adsorbent in batch equilibrium experiments and frontal loading experiments can conceptually be characterised by this displacement slope parameter B, where the magnitude of the B value of a protein with a defined ion-exchange adsorbent reflects the average number of charge groups on the protein involved in the interaction and the physical and electronic characteristics of the particular displacing ion used to affect desorption. On the other hand with zonal elution experiments, it has been commonly proposed in various publications (see for example Refs. [6]–[11]) that the experimentally derived stoichiometric displacement parameter Z_c calculated from the ion-exchange gradient elution chromatographic data of proteins can also be used to define the number of effective charged binding sites established between a protein molecule and the ion-exchange ligands.

In this paper, investigations have been carried out of the possible relationship between the displacement slope parameter B and the stoichiometric displacement parameter Z_c. A formal relationship between B and Z_c has been derived, based on assumptions implicit to the binary ion-exchange model with the number of the interacting binding sites assumed to be independent of the salt concentration. Studies were carried out with several commonly available proteins and an amino acid derivative, dansyl-L-arginine. The adsorption capacities at zero concentration of the displacing ion and their relationship to the values of the corresponding parameter Z_c were also investigated.

2. Experimental

2.1. Materials and chemicals

The tentacle-type ion exchanger used was LiChrospher 1000 SO_3^- (particle size 5 μm), which was obtained from E. Merck (Darmstadt, Germany). The displacing salt was sodium chloride obtained from Ajax (analytical reagent, N.S.W., Australia). Glacial acetic acid ($d = 1.05$ mg/ml at 20°C) obtained from May and Baker (a division of Rhone-Poulenc Australia, Sydney, Australia) was used to prepare the adsorption buffer solutions. All five proteins and the amino acid derivative were purchased from Sigma (St. Louis, MO, USA). These test solutes were horse heart cytochrome *c*, hen egg white lysozyme, hen egg white ovalbumin, soybean trypsin inhibitor, bovine pancreas insulin and synthetic dansyl-L-arginine.

An Orion pH meter (Model SA520) calibrated against standard buffer solution was employed to prepare the buffer solution. A UV–visible spectrophotometer (Type 4050, LKB/Biochrom, UK) was used to determine the protein concentration in solution. The adsorption experiments were carried out at 25 ± 1°C in a temperature-controlled incubator (Thermline, N.S.W., Australia) except for the experiments

with ovalbumin where a temperature of 2 ± 0.5°C in a cold storage room was used.

2.2. Preparation and determination of concentration of protein solutions

The experimental buffer solutions were freshly prepared each time. Glacial acetic acid (1.5 ml) was added to distilled water (500 ml) to make a solution of concentration [HOAc] = 0.05 *M*. The pH value of this solution was then adjusted to 4.00 with a 5 *M* NaOH solution. The sodium ion concentration of this final buffer solution, calculated to be 0.0076 *M*, was taken into account as part of the displacing ion concentration in the experiments. Protein solutions for the adsorption experiments were prepared directly by dissolving the proteins in this buffer solution. The protein concentrations were controlled between 0.8 and 1.0 mg/ml which was found appropriate in the adsorption studies for the proteins to saturate the ion exchanger.

The determination of the protein concentration in solution was carried out using optical density measurements with the UV–visible detector set at 215 nm. All measurements were carried out at room temperature. The calibration curve and the determination of the protein concentrations in the test solutions were achieved by comparing the optical absorbance of the sample solution with the initial buffer solution as a blank reference. In order to determine the protein concentration in the adsorption solutions, the initial protein solutions or the supernatants after the adsorption experiments were completed were diluted according to the value of the extinction coefficients of the different proteins. The protein solution was typically diluted to provide an optical density measurement between 0.4 and 0.7 A.U. by the spectrophotometer.

2.3. Determination of the displacement slope parameter B and the adsorption capacity at zero concentration of displacing ion, $q_{m,i}$

The batch adsorption experiments were carried out in the following way and repeated at least in duplicate. A precisely known weight of about 10 mg of LiChrospher 1000 SO_3^- particles was weighed in a plastic centrifuge tube. The desired amount of the displacing salt NaCl in solid form was then introduced into the tube, followed by the appropriately prepared protein solution (9 ml). These experimental tubes were then quickly placed into a temperature-controlled incubator with continuous gentle agitation which lasted at least 15 h to ensure that the adsorption equilibrium had been reached. The temperature of the incubator was held at 25 ± 1°C. After the adsorption was terminated the supernatant was separated by centrifugation using a Sorvall RT-6000 benchtop centrifuge at the same temperature. The adsorption capacity was calculated based on the protein concentration in the supernatant.

The displacing slope parameter *B* was determined by linear regression analysis of the plots of the adsorption capacity versus the displacing ion concentration, C_i. Experimental adsorption data corresponding to five or six concentration points were measured at different displacing ion concentrations below $[Na^+] = 0.12$ *M*. The adsorption capacity at zero concentration of the displacing ion was obtained by linear regression analysis and extrapolation. Sigmaplot version 5 software was used for these computational analyses.

3. Results and discussion

3.1. Theoretical relationship between the displacement slope parameter B and the stoichiometric parameter Z_c

It has been previously shown [5] with the silica-based tentacle-type ion exchanger LiChrospher 1000 SO_3^- that the value of the adsorption capacity of horse heart cytochrome *c* decreases linearly with increasing displacing ion concentration over a particular range, provided the protein concentration conditions were chosen so as to allow the cation exchanger to reach saturation in batch equilibrium experiments without the formation of protein aggregates. This dependency between the adsorption capacity and the displacing ion concentration was found [5] to follow the empirical relationship given below:

$$q_m = q_{m,i=0} - BC_i \quad (1)$$

where q_m is the adsorption capacity at the displacing ion concentration C_i and $q_{m,i=0}$ is the adsorption capacity at zero concentration of displacing ion. The slope of the plot q_m versus C_i is referred to as the displacement slope parameter, B, which is a characteristic parameter of the interaction between the protein and the adsorbent in the presence of the particular displacing ion and under well defined conditions of pH, temperature and adsorbent saturation, i.e. at relatively high concentrations of adsorbed protein in the batch adsorption experiment. Implicit to this dependency is the assumption that the adsorption phenomenon established between the protein and the ion exchanger is dominated by electrostatic interactions. The general form of the mass action expression for such an ion-exchange process involving a protein P_i which binds to a charged ligand L can be represented in the following manner:

$$P_{i,f} + Z_c L_f \rightleftharpoons P_{i,b} + Z_c L_{(f-a)} \quad (2)$$

where $P_{i,f}$ represents the protein concentration in the free solution, $P_{i,b}$ represents the protein concentration in the bound state, L_f the free ligand concentration and $L_{(f-a)}$ the concentration of the ligands remaining accessible to the solvent after the protein has bound to the ion exchanger. The term Z_c, often called the stoichiometric binding parameter, represents the average number of binding sites established between the protein and the ion-exchange adsorbent. It can be noted that Z_c does not equate with the total charge on the protein, Z_i, but rather to the charge composition within the contact region(s) of the protein which interacts with the electrostatic ligand(s). When the near-equilibrium approximation applies under linear elution chromatographic conditions, i.e. when the number of binding sites, Z_c, is independent of the protein concentration and the ligands L interact with the protein independently of each other, then the retention behaviour of the protein, P_i, over a limited range of salt concentrations can be described in terms of the retention or capacity factor, k', such that

$$\log k_i' = \log K_{0,1} + Z_c \log(1/C_i) \quad (3)$$

where $K_{0,i}$ is the distribution coefficient, and C_i is the concentration of the displacing salt at which the protein desorbs. The Z_c term can be conveniently evaluated from the plots of log k' versus log $(1/C)$ and used to characterise the physicochemical basis of the protein–ion-exchange interaction in this type of chromatographic elution. In addition, under these so called linear or near-equilibrium zonal elution chromatographic conditions, the concentration of the bound protein can be evaluated [6] from the expression:

$$P_b = K_{0,1} P_f (P_{b,max}/C_0)^{Z_c} \quad (4)$$

where $P_{b,max}$ represents the maximum concentration of the protein, i.e. q_m, that can be bound to the ion exchanger under the particular buffer, pH and temperature conditions. Moreover, from Eqs. 1 and 4 it is evident that the capacity factor k' can be directly related to $K_{0,i}$ since k' is defined by the ratio of the bound to free protein concentration, i.e. $k' = \Phi[P_b]/[P_f]$, where Φ is the phase ratio of the system.

When similar considerations are applied to the batch equilibrium experiments, the quantity of the protein desorbed from an ion exchanger by increasing the displacing ion concentration will also be dependent on the number of effective binding sites associated with the protein–ligand interaction, e.g. on the parameter Z_c. In particular, the Z_c and B parameters both represent proportionality terms which link an interactive property of the protein (such as the k' or q_m) and the concentration of the displacing salt. In batch equilibrium experiments the quantity of the protein desorbed from the ion exchanger must be replaced by the same quantity of displacing ions in terms of the number of their equivalent charges, i.e.

$$(q_{m,i=0} - q_m)Z_c = \Psi Q_i \quad (5)$$

where Q_i is the equivalent quantity of the displacing ions substituted on the ion exchanger, Ψ is the charge number of the displacing ion and has a value of one for monovalent ions such as the sodium ion used in the present experiments.

In addition, it can be predicted that the equivalent quantity Q_i of displacing ion is proportional to the concentration of this ion in the solution (C_i) and can be represented by a constant K_q, such that:

$$Q_i = K_q C_i \quad (6)$$

By substituting Eq. 5 with Eqs. 1 and 6 the following relationship between B and Z_c can be obtained:

$$BZ_c = \Phi K_q \quad (7)$$

and hence

$$Z_c = \Psi K_q B^{-1} \quad (8)$$

According to Eq. 8 a reciprocal linear relationship between the parameter Z_c and the displacement slope parameter B is predicted for the interactions of different proteins with the ion exchanger provided the assumptions made above apply. In order to establish the validity of this dependency, the values of the B parameters were determined for several proteins and the results correlated with the Z_c values of the same proteins determined from elution studies using the same strong tentacle-type cation exchanger.

3.2. Protein adsorption onto the tentacle-type LiChrospher 1000 SO_3^- ion exchanger

On the basis of our previous experiments [5], a concentration range of 0.8—1.0 mg/ml for the protein solutions was selected for each of the batch adsorption experiments in order to determine the adsorption capacities of the proteins with the LiChrospher 1000 SO_3^- at different displacing ion concentrations. The dependencies of the adsorption capacities of each solute on the displacing ion concentration are shown in Figs. 1 and 2. Under the experimental conditions used, four out of the six samples showed a decrease in their adsorption capacities with increasing displacing ion concentration. With lysozyme and insulin the results showed a positive slope, indicating that the adsorption capacity increased with increasing displacing ion concentration present in solution. The phenomenon observed for

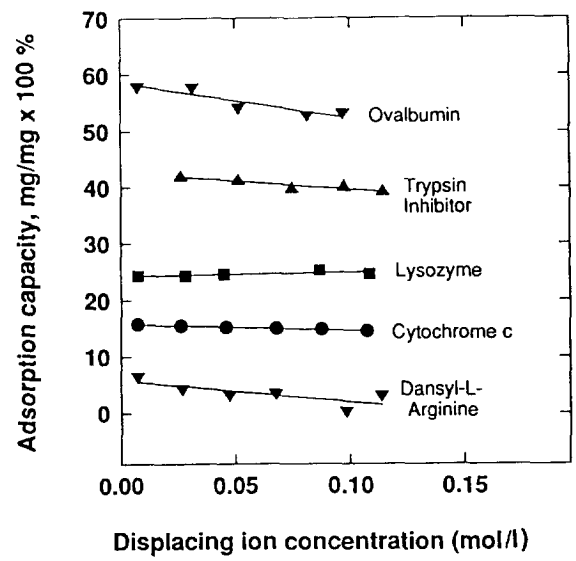

Fig. 1. Plot of the adsorption capacity of the different solutes with the 'tentacle-type' ion exchanger LiChrospher 1000 SO_3^- as a function of the displacing ion concentration.

insulin (Fig. 2) is very noteworthy. The calculated displacement slope parameters B and the derived adsorption capacity at zero concentration of displacing ion ($q_{m,i=0}$) for the various

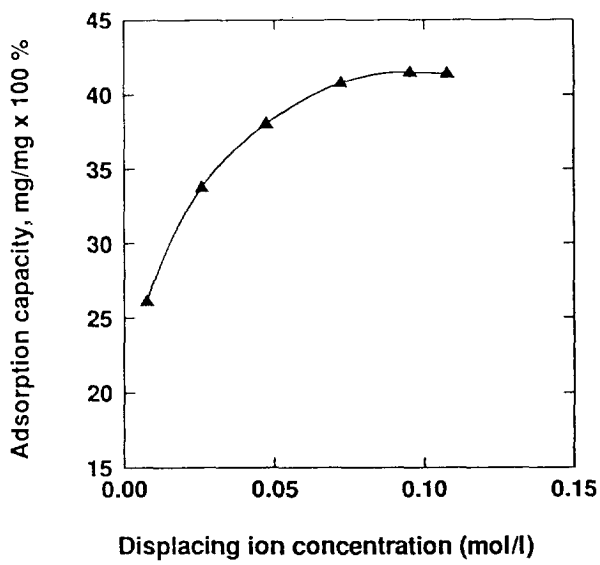

Fig. 2. Variation of the adsorption capacity of bovine insulin with the 'tentacle-type' ion exchanger LiChrospher 1000 SO_3^- with the displacing ion concentration.

Table 1
Physical properties and experimentally derived parameters of the biosolutes

Solute	MW	H_c b[a]	Z_c[b]	$q_{m,i=0}$	B
Dansyl-L-arginine	444	≤100	1	13.02	86.5
Insulin	5734	996	2.33	3.59	--
Cytochrome *c*	12 400	1092	5.85	1.27	0.9
Lysozyme	14 300	893	4.59	1.69	−0.43
Trypsin	21 500	1031	3.68	1.98	1.4
Ovalbumin	46 000	992	4.66	1.27[c]	1.4[c]

[a] Data from Ref. [13].
[b] Data from Ref. [12].
[c] Data obtained at 2°C; all other data were acquired at 25°C.

proteins and dansyl-L-arginine are listed in Table 1 together with the adsorption capacities expressed as millimole protein per gram ion exchanger (mmol/g) and the slope B expressed as the unit decrease of the adsorption capacity per mole displacing ion (mmol/g mol). The corresponding experimentally derived values of the parameter Z_c, determined for the same proteins and dansyl-L-arginine from the corresponding gradient elution high-performance liquid chromatographic data using the same cation-exchange adsorbent and the same buffer-displacing salt–temperature system [12], as well as their molecular mass (MW), are also given in Table 1. Since ovalbumin was found to readily aggregate in the low-ionic-strength solutions at the experimental temperature (25°C), the protein concentration in the supernatant was not used to deduce the quantity of ovalbumin adsorbed onto the particles. However, by employing analogous experimental procedures at 2°C, it was found that the self association of ovalbumin in solution was sufficiently reduced to allow the experiments to be conducted. Consequently, the data for the ovalbumin was obtained at 2°C instead of 25°C.

As already shown in our previous studies [4,5], the adsorption of proteins onto the tentacle-type ion exchangers involves a 'multilayer dissolution' binding mechanism. The adsorption capacity of a protein with this type of ion-exchange adsorbent arises from a combination of processes mediated by both electrostatic and hydrophobic interactions between the protein molecule and the linear grafted 'tentacular' ligands. In the normal ion-exchange situation, electrostatic interactions between the protein, the displacing ion and the ion exchanger dominate the retention process. In free energy terms this electrostatic interaction will tend to be stronger than the hydrophobic interaction between a protein molecule and the ion-exchange ligands whilst increasing concentrations of displacing ions will significantly decrease the adsorption capacity. As evident from the data shown in Fig. 1 and Table 1, cytochrome *c*, ovalbumin, trypsin inhibitor and dansyl-L-arginine all follow this trend. In particular, this group of test biosolutes demonstrate linear proportionality between q_m and C_i (with positive B values) in these experiments as shown in Fig. 1 and Table 1.

However, under certain circumstances hydrophobic interactions between a protein and the tentacular ligands can potentially become important. Due to the presence of higher concentrations of the displacing salt, the electrostatic charge potential distributed along the protein surface may be partly neutralised by the displacing ion Na^+ and its counter ion Cl^- through complementary dipole–dipole interactions involving the solvated ions and the amide bonds of the polypeptide backbone or the charged amino acid side-chains of the protein. Moreover, with tentacular ligand structures containing repeated monomer units each bearing a cationic functionality, the local co- and counter-ion concentrations may become appreciably higher than in the bulk solvent through participation of the Donnan equilibrium processes. As a result of these charge neutralisation effects involving both the tentacular ion-exchange ligands and the protein molecules, in regions of the ligands where more non-polar microenvironments exist, hydrophobic interaction between the protein and the ligands can become more favourable and could lead to salt-mediated retention processes which dominate the interaction depending on the particular conformation(s) adopted by the protein molecule in the solution and its surface charge/hydrophobicity properties. Consequently, when hydrophobic interactions represent an increasingly dominant phe-

nomenon, then the adsorption capacity is expected to rise as the ionic strength increases. This behaviour was evident in the results obtained with lysozyme and insulin. With such types of multimodal adsorbents it can be noted that the adsorption behaviour of proteins will be particularly responsive to the conformational status of both the protein and the tentacular ligands and will exhibit significant variation in response to relatively small changes in the elution/desorption conditions. As previously observed [4] from the analysis of Hill coefficient data derived for protein adsorption with tentacular-type adsorbents, these changes in the chemical and physical properties of the contacting regions established between the protein and the ligands can lead to time-dependent reorientation effects or the re-arrangement of the protein–ligand structures leading to closely packed protein–protein complexes on the adsorbent surface. As anticipated, the global hydrophobicity values ($H\varphi^{b}$ in Table 1) for lysozyme and insulin and the other proteins do not reveal any correlation relevant to the origin of these selective differences. Since the global hydrophobicity values were calculated [13] for these molecular structures without taking into account their conformational features in solution, the nature of their contact regions or the anisotropic distribution and accessibility to the solvent of charged and non-polar groups on the surface of the proteins, it is not unexpected that no correlation was found between q_m and H_{φ}^{b}. Similarly, it can be anticipated for long, linear polyelectrolyte chains such as the tentacular ligands that conformational effects will also play a significant role in determining their effective capacity behaviour in different salt or pH systems. As a consequence of their flexibility, these ligands may exhibit characteristics of a self-assembling clathrin-like structure when very large charged biomacromolecules are present but more dispersed structures with low-molecular-mass compounds where the charge density per unit area of the molecular surface may be higher. The opportunity for hydrophobic interactions to occur between the non-polar chemical moieties within the structures of the tentacular ligands and the protein solutes, involving, in particular, buried non-polar amino acid residues which become more accessible due to partial unfolding or re-orientation of the protein molecule as it associates with the ion-exchange ligands, would be very much more favoured with conformationally flexible proteins than for relatively rigid molecules. Thus, positive slope (negative B) values are to be anticipated with these adsorbents for conformationally more flexible proteins, particularly those proteins with a relatively high surface hydrophobicity/hydrophilicity ratios such as lysozyme or insulin.

As noted above, this inverse trend in the adsorption capacity behaviour mediated inter alia by hydrophobic processes will only occur over a relatively narrow range of ionic strength conditions which are characteristic for each protein. This component of the adsorption capacity mediated by hydrophobic interactions will thus appear to be independent of the concentration of the displacing salt until a certain critical ionic strength value is reached. Such behaviour has been noted previously by other workers investigating the salt-mediated chromatography of proteins. For example, the interplay between electrostatic and hydrophobic processes when proteins are in the presence of electrolytes and polyelectrolytes has been commented upon previously by Melander et al. [14] in terms of the propensity for salting-in and salting-out of proteins as well as determining the extent of interaction with hydrophobic interaction adsorbents. Like the behaviour noted with conventional ion-exchange adsorbents, as the displacing salt concentration continues to increase, the final adsorption capacity will depend on the electrostatic interactions between the protein molecules and the ion-exchange ligands. This controlling effect is shown by the tendency of the dependency to reach an asymptote as shown in Fig. 2.

3.3. Experimental relationships between the adsorption capacity at zero concentration of displacing ion $q_{m,i=0}$ and the slope B with the parameter Z_c

As shown in Fig. 3, a linear dependency was found between the reciprocal of the adsorption capacity $q_{m,i=0}$ and the parameter Z_c ($1/q_{m,i=0}$

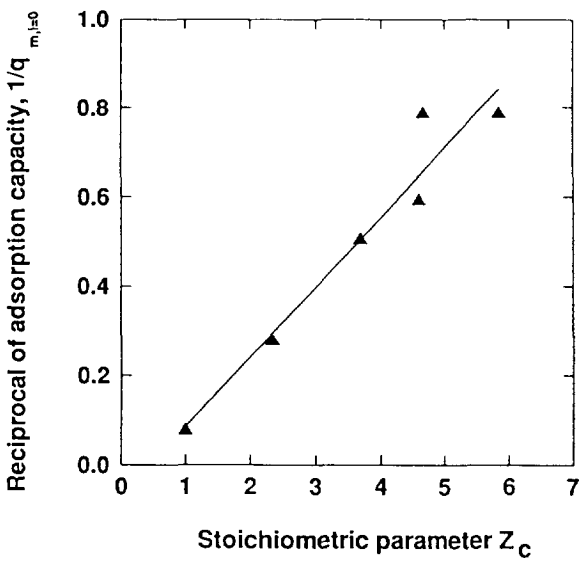

Fig. 3. Plot of the reciprocal of the adsorption capacities, $1/q_{m,i=0}$, versus the stoichiometric displacement parameter Z_c (data from Ref. [12]).

versus Z_c) for all the biosolutes studied here. Under our batch equilibrium experimental conditions where the adsorption of the protein onto the ion exchanger reaches saturation, the adsorption equilibrium is established principally on the basis of complementary neutralisation of electrostatic charges between the protein and the ion exchanger. The results obtained suggest that when the value of the experimental parameter Z_c of a protein molecule is small, then more molecules of this protein are needed to neutralize the same quantity of electrostatic charges on the ion-exchange ligands. A larger adsorption capacity and hence a larger B value thus corresponds proportionally to a smaller Z_c value, consistent with the general observations previously noted for the different adsorption behaviour of low- and high-molecular-mass solutes. In addition, the above findings confirm earlier proposals [15,16] as well as various empirical observations [10,11,17–19] that with proteins in solution or within an ion-exchange environment, many of the charged groups on the protein surface are associated as salt bridges and only a few charged amino acid side-chain groups accessible on the protein surface are involved at any one time in interactions with the electrostatic ligands. The establishment of regions or clusters of charged amino acid residues which involve the highest charge densities of the molecular surface [8,10,11] and the greatest accessibility could lead to the formation of a predominant contact interface which docks with the ion exchanger together with a range of other less favoured contact orientations some of which will represent kinetic rather than thermodynamic intermediates in the binding process. For this reason the displacement slope parameter B as well as the Z_c parameter will usually have a fractional value since both these proportionality terms represent averages of the various competing structures which can form an appropriate contact interface with the electrostatic ligands. Moreover, the nature of the dependency between q_m and Z_c provides further support to the generally held view that the parameter Z_c represents the effective number of binding sites for the interactions between the proteins and the ion exchanger and not the total number of charges Z_i associated with the ionised protein.

In physical terms, the displacement slope parameter B referred to in this paper is actually the parameter which represents the amount of bound protein molecules on the ion-exchange ligands desorbed per mole of displacing ion (e.g. Na^+) in solution. If the binding between a protein molecule and the ion-exchange ligand is governed solely by electrostatic interactions during the process (and provided the nature of the hydrophobic interactions are not significantly modified with the variation of the concentration of displacing ion), then the effective number of binding sites established between a protein molecule and the ion-exchange ligand will be directly reflected in this desorption behaviour as the number of moles of protein desorbed per mole of displacing ion, namely the value of the slope parameter B. Thus, the displacement slope parameter B is expected to have a inverse relationship with the experimentally determined stoichiometric displacement parameter Z_c derived from linear elution chromatography, i.e the dependency should take the form of Eq. 8. In Fig. 4, the experimental results of the displace-

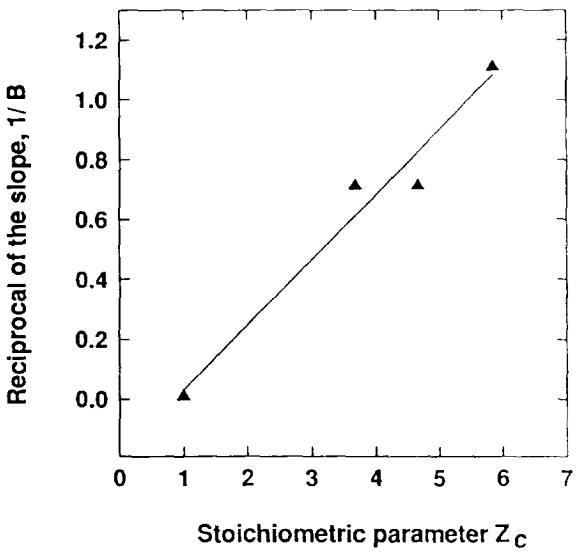

Fig. 4. Plot of the reciprocals of the displacement slope B values versus the stoichiometric displacement parameter Z_c (data from Ref. [12]).

ment slope parameter B and the parameter Z_c values are compared as a semi-reciprocal plot. Both insulin and lysozyme, which demonstrated strong hydrophobic interactions with the tentacular adsorbent in the presence of the displacing salt under the experimental condition used, diverge significantly in this linear dependency and their results are not shown in this figure. For the four biosolutes which showed more regular ion-exchange behaviour, it is evident that the Z_c parameter values are linearly related to the reciprocal of the displacement slope parameter B by a constant which is characteristic of the displacing ion and the adsorption system used for a particular temperature and pH condition.

4. Conclusions

The specific ON–OFF adsorption mechanism of the tentacle-type ion exchanger LiChrospher 1000 SO_3^- allows the relationship between the parameter Z_c and the adsorption capacity at zero concentration of displacing ion $q_{m,i=0}$ as well as the relationship between the parameter Z_c and the displacement slope parameter B to be studied quantitatively. The experimental results obtained confirm that the value of the parameter Z_c can be independently equated with the effective number of binding sites as derived from thermodynamic equilibrium measurements. Consequently, the displacement slope parameter B in association with the experimental parameter Z_c values can be used to study the nature of the interaction between a biomolecule and an ion exchanger in both batch adsorption equilibrium experiments as well as in zonal elution experiments.

Acknowledgement

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