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Adsorption of proteins on porous and non-porous poly(ethyleneimine) and tentacle-type anion exchangers[a]

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ABSTRACT

Adsorption isotherms of proteins [bovine serum albumin (BSA), soybean trypsin inhibitor and alcohol dehydrogenase] on anion exchangers were measured by on-line and off-line methods. The poly(ethyleneimine) (PEI) type and the tentacle-type materials exhibited principally different modes of adsorption. On thin layers of PEI, bonded to non-porous silica, BSA adsorption data corresponded to a monolayer of molecules, with 80% adsorbed side-on, with a high affinity constant for binding, and 20% adsorbed more weakly. With porous material, the amount of BSA bound per unit surface with high affinity was smaller.

With tentacle-type anion exchangers, adsorption exceeded a monolayer by far, and data corresponded to a Hill-type isotherm. The Hill coefficients, which were smaller than 1, indicated an approximately Gaussian affinity distribution of the binding sites for the protein. As a consequence, adsorption capacities at elevated ion strength decreased drastically, while the affinity distribution became narrower. Adsorption capacities and binding constants increased with temperature, while Hill coefficients remained unchanged.

INTRODUCTION

The introduction of macroporous ion-exchangers based on surface-modified silicas has substantially improved the analysis and isolation of proteins in terms of resolution, speed and biorecovery [1–3]. A new family of novel macroporous ion-exchangers specially designed for nucleic acid and protein analysis have been recently introduced by E. Merck, Darmstadt, F.R.G. (see ref. 4).

Although the wide-spread applicability of these ion-exchangers has been demonstrated, very few fundamental studies on the kinetics and the equilibria of

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proteins on ion-exchangers have been carried out [5–7]. The assessment of isotherms and kinetic data of proteins elucidates the type and strength of interactions and provides valuable hints for the use of ion-exchanger in isolation and purification.

This work examines the effect of the type of surface modification and the pore size of silica support on the adsorption capacity, the adsorption equilibria, the extent of non-specific adsorption and the kinetics of protein adsorption. The results were to be interpreted in the frame of existing models of adsorption. The aim was to contribute to a better understanding of the protein–ion exchanger interactions and to the questions which pore sizes and surface compositions are desirable for ion exchangers.

EXPERIMENTAL

Materials

Silicas (LiChrospher Si 100, 300, 1000 and 4000, with mean pore diameters of 10, 39, 105 and 280 nm, respectively) were obtained from and characterized by E. Merck (Darmstadt, F.R.G.). Non-porous glass beads (type 3000) were provided by Potters-Ballotini (Kirchheimbolanden, F.R.G.). γ-Glycidoxypropyltrimethoxysilane (GLYMO) and polyethyleneimine (PEI-600, mean mol. wt. 600) were purchased from Aldrich (Heidenheim, F.R.G.). Bovine serum albumin (BSA, 67 000 dalton), soybean trypsin inhibitor (STI, 21 500 dalton) and alcohol dehydrogenase from yeast (ADH, 141 000 dalton), all buffer components (reagent grade) and organic solvents (HPLC or reagent grade) were from E. Merck. Water was purified using a Mili-Q system (Millipore, Eschborn, F.R.G.).

Purity of proteins was determined by size-exclusion chromatography on a Zorbax GF-250 column (DuPont, Wilmington, DE, U.S.A.) with UV detection at 278 nm and 0.05 *M* phosphate buffer pH 7.0 containing 0.2 *M* NaCl. The BSA used was 98% pure, the water content was assumed to be 5% according to the data of the manufacturer. The purity of ADH and STI was >99% and 95%, respectively.

PEI modification

Bonded PEI anion exchangers were made using a procedure similar to the one introduced by Chang *et al.* [8]. Silicas were activated overnight at 423 K under vacuum. To 9 g of each material, 60 ml GLYMO were added and heated to 493 K for 2 h under nitrogen. GLYMO-modified silicas were washed with methanol and diethyl ether and dried at 333 K under vacuum. To obtain weak anion exchangers, they were suspended in 100 ml of a 1% PEI solution in methanol and reacted 24 h at 298 K in closed 200-ml erlenmeyer flasks at 140 strokes min^{-1} in a shaking water bath. The materials were collected on a frit, washed with methanol, 0.01 *M* HCl and, finally, water until the filtrate was neutral.

Preparation of the glass beads

The type 3000 glass beads, having a wide size distribution from 0.1 to 40 μm, were fractionated according to size by means of an Alpine 200 zig-zag classifier (Alpine, Augsburg, F.R.G.). A fraction containing particle sizes from 5–10 μm, with a mean value of 8 μm, was treated with an equal volume of *aqua regia* for 2 h at room temperature to remove organic impurities and increase the number of surface silanol groups. Finally, the material was washed to neutrality and dried under vacuum.

Tentacle-type strong anion exchangers

Pretreated glass beads and LiChrospher Si 1000 were subjected to a three-step procedure developed and described by Müller [4,9]. In short, the materials are silanized with GLYMO, the oxirane ring opened by acidic hydrolysis. Then, a radical grafting polymerization of an N-trimethylammoniumethyl(TMAE)–acrylamide salt is initiated by Ce^{4+} ions. The procedure results in individual, linear polyelectrolyte chains chemically bonded to the surface.

The porous (LiChrospher) material is equivalent to the commercial materials of E. Merck, which, according to the manufacturer, have an average chain length of 10 to 50 monomer units. Due to the very low surface area of the non-porous glass beads (the surface area was too low for exact surface determination by nitrogen adsorption, the geometrical surface area is *ca.* 0.3 m^2/g) the polymerization had to be done in the presence of excess monomer, probably resulting in significantly longer polymer chains.

On-line isotherm measurements

These experiments were done using apparatus similar to that previously described by other workers [10–12]. It consisted of a stirred, thermostated glass vessel containing the ion exchanger suspension, a Gelman Science (Ann Arbor, MI, U.S.A.) Acrodisk filter unit (1.2 μm porosity), a total of 40 cm of Tygon tubing with 1.4 mm internal diameter (*ca.* 18 cm^2 internal surface), an IN 4 peristaltic pump (Ismatec, Wertheim-Mondfeld, F.R.G.), a Merck-Hitachi F-1000 fluorescence detector equipped with a 40-μl cell and a Kipp and Zonen (Solingen, F.R.G.) chart recorder. In operation, the flow direction was frequently reversed for a few seconds to resuspend particles accumulating in the filter unit. The fluorescence detector was set to an extinction wavelength of 280 nm and an emission wavelength of 370 nm. To avoid non-specific adsorption of protein to the system components, the whole apparatus was flushed with a 1 mg/ml solution of BSA in 20 m*M* Tris–HCl buffer pH 8.3 for 24 h and subsequently for 8 h with the buffer only. After this procedure, no significant adsorption or desorption of protein to the tubing or glass vessel could be detected.

Isotherms were measured in a cumulative manner. To the stirred suspension of anion exchanger (porous materials: 100 mg in 25 ml, non-porous material: 200 mg in 25 ml), small volumes of BSA solution in buffer were added. After the equilibrium was established (indicated by a constant signal from the fluorescence detector —between 5 min and 2 h), the next portion of protein was added, and so on. Before starting each set of binding measurements the total system was calibrated in absence of ion exchanger, by at least 10 subsequent additions of protein, covering a concentration range from 0.4 μg/ml to 1 mg/ml. In the time scale of these experiments and within the precision limits of the method no proteolytic digestion or denaturation of the BSA was observed. Standards remained almost constant over hours of pumping through the system.

Off-line isotherm measurement

These experiments consisted of three steps.

Step 1. Wet ion exchanger (50–200 mg) was placed into 1.5 ml Eppendorf vials. To achieve conditioning to the buffer composition used in the experiment, vials were several times filled up with buffer, shaken, centrifuged and the supernatant decanted.

Step 2. Buffer and protein solution were added, the vials placed into 100-ml

erlenmeyer flasks filled with 40 ml of water, put into a thermostatted shaking water bath (SW-1P, Julabo, Bernkastel-Kues, F.R.G.). The suspension was shaken overnight (140 strokes per minute, amplitude 15 mm), which prevented particles from settling. Then the suspension was centrifuged in an Eppendorf centrifuge.

Step 3. Variable amounts of the supernatant protein solution were injected into a Merck-Hitachi gradient high-performance liquid chromatography (HPLC) system. The system was equipped with a column 36 × 8 mm, filled with 2.1 nm non-porous, monodisperse, C_{18} modified silica. The injection volume was 100 μl for higher protein concentrations, up to 500 μl for low concentrations. Eluents were: (A) 0.1% trifluoroacetic acid (TFA) in water, (B) 0.1% TFA in acetonitrile–water (80:20, v/v). Elution was performed by the following gradient: 1 min isocratically at 25% B, 5 min linear gradient from 25 to 100% B, 2 min isocratically at 100% B. Quantification was done through UV detection at 220 nm by peak height. Each sample was measured at least twice, with at least one calibration run using a similar BSA solution between the two runs.

To prevent the ion exchangers from drying, the particles were always kept in suspension. The exact weight of the ion exchanger was determined gravimetrically, after completing the measurements, by washing with distilled water and drying overnight at 353 K. Non-specific adsorption to the vials was minimized by preincubation of the vials with 1.5 ml of a 0.1 mg/ml BSA solution in Tris–HCl buffer pH 8.0 overnight and rinsing 3 times for 1 h with 1.5 ml of the buffer used in the particular adsorption experiment.

Desorption experiments

Desorption by dilution was measured using the same off-line system. Adsorption was allowed to take place for 24 h. After centrifugation, the protein solution was decanted and the concentration determined by reversed-phase HPLC as described. Then the vial was weighed, 1.2 ml of fresh buffer were added and the ion exchanger resuspended in the shaking water bath. After each 24 h, the procedure was repeated, finally to give the desorption isotherm, consisting of 4 to 5 points.

Dynamic load capacities

Adsorption capacities of the PEI-bonded silicas were determined by measuring breakthrough curves. HPLC cartridges (E. Merck) 25 × 4 mm I.D. were packed at 250 bar maximum pressure. After conditioning of the column and running a test chromatogram, a 1 mg/ml protein solution in 20 m*M* Tris–HCl buffer pH 8.0 (buffer A) was pumped through the cartridge at a flow-rate of 0.5 ml/min. The effluent concentration was monitored at 278 nm with a UV detector (LKB 2150). The whole area above the recorded curve and below the line representing the final concentration of 1 mg/ml was integrated to give the total adsorption capacity at this BSA concentration.

After loading, the protein was eluted by application of a step gradient: 15 min 0.5 *M* NaCl in buffer A, 15 min 1 *M* NaCl in buffer A, 15 min reconditioning with buffer A, flow-rate 0.5 ml/min.

Adsorption kinetics

The apparatus and method used for on-line isotherm measurements was modified for the adsorption kinetic experiments. The total suspension volume was reduced to 11 ml in a stirred, thermostatted vessel. The concentration was determined by aspiration of adsorbent-free solution through an LKB solvent filter (sinter metal) and the detector (LKB 2150, 278 nm) by means of a peristaltic pump (Ismatec IN 4) and returning it into the adsorption vessel. The total volume of this detection loop was *ca.* 1 ml. The system was filled with 10 ml of protein solution (1 mg/ml in buffer A). A 100-mg amount of ion exchanger was suspended in 1 ml of buffer A and put into an ultrasonic bath for 5 min to break up any agglomerates. The suspension was added to the vigorously stirred protein solution by means of a 5-ml syringe (the addition took not more than 1 s), while the peristaltic pump worked at a flow-rate of 2 ml/min.

RESULTS AND DISCUSSION

Dynamic load capacities of ion exchangers

The load capacities measured varied significantly with the pore size of the material, with the size of the protein (Table I), and with the number of repetitive loadings onto the same column (Table II). Especially the last finding, namely a successive decrease in loading capacity after every loading/elution cycle, made it impossible to use the same packed cartridge for a series of experiments. Such a series would, for example, be required for an isotherm calculated from breakthrough experiments. The full adsorption capacity could not be reestablished even by an extensive washing cycle, including overnight washing with 1 *M* NaCl in buffer A and a gradient from 0–80% isopropanol in 0.1% aqueous TFA. It seems unlikely that this fouling process is caused by a significant decomposition of the anion-exchange

TABLE I

DYNAMIC LOAD CAPACITY OF PEI-BONDED ION EXCHANGERS FOR SELECTED PROTEINS

Pore diameter and surface area as reported by supplier, E. Merck. Buffer 1: 20 m*M* Tris–HCl, pH 8.0; buffer 2, 50 m*M* NaCl in buffer 1.

Support material	Pore diameter (nm)	Surface area (m^2/g)	Load capacity (mg/ml; in parentheses: mg/m^2)			
			Buffer 1			Buffer 2
			ADH	STI	BSA	BSA
LiChrospher Si 100	10	370	21.9 (0.08)	64.3 (0.24)	17.3 (0.07)	11.8 (0.04)
LiChrospher Si 300	39	58	41.0 (0.9)	126.1 (2.8)	70.9 (1.6)	51.1 (1.1)
LiChrospher Si 1000	105	25	84.8 (4.4)	53.1 (2.8)	43.3 (2.2)	28.3 (1.5)
LiChrospher Si 4000	280	9	41.8 (5.8)	18.5 (2.6)	19.5 (2.7)	13.0 (1.8)
Non-porous silica	–	1.8	13.1 (5.4)	6.1 (2.5)	6.5 (2.7)	5.5 (2.3)

TABLE II

DECREASE IN DYNAMIC BSA LOAD CAPACITY ON POROUS AND NON-POROUS PEI-BONDED ION EXCHANGERS AFTER SIX CONSECUTIVE FRONTAL LOADINGS

For buffers, see Table I.

Support material	% decrease in BSA load capacity	
	Buffer 1	Buffer 2
LiChrospher Si 100	29	32
LiChrospher Si 300	33	37
LiChrospher Si 1000	30	28
LiChrospher Si 4000	28	27
Non-porous silica	26	17

modification, because (i) several empty cycles, *i.e.* pumping of loading and elution buffers in the absence of protein did not cause a further decrease of the adsorption capacity, (ii) the "fouling rate" was slightly dependent on the pore size (Table I), and (iii) the non-porous surface, which should be most exposed to hydrolytic or other degrading mechanisms, showed the smallest decrease in adsorption capacity; the "fouling rate" was highly dependent on the protein. With ADH, capacities dropped, in some cases, more than 50% between the first and the second cycle.

The dependence of the absolute load capacities (Table I) on the pore size is consistent with the findings of other authors [13,14]. The calculation of the load capacity per unit surface (Table 1) allows more insight into the surface accessibility of materials with different pore sizes. The results suggest that the surface of the 39-nm material and, in the case of ADH, even the 105-nm material can not adsorb protein at the same density as a non-porous or 280 nm porous anion exchanger. It needs to be mentioned that dynamically measured load capacities are not identical with the equilibrium adsorption capacities q_m obtained from batch experiments [14]. Adsorption kinetic experiments in suspension with the same PEI-bonded materials used in this study [15] showed that for the 105- and 280-nm porous materials adsorption of proteins (initial concentration *ca.* 1 mg/ml) is more than 90% complete after 1 min. For the 39- and 10-nm porous ion exchangers, 90% saturation required between 10 min and several hours, depending on the protein and the specific material. The differences between dynamic and equilibrium load capacity are, therefore, larger for the materials with smaller pores than for the macroporous and non-porous materials. However, they are not large enough to explain the drastic decline of load capacity per unit surface with increasing protein size and decreasing pore diameter. This appears to be more of a steric rather than a kinetic effect.

Adsorption isotherms

All isotherms measured by each method were of type 1 [16]. This means that they were singlestep, hyperbolic isotherms with a relatively well-defined saturation value.

Adsorption isotherms for BSA on PEI-bonded weak anion exchangers

These isotherms are illustrated in Fig. 1a. For better comparison, the data for

bound BSA (concentration on adsorbent), q^*, in Fig. 1a were reduced to relative saturation data, θ ($\theta = q^*/q_{max}$). In view of the non-linearity of the Scatchard and Hill plots the q_{max} values used were determined by extrapolation from a double reciprocal plot. The Scatchard and Hill plots (Fig. 1b and c) show clearly that the overall adsorption behavior does not fit a simple Langmuir isotherm.

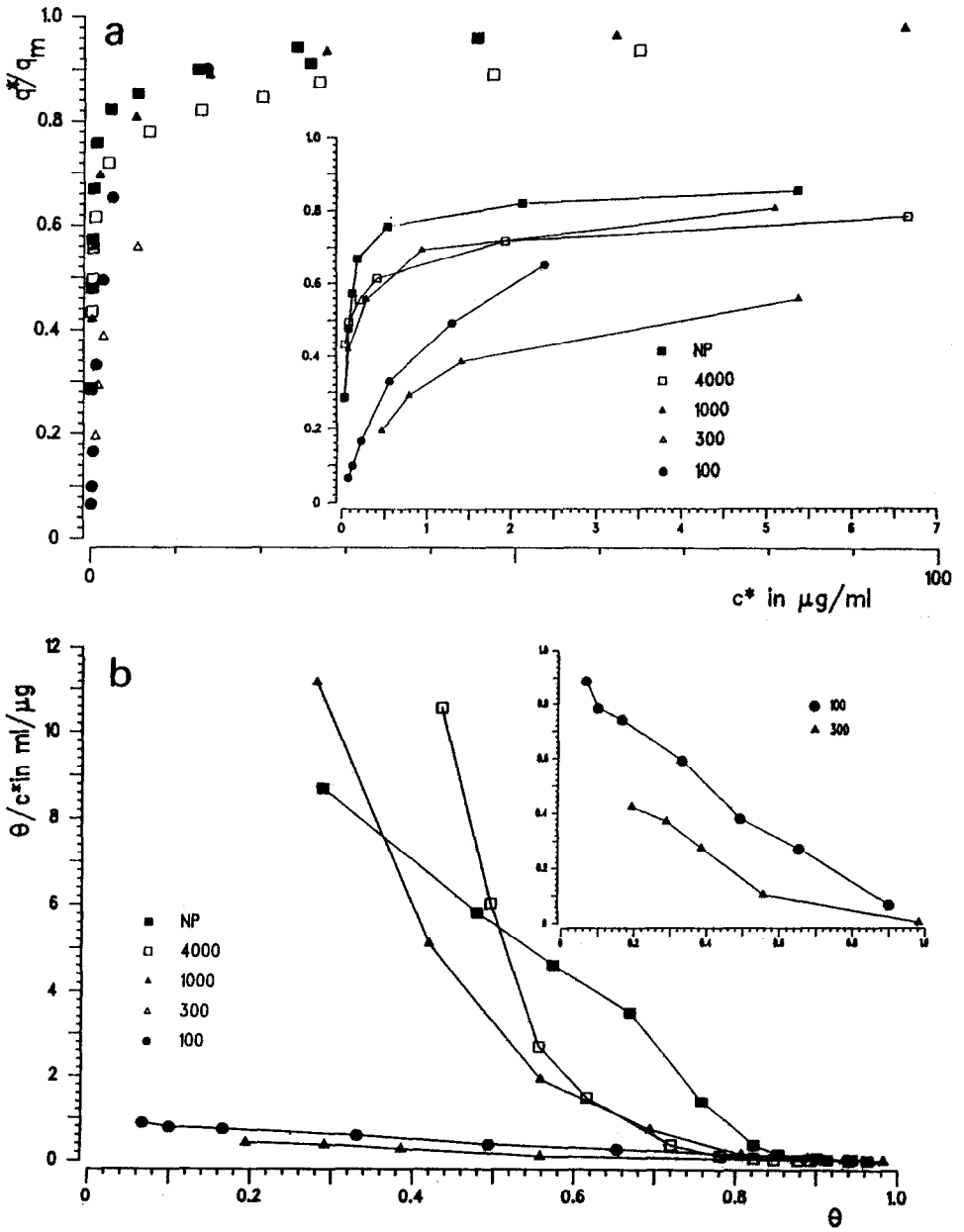

Fig. 1.

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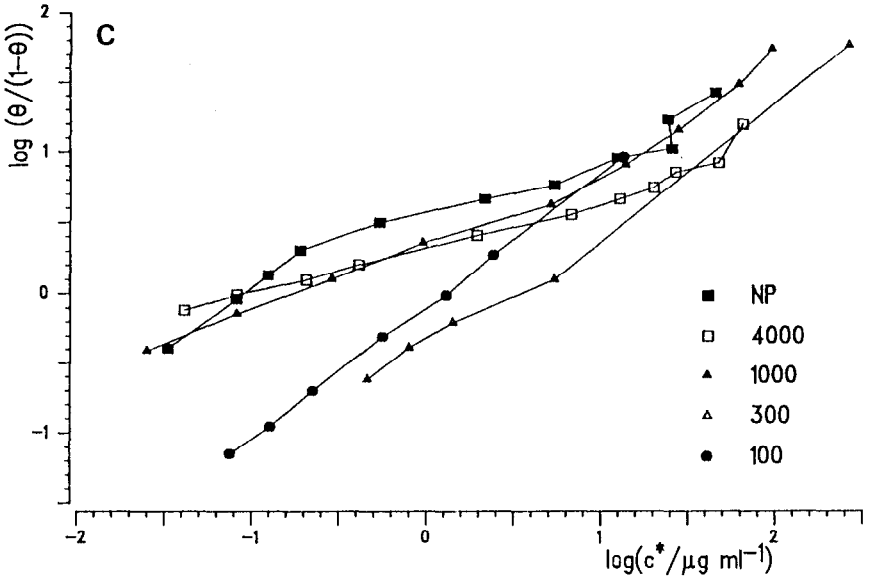

Fig. 1. Adsorption isotherms for BSA on PEI-bonded silicas of varying pore diameter (for conditions see Experimental). (a) Isotherm plot; (b) Scatchard plot; (c) Hill plot. Abbreviations: NP = non-porous silica, PEI coated; 4000 = LiChrospher Si 4000, PEI coated; 1000 = LiChrospher Si 1000, PEI coated; 300 = LiChrospher Si 300, PEI coated; 100 = LiChrospher Si 100, PEI coated. c* = Equilibrium concentration of BSA in solution.

The data for 39- and 10-nm silicas indicate a much lower binding constant compared to the macroporous and non-porous materials. However, in the case of LiChrospher Si 300, this lower association constant does not correspond with the chromatographic data (not shown), where this 39-nm material exhibited the strongest retention of all PEI-bonded ion exchangers. Because the adsorption time for every point in the isotherm was limited, it is likely that the data for LiChrospher Si 300 (and Si 100) do not represent a real equilibrium. This is further supported by kinetic data [15].

The non-porous material behaves ideally up to a saturation of *ca.* 80% of the total capacity, as can be seen from the low concentration part of the Scatchard plot. With LiChrospher Si 4000 and especially with Si 1000, this tendency is weaker, meaning that a Langmuir-like adsorption occurs only at lower degrees of saturation, if at all. Nevertheless, all materials exhibit a final load capacity that is higher than predictable through linear extrapolation of the data at low saturation, which indicates a change in adsorption mechanism. The Hill plots obtained from the data are S-shaped.

Data of Reynaud *et al.* [17] on the adsorption of BSA to graphite powder show an adsorption density of 2 mg/m^2. This was interpreted as a monolayer adsorption of the ellipsoidal BSA molecule in side-on position and further confirmed by voltammetric data. At higher BSA concentration an additional adsorption step of *ca.* 0.5 mg/m^2 occurred. The authors also found that in the adsorbed state the protein layer was only 2.4 nm thick, compared to a minimum of 38 nm as expected from

crystallographic data. This implies a strong conformational change or denaturation of BSA in the adsorbed state. On the chromatographic ion exchangers used in the present study the conformation is not expected to change so much, and the molecule should

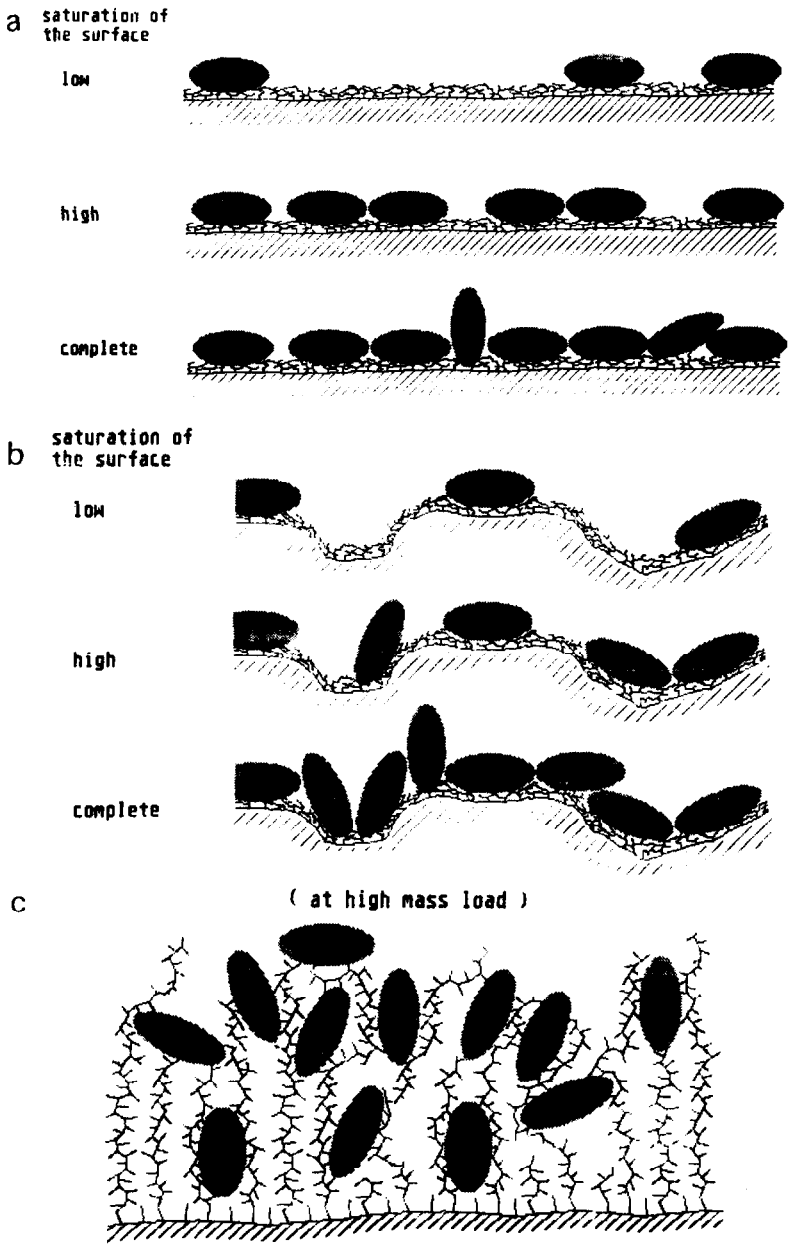

Fig. 2. Illustration of the adsorption of BSA molecules on the surface of different types of anion exchangers, (a) Non-porous PEI-bonded silica; (b) porous PEI-bonded silica; (c) silica or glass with "tentacle"-type modification.

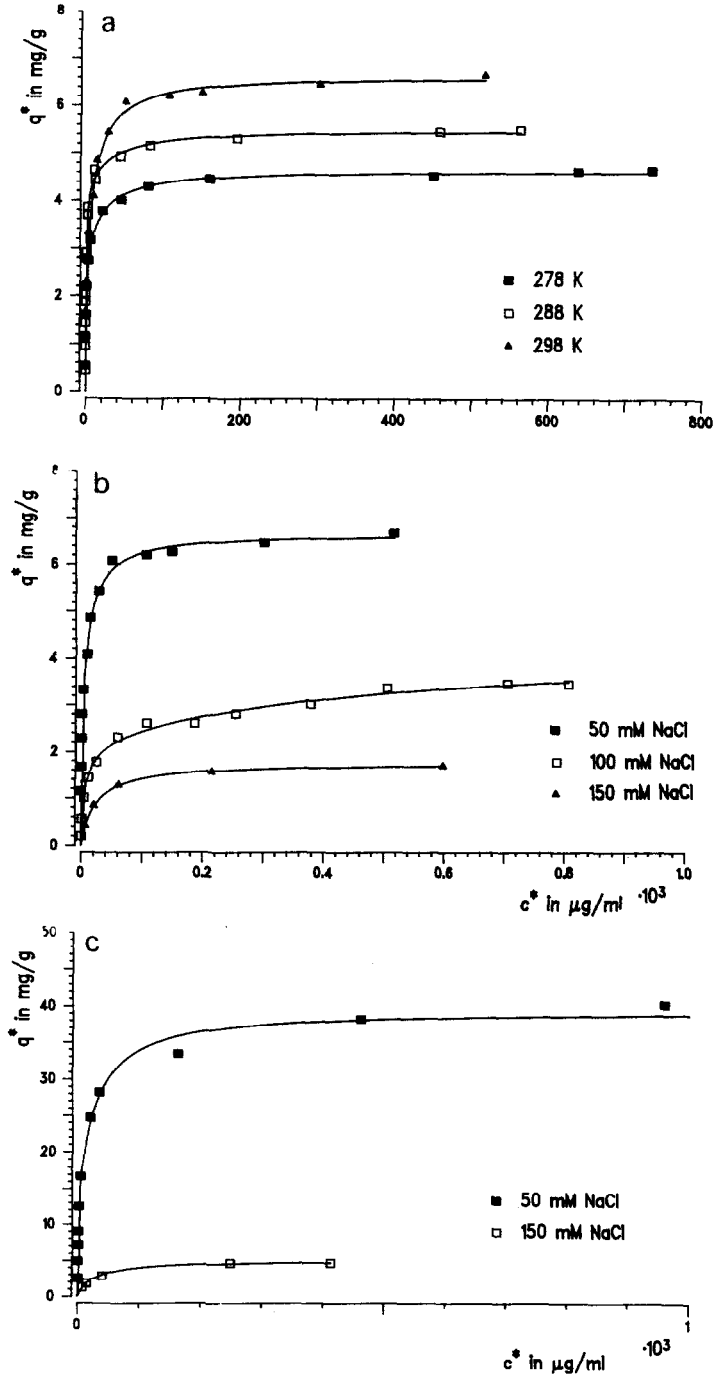

Fig. 3. Adsorption isotherms for BSA on TMAE "tentacle"-type anion-exchanger. (a) Non-porous support material, 50 m*M* NaCl, variation of temperature; (b) non-porous support material, 298 K, variation of NaCl concentration; (c) 105-nm porous support material, 298 K, variation of NaCl concentration. c* = Equilibrium concentration of BSA in solution.

occupy less space. The values obtained in the present study are 2.3 ± 0.1 mg/m^2 for the Langmuir-type adsorption limit and 2.8 ± 0.05 mg/m^2 for the total adsorption capacity (non-porous material). They may therefore very well reflect the capacity for side-on adsorption and the filling of remaining binding sites by molecules adsorbed in less favorable positions (Fig. 2a). The values are also consistent with data from other recent studies of methacrylate-based anion exchange packings [12].

Porous silicas are built up from primary particles in the diameter range of 2–10 nm [18], depending on the specific production process. Thus their pores can not have smooth walls and an ideally cylindrical shape. Hydrothermal processes used to convert mesoporous into macroporous materials are likely to smooth minor irregularities without producing a really flat surface. The non-porous silica wich is produced by a continuous process without or with extremely small ($\leqslant$1 nm) primary particles, shows no surface irregularities in a range observable by electron microscopy [19]. A thin, tightly bound modification applied to porous and non-porous silicas should retain these characteristics. The smoother, more homogeneous surface offers more adsorption sites with identical environment per unit surface (as illustrated in Fig. 2a and b). This can explain the greater extent of linearity in the Scatchard plots for the non-porous ion exchanger compared with the 280- and 105-nm porous materials.

Adsorption isotherms on tentacle-type ion exchangers

These results are given in Figs. 3a–c and 4a–c and Tables III–V. All isotherms on both tentacle-type materials fit the Hill isotherm quite well. According to Sips [20], this can be interpreted as a Gaussian-like distribution of binding sites, *e.g.* the Sips coefficient, r, approaches a value of 1.

The Hill coefficients at a sodium chloride concentration lower than 150 m*M* were substantially smaller than unity (see Fig. 3) which always corresponds to concave Scatchard plots (not shown). This type of behavior is often interpreted as a sign for negative cooperativity between the binding sites.

From the data shown in Figs. 3 and 4 an increase of adsorption capacity with temperature is obvious. Also the dissociation constant at lower saturation, as indicated by the distribution constant (K_d) (2-site Langmuir fit) is dependent on the temperature. In accordance with published chromatographic data on PEI-coated ion exchangers [21], the binding strength increases with temperature (Table III, Fig. 4a). The calculated Hill coefficients are all in the same range.

TABLE III

CHARACTERISTIC PARAMETERS OF BSA ADSORPTION ON NON-POROUS TMAE "TENTACLE"-TYPE ION EXCHANGER AT DIFFERENT TEMPERATURES (ALL AT 50 m*M* NaCl)

q_m extrapolated from double reciprocal plot; q_{m1}, q_{m2}, K_{d1} and K_{d2} values derived from two-center Langmuir isotherm (simplex fit).

Temperature (K)	q_m (mg/g)	Hill coefficient (n)	Hill constant (K)	q_{m1} (mg/g)	q_{m2} (mg/g)	K_{d1} (μg/ml)	K_{d2} (μg/ml)
278	4.9	0.53 ± 2%	0.61 ± 10%	2.8	2.0	0.4	20
288	5.8	0.52 ± 5%	0.89 ± 11%	4.2	1.4	0.3	28
298	7.1	0.56 ± 3%	0.49 ± 8%	2.4	4.3	0.2	12

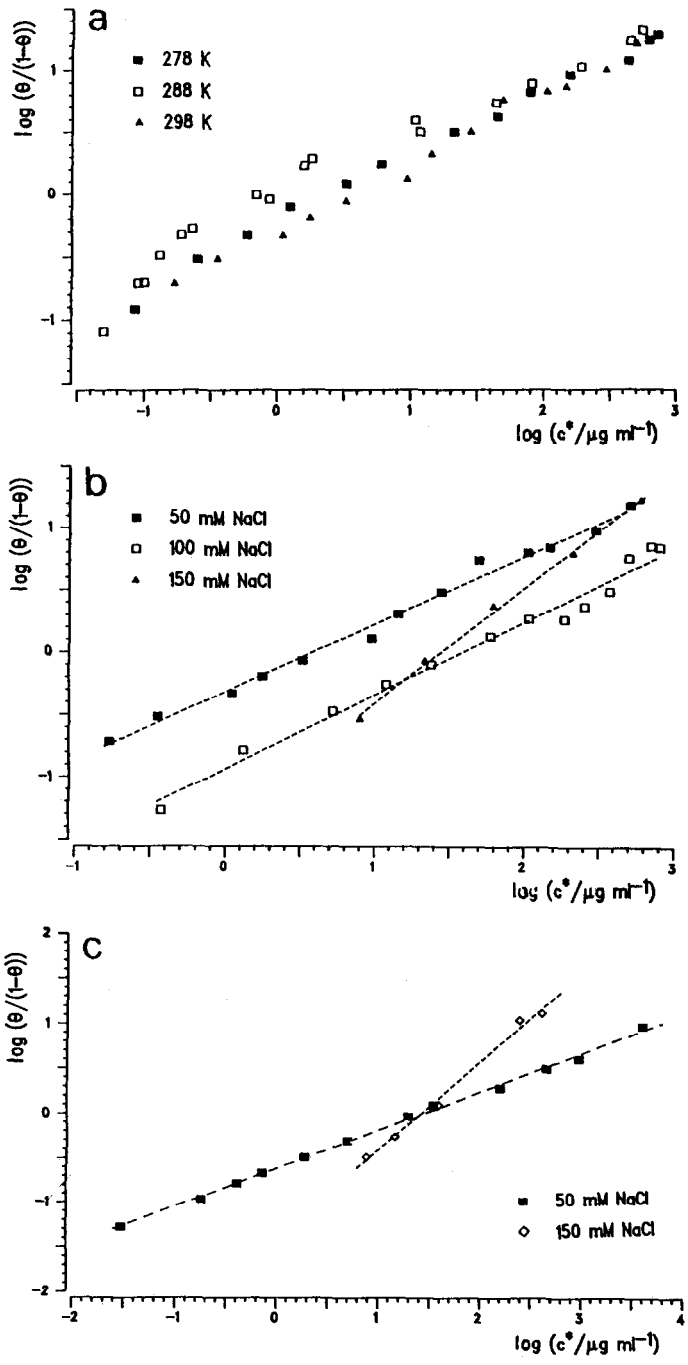

Fig. 4. Hill plots of the data in Fig. 3. (a) Non-porous support material, 50 m*M* NaCl, variation of temperature; (b) non-porous support material, 298 K, variation of NaCl concentration; (c) 105-nm porous support material, 298 K, variation of NaCl concentration.

TABLE IV

CHARACTERISTIC PARAMETERS OF BSA ADSORPTION ON NON-POROUS TMAE "TENTACLE"-TYPE ION EXCHANGER AT DIFFERENT NaCl CONCENTRATIONS (ALL AT 298 K)

For q_m, q_{m1}, q_{m2}, K_{d1} and K_{d2} see Table III.

Concentration (mol/l)	q_m (mg/g)	Hill coefficient (n)	Hill constant (K)	q_{m1} (mg/g)	q_{m2} (mg/g)	K_{d1} (μg/ml)	K_{d2} (μg/ml)
0.05	7.1	0.56 ± 3%	0.49 ± 8%	2.4	4.3	0.2	12
0.10	3.9	0.61 ± 4%	0.11 ± 5%	2.2	2.2	6.0	500
0.15	1.8	0.95 ± 3%	0.05 ± 12%	1.8		25	
0.4	0	–	–	–		–	

Increased salt concentrations lead to lower binding strength (higher K_d) and lower binding capacity. The first observation could be foreseen from the theory of ion exchange and the practice of ion exchange chromatography. However, the decrease in binding capacity is not to be expected in the case of Langmuir-type adsorption, but is a natural consequence, when the linear Hill plots are interpreted as a distribution of binding affinities. The sites with low affinity will only bind at low ionic strength. The decrease is more drastic with the porous material than with the non-porous beads. With increasing ionic strength and decreasing binding capacity, the slope of the Hill plots gets steeper. At 150 mM NaCl content, both materials exhibit a Hill coefficient close to one, indicating a Langmuir-type behavior. This adsorption behavior has been documented for biomimetic affinity chromatography of proteins [12]. These data indicate that at last two interaction modes occur at low salt concentrations.

The differences betweeen the PEI-bonded weak anion exchangers and the tentacle-type quaternary anion exchangers involve not only the form of the isotherm but also the adsorption capacity. With the latter materials, the apparent surface coverages exceed the expected monolayer density of side-on adsorbed molecules. On the 105-nm tentacle-type material, the capacity is 2 mg/m^2 at 50 mM NaCl in the buffer (nearly 3 mg/m^2 without NaCl, not shown) compared to 1.5 mg/m^2 (50 mM NaCl) and

TABLE V

CHARACTERISTIC PARAMETERS OF BSA ADSORPTION ON POROUS TMAE "TENTACLE"-TYPE ION EXCHANGER AT DIFFERENT NaCl CONCENTRATIONS (TEMPERATURE 298 K)

For q_m, q_{m1}, q_{m2}, K_{d1} and K_{d2} see Table III.

Concentration (mol/l)	q_m (mg/g)	Hill coefficient (n)	Hill constant (K)	q_{m1} (mg/g)	q_{m2} (mg/g)	K_{d1} (μg/ml)	K_{d2} (μg/ml)
0.05	50	0.43 ± 2%	0.25 ± 3%	23	22	1.3	163
0.15	5.0	1.0 ± 5%	0.04 ± 20%	5		26	
0.4	<0.1	–	–	–		–	

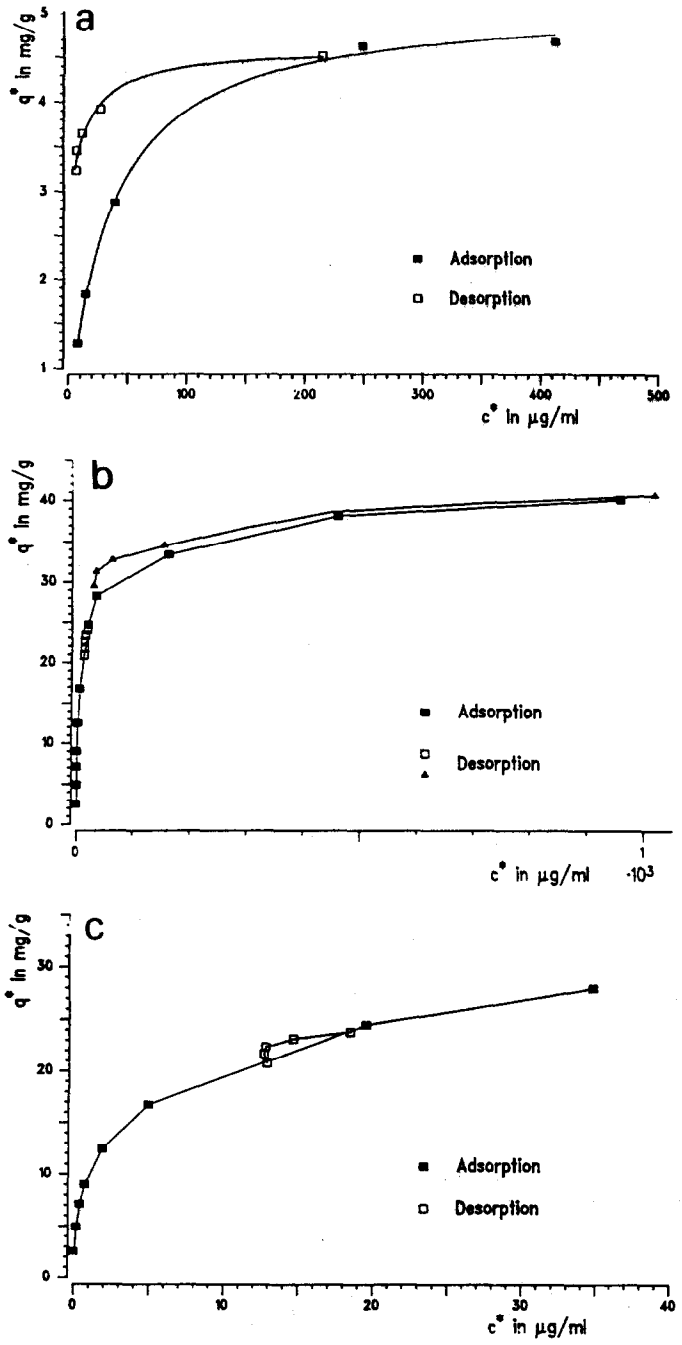
a
q* in mg/g
c* in µg/ml
Adsorption
Desorption
b
q* in mg/g
c* in µg/ml
·10³
Adsorption
Desorption
c
q* in mg/g
c* in µg/ml
Adsorption
Desorption

Fig. 5.

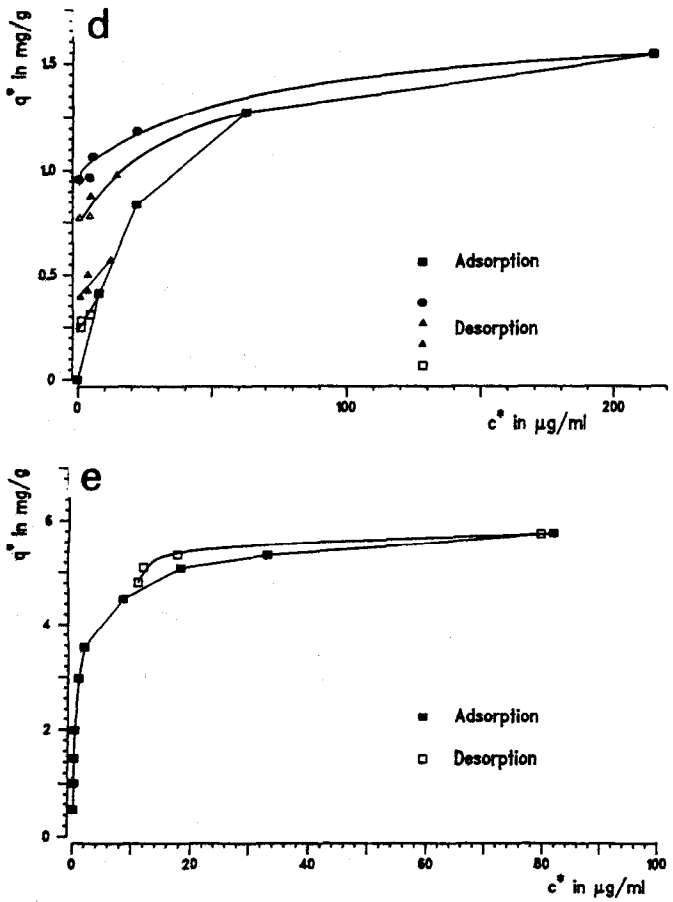

Fig. 5. Desorption of BSA from "tentacle"-type anion exchangers by dilution of the protein solution. Samples were taken 24 h after dilution (for other conditions see Experimental). (a) 105-nm porous material, 150 m*M* NaCl; (b) 105-nm porous material, full concentration range 50 m*M* NaCl; (c) 105-nm porous material, low concentration range 50 m*M* NaCl; (d) non-porous material, 150 m*M* NaCl; (e) non-porous material, 50 m*M* NaCl.

2.2 mg/m^2 (without NaCl) on the PEI-bonded material. The non-porous tentacle-type material exhibited an extremely high load capacity. Based on the estimate of 0.3 m^2/g specific surface area, the measured value of 7.1 mg/g gives a specific load capacity of 23.7 mg/m^2. This value exceeds the monolayer density in any orientation of the BSA molecules by far. Hence the protein must be adsorbed in multilayers or "dissolved" in the polymer layer (as illustrated in Fig. 2c).

The reason why all these isotherms on the tentacle-type adsorbents fit the Hill equation is not clear. An actual Gaussian-like distribution of discrete binding sites is not probable in this highly flexible polymer layer. Due to the dynamic character of the stationary phase it appears much more likely that the actual sites of binding form during the interaction with the protein, and their shape is largely determined by the properties of the protein. Negative binding cooperativity is a better explanation for the

isotherm shape. Thus, a BSA molecule adsorbed into the layer decreases the affinity for the uptake of more molecules in its immediate surrounding, *e.g.* by occupying the charged groups or changing the conformational structure of the "tentacle" layer. At higher salt content and therefore, weaker interaction, the adsorbed BSA molecules would be more isolated, not influence each other very much, but only permit a relatively low density of adsorption.

Desorption measurements

Desorption "isotherms" were done with the 105-nm porous and the non-porous tentacle-type ion exchanger, at two different ionic strengths and starting from different points in the adsorption isotherm. In all cases, significant adsorption hysteresis was observed (Fig. 5a–e) characteristic of extremely slow desorption phenomena. If the isotherms represent a real equilibrium state, this is impossible [10]. The reason for the behaviour must therefore be sought in kinetics.

Considering the fast uptake rates (adsorption kinetics), with an equilibrium constant of 26.3 mg/ml (porous material, 150 m*M* NaCl), the desorption rate equals the adsorption rate at a solution concentration of 26.3 mg/ml. Control experiments showed that the protein adsorption from 40 μg/ml initial concentration was very probably diffusion controlled and equilibrium was virtually established within 2 min. The actual surface interaction step is probably even faster. The desorption from the surface should, according to these facts, only take minutes till equilibrium is reached. The extremely slow desorption leading to the hysteresis must therefore be due to secondary equilibria, as other authors have proposed [22].

CONCLUSIONS

The experimental data obtained in this study strongly suggest that the manner of protein adsorption to anion exchangers can be different with different kinds of surface properties and chemical modifications of the support material. At moderate and high values of relative saturation, low surface area materials with thin layers of modification exhibit less negative cooperativity than those with either higher surface area or thick layers of modification. At high relative saturation the amount of protein adsorbed can range from less-than-monolayer (thin modification layer, rough surface) over monolayer density (thin modification layer, smooth surface) to far more than monolayer density (thick tentacle-type modification layer). Both the thin layer and the tentacle-type of anion exchangers seem to be principally useful for the preparative separation of proteins. Hill plots and especially the Hill coefficient *n* obtained from the measurement of adsorption isotherms can in either case be useful. They allow to estimate how much of the total load capacity is actually available for interaction in both isocratic and gradient separations. Similar criteria have been documented for immunoaffinity chromatography of proteins [23].

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