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Comparison between mass transfer properties of weak-anion-exchange resins with graft-functionalized polymer layers and traditional ungrafted resins

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Abstract

Glycidylmethacrylate was grafted to Toyopearl HW-65M and subsequently modified with diethylamine to obtain a weak anion exchanger. The degree of grafting was varied from 11 to 50%. The binding capacity for bovine serum albumin was 11 mg/ml for the lowest degree of grafting and 97 mg/ml for the highest degree of grafting. The maximum binding capacity was observed at 27% degree of grafting. The mass transfer properties of the grafted resins and an ungrafted resin (Toyopearl DEAE 650M) were investigated assuming rectangular isotherms. Simple models for reaction kinetics, pore- and surface diffusion and film diffusion were used to describe the concentration–time data in batch mode. The data were best fitted by a pore diffusion model. The estimated pore diffusion coefficients (D_p) for bovine serum albumin were fitted by a polynome to the degree of grafting with an maximum value at 27% of $D_p = 1.95 \cdot 10^{-11}$ m^2/s. Compared to published data of other ungrafted resins and to the molecular diffusion coefficient of bovine serum albumin in free solution of $D_p = 5.6 \cdot 10^{-11}$ m^2/s, the diffusion in grafted layers seems to be accelerated. The breakthrough curves for columns packed with various resins showed a decrease in sharpness with increasing degree of grafting which could not be described by a simple pore diffusion model using the calculated transport coefficients from batch mode. The shape of the breakthrough curves could be well described by a combined film and pore diffusion model. For the ungrafted Toyopearl DEAE 650M resin the breakthrough curve is more favorable and the influence of film diffusion to the mass transfer is reduced. It can be concluded that grafting will increase the capacity and the pore diffusion in batch mode but in column operation the grafting layer has a film resistance which plays an important role in the overall mass transfer.

Keywords: Mass transfer; Anion exchangers; Stationary phases, LC; Grafting; Breakthrough curves; Glycidylmethacrylate

1. Introduction

Many adsorption resins are currently in use for the purification of biomolecules. They differ with respect to the base resin as well as to the surface chemistry. Base materials can be divided into synthetic polymers (such as polystyrene-divinylbenzene, methacrylates, acrylamides) and natural polymers (among them dextran and cellulose) [1–4]. The functionality of a resin is conferred to the surface chemistry with ionic, hydrophobic and affinity ligands. The performance of a resin is influenced by the ligand density and spatial accessibility [5]. The methods for ligand immobilization can be classified into three areas: derivatization with low-molecular-mass lig-

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ands [6], derivatization with high-molecular-mass ligands (polymers) [7] and graft- and block polymerizations [8,9].

All modification methods are in use and can be applied to different resins. None of these is necessarily the most effective approach. It is often observed when solving a particular separation problem that resins with different immobilization chemistries and various protein binding capacities for a standard protein exhibit comparable results.

The performance of a resin is often best characterized by using the dynamic binding capacity. The dynamic binding capacity is the achievable binding capacity under operation conditions [10]. Albumin and lysozyme are often used for the determination of the dynamic binding capacities of ion exchangers.

Macroporous resins, in which mean pore sizes are greater than 50 nm, are used for the purification of proteins. The available surface areas are low, in the range of 10–50 m^2/g. Large pores are essential to overcome restriction of diffusion towards high-molecular-mass proteins but this results in lower ligand density and reduced total binding capacity [11]. With such macroporous resins, surface modification using graft-functionalized polymers is a major advantage as it improves the protein binding capacity independently of the specific surface area.

It is shown that resins with polymer grafted layers offer improved higher mass transfer kinetics due to a parallel mechanism of surface and pore diffusion coupled with higher ligand density [12,20]. This assumption is however not well defined with respect to the influence of the surface chemistry

The influence of the surface chemistry (using a grafting reaction) on maximum binding capacity and on mass transfer properties of biochromatography resins is investigated using a model system in this work.

2,3-Epoxypropylmethacrylate was grafted to Toyopearl HW-65M by a Ce^{4+}-initiator system. The grafted epoxypolymer was converted to a weak anion exchanger by subsequent reaction with diethylamine. Accessibility of the internal pore space was checked by gel filtration with different proteins and mass transfer coefficients of the resins were calculated from simple model equations assuming irreversible binding isotherms.

2. Material and methods

2.1. Chemicals

2,3-Epoxypropylmethacrylate (glycidylmethacrylate), diethylamine, ammoniumcer(IV) nitrate, bovine serum albumin, lysozyme from chicken egg white and blue dextran were all purchased from Merck. Toyopearl HW-65M, a hydrophilic, hydroxy group containing methacrylate polymer, was obtained from Tosoh. The work described herein with grafted resins is with research samples which are not commercially available. The ungrafted anion-exchange resin Toyopearl DEAE 650M was purchased from Tosoh Bioscience. The mean particle size of both resins is in the range of 40–90 μm and the mean pore size is ~90 nm. The density of unmodified Toyopearl HW-65M is 1.09 g/cm^3 and the mean total porosity is ~80%.

2.2. Concentration–time curves

The concentration–time curves for protein adsorption were determined as follows: ~1 g of dried resin was suspended in a solution of 20 m*M* Tris buffer, pH 8 with 0.1, 0.25, 0.5, 1 and 2 mg/ml of bovine serum albumin. The solutions were shaken for a fixed time and the concentration in the supernatant was then determined by UV at 280 nm.

2.3. Breakthrough curves

The breakthrough curves of the ion-exchange resins were determined with a Merck–Hitachi HPLC system using Superformance® glass columns of 50 mm×10 mm I.D. The column volume for all experiments was 3 ml. Columns were flow packed with a flow rate 20% higher than the maximum flow rate used in experiments. Following packing, none of the columns showed any change in packed bed height. The following buffers were used: (i) equilibration: 20 m*M* Tris buffer, pH 8; (ii) loading: 2 mg/ml bovine serum albumin in 20 m*M* Tris buffer, pH 8. The breakthrough value was calculated

at 10% of the load concentration. The maximum binding capacity was determined by elution of bound protein with a 1 *M* sodium chloride solution in 20 m*M* Tris buffer, pH 7. The protein content was measured by UV absorption at 280 nm.

2.4. Isotherms

The binding isotherms were evaluated by shaking the resins (~0.5 g) with bovine serum albumin solutions of each concentration in 20 m*M* Tris buffer, pH 8. The equilibrium values were determined after 3 h. Bound protein was eluted and determined by UV adsorption at 280 nm.

2.5. Determination of the accessible pore volume by gel filtration

The accessible pore volumes of grafted resins compared to the unmodified Toyopearl HW-65M and ungrafted Toyopearl DEAE 650M were determined using gel filtration. Experiments were carried out in a Superformance glass column of 300 mm×10 mm I.D. Linear velocity was 1 cm/min. Samples for determining the accessible pore volume were blue dextran, bovine serum albumin, lysozyme and acetone. The difference between elution volumes of acetone and dextran, which corresponds to the total pore volume, was used to calculate the accessible pore volume for the resins and was found to be 10 ml which was 50% of the total resin volume in the column. This experiments were carried out in 20 m*M* Tris buffer, pH 7 and 0.25 *M* sodium chloride.

The fraction of the stationary gel volume K_{av} (%) which was available for diffusion of a given solute species was calculated according to [1]:

$$K_{av} = 100 \cdot \frac{V_e - V_0}{V_t - V_0} \quad (1)$$

where V_e is the elution volume of bovine serum albumin or lysozyme, V_0 is the elution volume of blue dextran, and V_t is the elution volume of acetone. A value of 100 corresponds to free accessibility, whilst a value 0 is total exclusion from the inner pore space.

2.6. Investigation of the total pore volume of the weak anion exchanger by the repulsion of the similarly charged lysozyme

Lysozyme at pH 7 is a negatively charged molecule which should be excluded from anion-exchanger resin under salt-free conditions. Ion exclusion is a long established technique for the separation of small ions [13]. A similar effect can be expected for biomolecules in highly charged macroporous resins but this has so far not been described in the literature. This phenomenon was used to investigate the size of the charge repulsion for the different modified resins. The elution buffer in this case was a 20 m*M* sodium dihydrogenphosphate buffer solution at pH 7. A 200-μl aliquot of 1-mg/ml sample solution was loaded onto the column.

2.7. Graft polymerization procedure

The graft polymerization onto Toyopearl HW-65M was carried out by using Ce^{4+} salt in strong acid solution with glycidylmethacrylate as monomer using the procedure of Müller [14]. The graft polymerization is initiated by oxidation of –OH groups, creating a carbon radical at the backbone of the base resin [15]. The resulting polyglycidylmethacrylate is covalently bound to the surface of the base polymer. Different degrees of grafting were achieved by varying the monomer concentration. The degree of grafting (DG) was calculated according to the following relationship [16]:

$$DG = 100 \cdot \frac{(M_p - M_S)}{M_S} \quad (2)$$

The dependence of degree of grafting upon the monomer concentration is shown in Fig. 1.

The degree of grafting showed a close linear relationship to monomer concentration. Degrees of grafting above 50% were easy to achieve but the permeability of such resins was too low when used in a flow through mode so the upper limit was set to

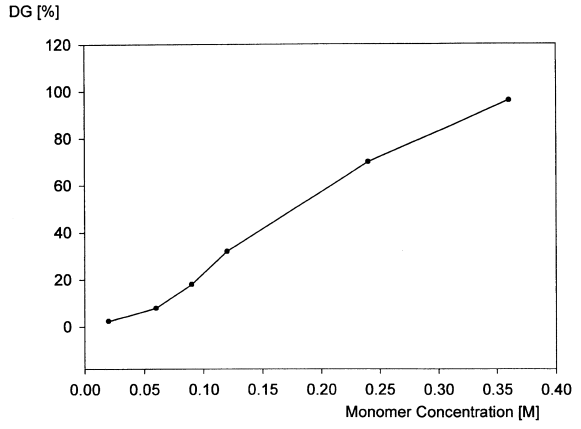

Fig. 1. Degree of grafting of 2,3-epoxypropylmethacrylate on Toyopearl HW-65M as function of the monomer concentration.

50%. Degrees of grafting from 11, 19, 27 and 50% were used for evaluation.

The resulting activated resins were modified by further reaction with diethylamine to prepare a weak anion exchanger [14]. As the reaction of the epoxy groups with diethylamine was not quantitative the residual groups were hydrolyzed in 1 *M* sulfuric acid. The epoxy group density was determined by non-aqueous titration according to the procedure of Pribl [17]. After hydrolysis, no remaining epoxy groups could be measured.

The ligand density of the weak anion exchangers was determined by titration with hydrochloric acid but not found proportional to the degree of grafting. The ligand density for the different grafted resins was in the range from 78 to 117 μmol/ml anionic groups. This does not correspond to the mass weight increase by surface grafting. A degree of 11% equates to a theoretical ligand density of 200 μmol/ml oxirane groups, whereas for a 50% degree of grafting this value is 1 mmol/ml. Of the oxirane groups, 39% of the resin with the low degree of grafting and 12% of the resin with the highest (50%) degree of grafting were converted to basic groups. With increasing amounts of graft polymer the relative ligand density decreased, despite reaction conditions being constant. This may either be the result of the increased total hydrophobicity of the polymer modified resin or an increased level of crosslinking. This was not further investigated.

3. Results and analysis

3.1. Isotherms

The isotherms of resins with different degrees of grafting are shown in Fig. 2.

Within the concentration range 0.1–2 mg/ml the shape of all curves was almost rectangular. This was also the case for the ungrafted Toyopearl DEAE 650M resin.

In general the protein binding capacity increases with the degree of grafting. The highest binding capacity was obtained at 27% degree of grafting with a value of 97 mg/ml. Maximum binding capacity is not linearly dependent on the degree of grafting. The binding capacity shows a very sharp increase at 19% degree of grafting, displays a maximum at 27%, but is not further changed by increasing the degree of grafting to 50%. Protein binding capacities and the corresponding ligand densities of the basic diethylamino groups are shown in Fig. 3.

There is no apparent simple relationship between ligand density and protein binding capacity. Ligand densities change by only 25% between 11 and 27% degree of grafting whereas protein binding capacity increases more than 8-fold from 11 to 97 mg/ml. The ligand density of the ungrafted Toyopearl DEAE 650M is within the range of 80–120 μmol/ml, which is similar to the ligand densities of grafted resins; however maximum binding capacity is three times lower. The increased total value of protein binding capacity for the polymeric modifications can be explained by the following assumptions.

(i) Graft functionalization in general improves the accessibility of the ion ligands to the solute.

(ii) At a ‘‘critical accessible ion ligand density’’ corresponding to a ‘‘critical degree of grafting’’ value a sudden increase in protein binding capacity can be obtained. This observation is similar to the results described from Jennissen with hydrophobic interaction chromatography. This phenomenon was called ‘‘critical hydrophobicity’’ [27]. The ‘‘critical hydrophobicity’’ is the minimum amount of immobilized hydrophobic ligands at which adsorption of protein takes place. The amount of adsorbed protein is a sigmoidal function of the surface concentration of immobilized ligands. The curve shape and the ‘‘critical hydrophobicity’’ can be explained

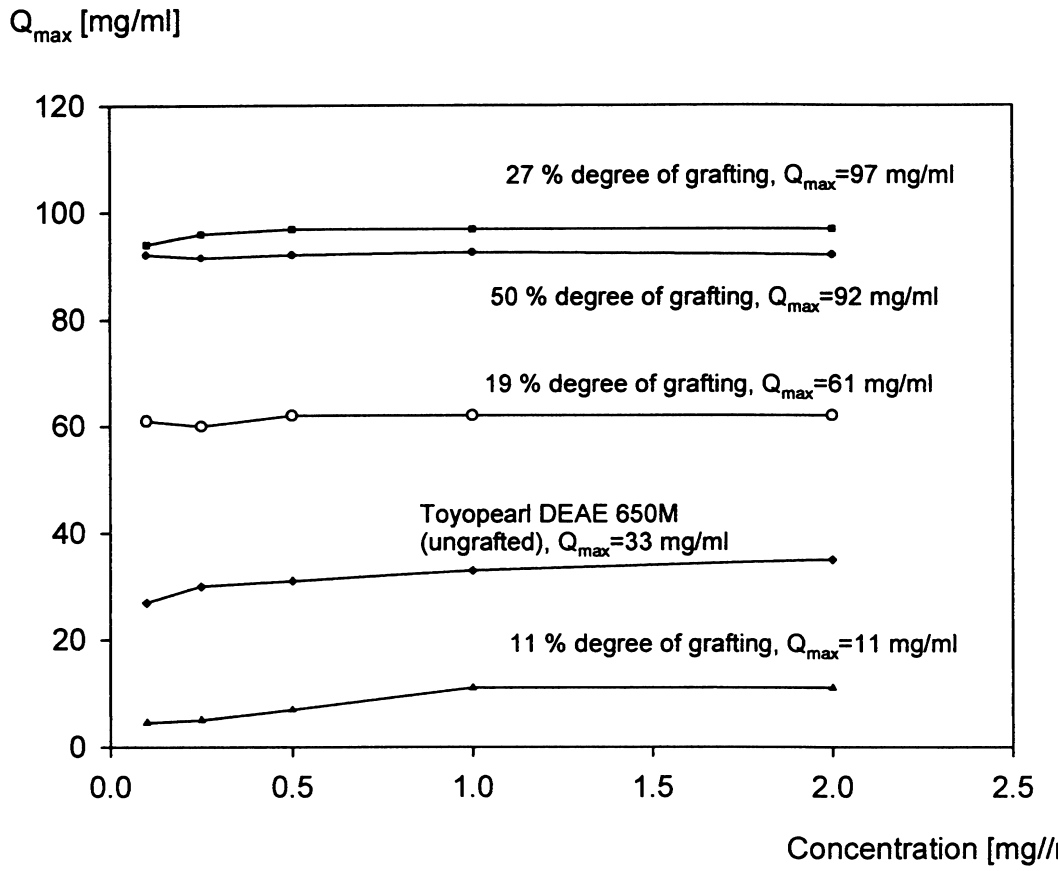

Fig. 2. Equilibrium uptake of bovine serum albumin in 20 m*M* Tris buffer, pH 8 for graft-functionalized resins and ungrafted Toyopearl DEAE 650M.

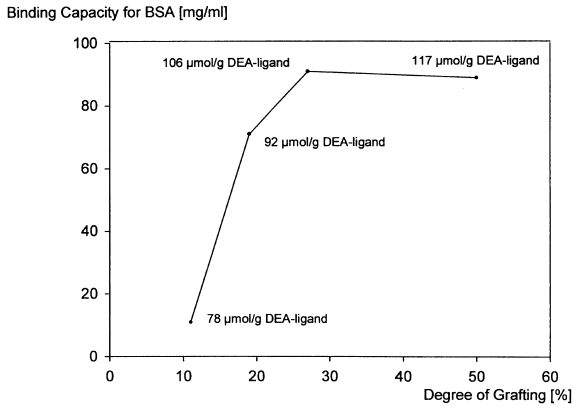

Fig. 3. Protein capacity as a function of the degree of grafting and the density of basic ligand.

by the concept of multivalence and cooperativity of protein adsorption. In contrast to ''critical hydrophobicity'' where the multivalence interaction can be obtained by straightforward surface modifications, a graft-polymerization is necessary to achieve multivalent ion-exchange interactions.

A possible explanation for the above observations is derived from gel filtration experiments.

3.2. Results of gel filtration

The pore volume of unmodified Toyopearl HW-65M and the Toyopearl DEA 650M grafted resin with 50% degree of grafting was determined and shows the influence of the surface grafting upon pore volume. The resin with the highest surface coverage

should display the greatest reduction in pore volume. Pore volume and accessible space were determined by gel filtration with dextran blue, bovine serum albumin (BSA) and acetone: the K_{av} values for Toyopearl HW-65M and DEAE 650M (grafted) were 0.525 and 0.486, respectively. Even a high degree of surface grafting has only a minor influence upon the total pore volume or on adsorbate accessibility as the elution volume of BSA is unchanged by grafting. The structure of the grafted resin appears to retain full accessibility for BSA.

A similar experiment was carried out with lysozyme with no added sodium chloride and the starting buffer alone (20 m*M* sodium dihydrogenphosphate buffer, pH 7). The accessible pore volume for lysozyme is shown in Fig. 4. The results are different to those for BSA. Only the pore space of the ungrafted resin (Toyopearl DEAE 650M) and the resin with 11% degree of grafting remained accessible for lysozyme. Degrees of grafting above 11% displayed restricted pore access reducing to zero at 50%. The explanation for this behavior is the repulsion of lysozyme from a pore filling polymer at higher degrees of grafting which restricts access to the inner pore space. The structure of the polymer is illustrated in Fig. 5.

It is possible that structures of Hyper D resins (from Biosepra) may be compared [18]. There is however a difference in the preparation procedure. Hyper D resins are prepared by filling ionic hydrogel into the pores of an existing resin, but for a resin with grafted polymers the entire structure of the hydrogel is constructed during preparation. There is one major difference in structure, which may have an important influence. The grafted polymer layer of the resins described here exists within the pores and also around the outer surface of the particles, whereas Hyper D particles contain the hydrogel within the pores alone. It is not possible to obtain evidence of a pore filling hydrogel by direct observation (scanning electron microscopy under dry conditions) as the water-rich structure of the hydrogel is not present.

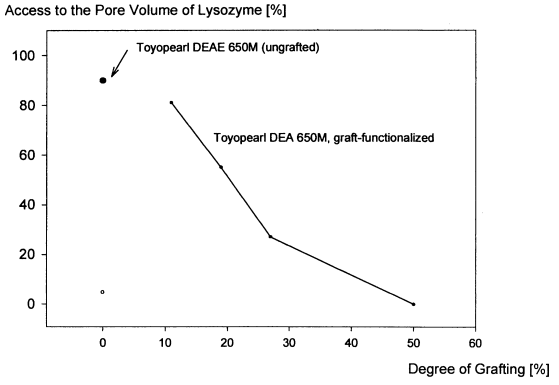

Fig. 4. Ion exclusion of lysozyme in 20 m*M* NaH_2PO_4, pH 7 from inner pore volume of grafted and ungrafted resins.

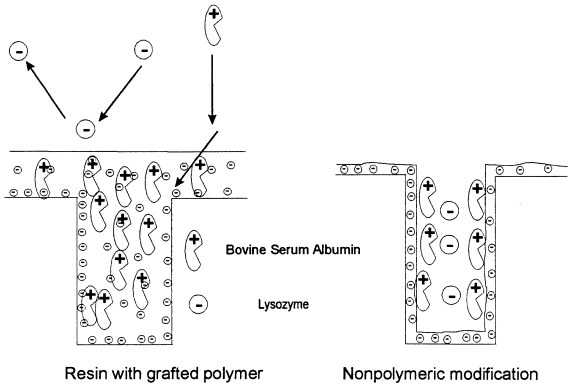

Fig. 5. Schematic drawings of the ionic hydrogel structure at the surface of a grafted resin compared to the ungrafted Toyopearl DEAE 650M (the ionic hydrogel is accessible for BSA whereas lysozyme is excluded at low salt concentration compared with ungrafted resin where both proteins can enter the inner pore space).

The pore filling hydrogel is in some way responsible for the increase in capacity at a similar ligand density. The accessibility to the ligands and the strength of the electrical field in the pores are both increased by formation of the hydrogel. So it is not clear which is the main reason for the resultant higher capacity of such resins.

The effect of the grafted polymer layer at the outer surface and in the pores was further evaluated by the influence on mass transfer properties.

3.3. Mass transfer properties

3.3.1. Batch experiments

The following issues have been described in the literature in relation to mass transfer of proteins in

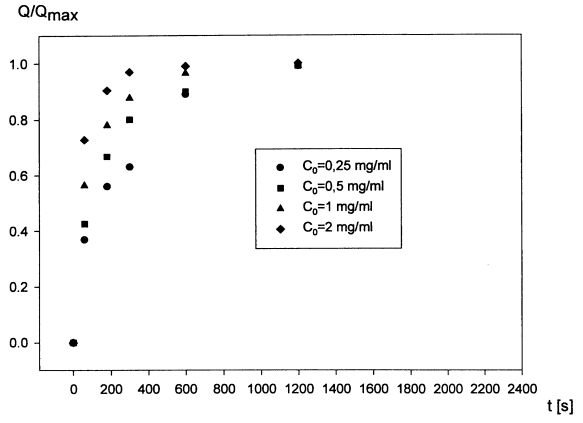

Fig. 6. Batch uptake kinetics for bovine serum albumin on ungrafted Toyopearl DEAE 650M in 20 m*M* Tris buffer, pH 8 at different initial concentrations.

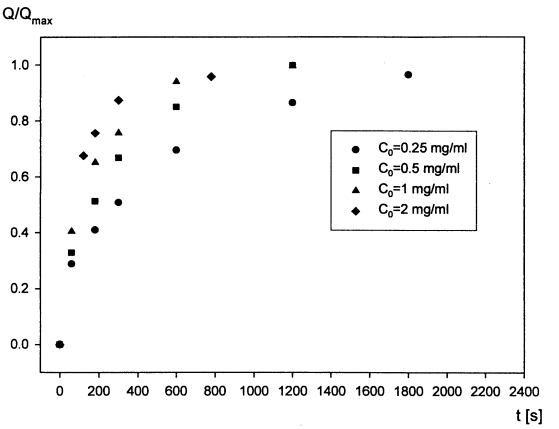

Fig. 8. Batch uptake kinetics for bovine serum albumin on graft-functionalized Toyopearl DEA 650M (19% degree of grafting) in 20 m*M* Tris buffer, pH 8 at different initial concentrations.

bioadsorbents [19,20]: (i) pore diffusion, (ii) surface diffusion, (iii) film diffusion, and (iv) reaction kinetics.

The reaction kinetics of ion exchange are so fast that they play no significant role [19], and will not be considered here. The concentration–time curves for the five different sorbents are shown in Figs. 6–10. Uptake is fastest for the ungrafted Toyopearl DEAE 650M and the resin with a degree of grafting from 11% at all concentrations, but these resins are low in total binding capacity. The uptake seems to be slower for resins with higher degrees of grafting (especially at low protein concentrations) but despite this, the total capacity is much higher. To make a practical comparison the mass transfer coefficients must be be calculated.

For the calculation of the mass transfer coefficients from the concentration–time data, models were used based on the assumption of rectangular isotherms. In the literature, models are often used

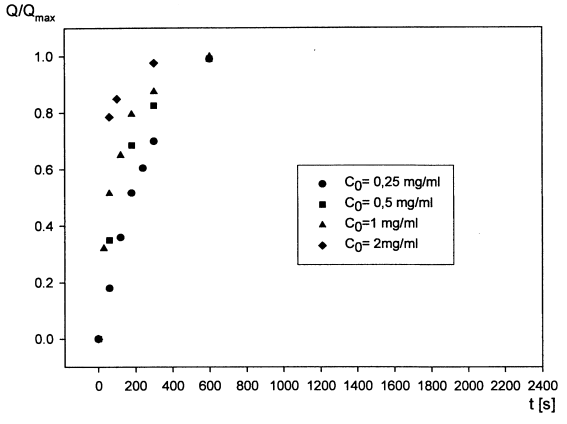

Fig. 7. Batch uptake kinetics for bovine serum albumin on graft-functionalized Toyopearl DEA 650M (11% degree of grafting) in 20 m*M* Tris buffer, pH 8 at different initial concentrations.

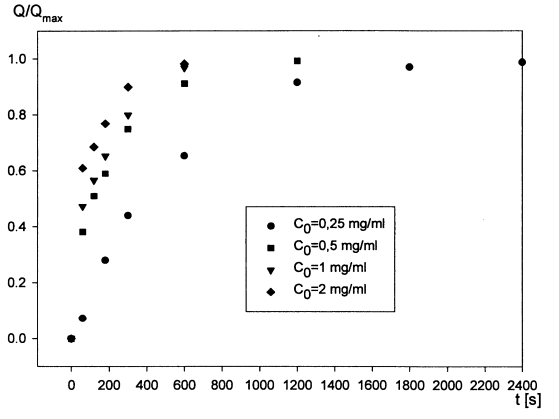

Fig. 9. Batch uptake kinetics for bovine serum albumin on graft-functionalized Toyopearl DEA 650M (27% degree of grafting) in 20 m*M* Tris buffer, pH 8 at different initial concentrations.

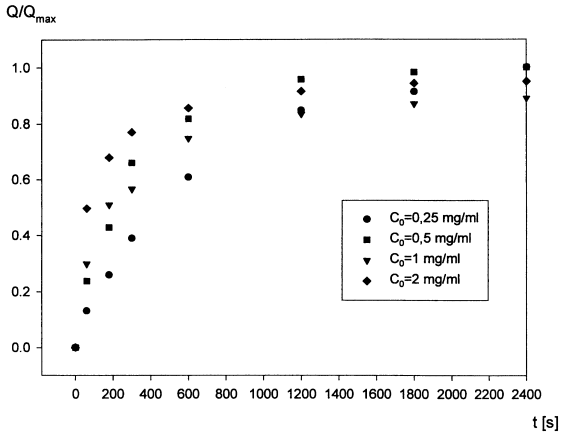

Fig. 10. Batch uptake kinetics for bovine serum albumin on graft-functionalized Toyopearl DEA 650M (50% degree of grafting) in 20 m*M* Tris buffer, pH 8 at different initial concentrations.

which attempt to relate the combined influence of all three resistance factors. In such an approach, very accurate data are needed and the interpretation of the obtained value is not easy. We tried a different approach. Single parameter equations were used and the model with the best fit of the experimental values was considered as the rate determining mechanism. The following models have been used.

(i) Pore diffusion [19,21]

$$t = \left(\frac{R_P^2 Q_{max}}{D_P C_0} - \frac{Q_{max} R_P}{C_0 k_f}\right) \cdot \frac{1}{3M} \cdot \ln\left(\frac{\lambda^3 + \eta^3}{\lambda^3 + 1}\right) - \frac{R_P^2 Q_{max}}{D_P C_0} \cdot \left\{\frac{1}{6\lambda M} \cdot \ln\left[\left(\frac{\lambda^3 + \eta^3}{\lambda^3 + 1}\right) \cdot \left(\frac{\lambda + 1}{\lambda + \eta}\right)^3\right] + \frac{1}{\lambda M\sqrt{3}} \cdot \left[\arctan\left(\frac{2\eta - \lambda}{\lambda\sqrt{3}}\right) - \arctan\left(\frac{2 - \lambda}{\lambda\sqrt{3}}\right)\right]\right\} \quad (3)$$

with

$$\eta = \left(1 - \frac{\bar{Q}}{Q_{max}}\right)^{\frac{1}{3}} \quad (4)$$

$$M = (1 - \varepsilon_{ext}) \cdot \frac{Q_{max}}{C_0} \quad (5)$$

and

$$\lambda = \left(\frac{1}{M} - 1\right)^{\frac{1}{3}} \quad (6)$$

(ii) Surface diffusion [22]

$$\frac{\bar{Q}}{Q_{max}} = 1 - \frac{6}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{\exp\left(-\frac{D_0 p_n^2 t}{R_P^2}\right)}{9\Lambda/\left[(1 - \Lambda) + (1 - \Lambda) p_n^2\right]} \quad (7)$$

where the p_n values are the roots of the following equation:

$$\tan p_n = \frac{3p_n}{3 + \left(\frac{1}{\Lambda} - 1\right) p_n} \quad (8)$$

and the Λ is:

$$\Lambda = C_0 - \frac{(1 - \varepsilon_{ext})}{\varepsilon_{ext}} \cdot Q_{max} \quad (9)$$

(iii) Film resistance [23]

$$\frac{\bar{Q}}{Q_{max}} = \frac{C_0 \varepsilon}{(1 - \varepsilon_{ext}) Q_{max}} \cdot \left\{1 - \exp\left[-\frac{3k_f}{R_P} \cdot \frac{(1 - \varepsilon_{ext})}{\varepsilon_{ext}} \cdot t\right]\right\} \quad (10)$$

All equations are valid for batch adsorption experiments in which the fluid phase concentration varies with time.

Eqs. (3)–(6) for pore diffusion form in principle a two-parameter equation which describes the combined pore diffusion and film resistance. For the adjustment of the pore diffusion coefficient the parameter for k_f was held constant at a value of $k_f = 0.0025$ cm/s [20].

Non-linear regression was performed using the program package ''Sigmaplot 2000'' from SPSS Science Software (Erkrath, Germany). The resulting mass transfer coefficients are summarized in Table 1.

The RSDs of the fitted transport coefficients were 8% for pore diffusion, 15% for surface diffusion and 56% for film diffusion. Pore diffusion was considered to be dominant mass transfer resistance within the resins. However surface diffusion may also play a role. Film diffusion shows less importance as a rate

Table 1
Mass transfer coefficients in graft-functionalized resins (calculated by non-linear regression)

Resin surface chemistry	Pore diffusion coefficient D_P (m^2/s) calculated with Eqs. (3)–(6)	Surface diffusion coefficient D_S (m^2/s) calculated with Eqs. (7)–(9)	Film resistance coefficient k_f (cm/s) calculated with Eq. (10)
Toyopearl DEAE 650M (ungrafted)	$6.7\cdot10^{-12}$	$3.1\cdot10^{-13}$	$1.2\cdot10^{-4}$
11% Degree of grafting	$3.2\cdot10^{-12}$	$1.8\cdot10^{-13}$	$4.5\cdot10^{-4}$
19% Degree of grafting	$1.2\cdot10^{-11}$	$1.8\cdot10^{-13}$	$1.65\cdot10^{-4}$
27% Degree of grafting	$1.95\cdot10^{-11}$	$2.1\cdot10^{-13}$	$2.3\cdot10^{-4}$
50% Degree of grafting	$9\cdot10^{-12}$	$1\cdot10^{-13}$	$1.3\cdot10^{-4}$

determining step. The pore diffusion coefficient as a function of the surface grafting has a maximum at 27% as shown in Fig. 11.

Pore diffusion within the ionic hydrogel appears to be accelerated. The reason for this may be explained along with the results from the gel filtration experiments as resulting from an overall higher charge density or a shortening of the path length for diffusion (Fig. 12), which both increases the capacity and enhances the diffusion of proteins within the pores.

The obtained values for pore diffusion coefficients from batch experiments were used to calculate breakthrough curves.

3.4. Breakthrough curves

The shape of the breakthrough curves of the different resins are shown in Fig. 13.

The slope of the breakthrough curves becomes shallower with increasing degree of grafting. The grafted polymer layer appears to increase mass transfer resistance. This is in accordance with the investigations of Hansen and Mollerup [24] on the influence of film resistance for mass transfer in different anion-exchange media (Q HyperD, Source and Poros). They showed that an overall driving force model failed to described the slope of the breakthrough curves. The film resistance was significant for Reynolds numbers less than 1.

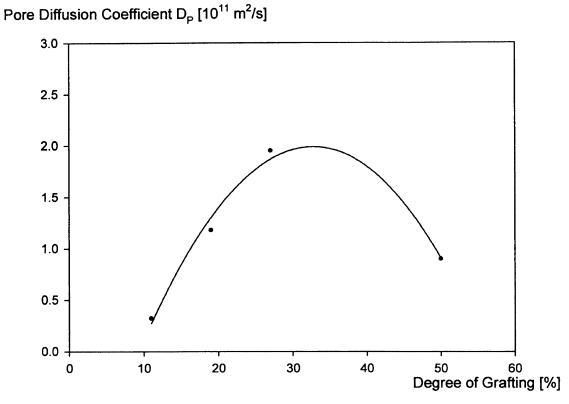

Fig. 11. Diffusion coefficient as a function of degree of grafting.

The influence of external film resistance upon mass transfer in graft-type resins was not as strong in batch adsorption experiments. This difference becomes more pronounced when comparing the shape of the breakthrough curve of the ungrafted resin Toyopearl DEAE 650M with that of the grafted resins. The ungrafted resin a has lower capacity than the grafted resin but the shape of the breakthrough curve is much steeper.

A simple pore model and a combined pore and film diffusion model were used to describe the breakthrough curves [25].

The models were transformed in dimensionless equations by use of the following definitions [26]:

$$T_r = \frac{C_0}{Q_{max}} \cdot \frac{u_S}{L} \cdot \left(t - \frac{L\varepsilon}{u_S} \right) \quad (11)$$

$$N_P = \frac{15\varepsilon(1-\varepsilon)D_P}{R_P^2} \cdot \frac{L}{u_S} \quad (12)$$

$$N_F = k_f a \cdot \frac{L}{u_S} \quad (13)$$

were T_r is the dimensionless throughput and N_P and N_F are the transfer units for pore diffusion and film

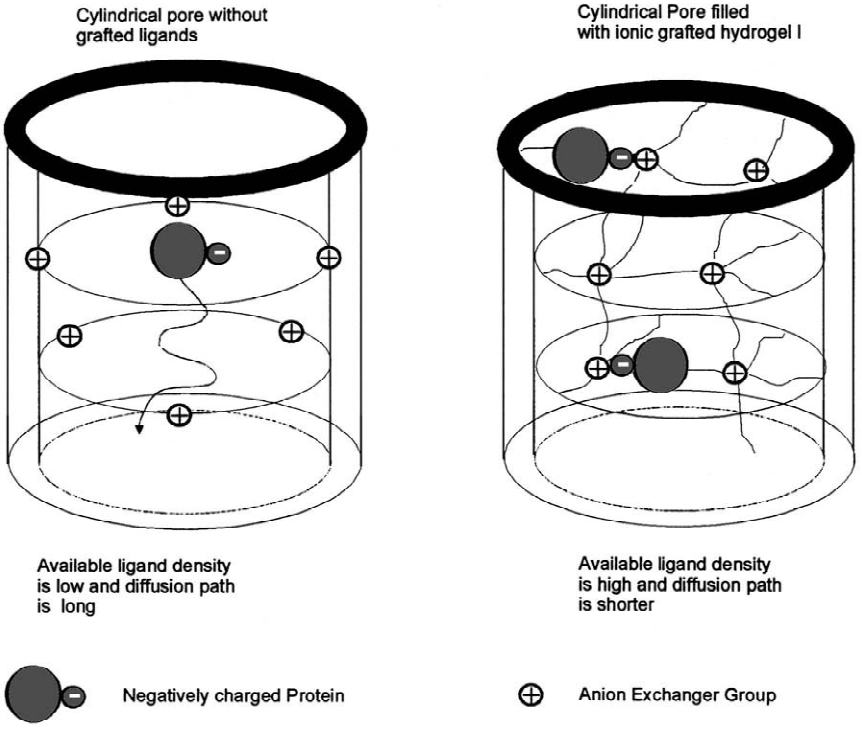

Fig. 12. Schematic drawings of mass transfer in pores with traditional and grafted surface modifications.

diffusion. The value a is the specific surface area, $6/d_p$. Using these definitions the equation for pure pore diffusion is:

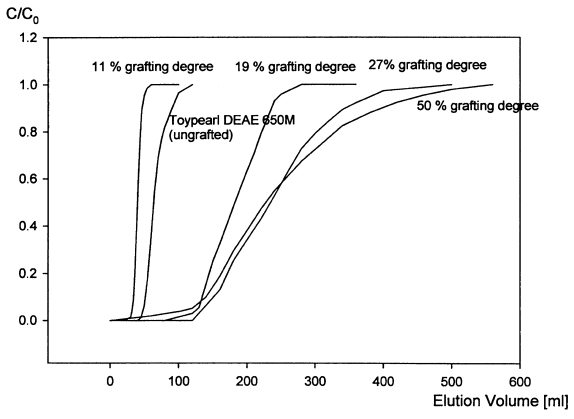

Fig. 13. Breakthrough curves of bovine serum albumin for different resins (2 mg/ml in 20 m*M* Tris buffer at pH 8, Superformance column 50–10 mm, 3-ml bed volume, flow rate 1 ml/min).

$$\frac{C}{C_0} = 1 - \left(\frac{1}{3.59}\right)^2 \cdot [2.39 - N_P(T_r - 1)]^2 \qquad (14)$$

and for the combined film and pore diffusion:

$$N_P(T_r - 1) = \frac{N_P}{N_F} \cdot [\ln(C/C_0) + 1] + 2.39 - 3.59[1 - (C/C_0)]^{\frac{1}{2}} \qquad (15)$$

By using the values of $\varepsilon = 0.8$ (bed porosity), $d_p/2 = 32.5$ μm (mean particle diameter), $L = 3$ cm for the bed length, $u_S = 1$ cm/min and D_p from Table 1, the N_P values of the different resins calculated with Eq. (12) are shown in Table 2.

With Eq. (14) using the parameters from Table 2 it was not possible to describe the breakthrough curves properly. The modified model (Eq. (15)) was used by setting N_P constant to the values from Table 1 and fitting N_F to the experimental data. The obtained N_F values are shown in Table 3.

The influence of film diffusion increases with

Table 2
Mass transfer values for diffusion

Resin surface chemistry	N_P
Toyopearl DEAE 650 (M) (ungrafted)	2.7
11% Degree of grafting	1.3
19% Degree of grafting	4.8
27% Degree of grafting	8
50% Degree of grafting	3.7

Table 3
Mass transfer values for the film resistance

Resin surface chemistry	N_F
Toyopearl DEAE 650 (M) (ungrafted)	0.12
11% Degree of grafting	0.109
19% Degree of grafting	0.11
27% Degree of grafting	0.0377
50% Degree of grafting	$2.5 \cdot 10^{-6}$

increasing degree of grafting. The breakthrough curve could be easily calculated and is shown in Fig. 14 for 27% degree of grafting.

4. Conclusions

Anion-exchange resins with different degrees of surface grafted polyglycidylmethacrylate modified with diethylamine prepared on Toyopearl HW-65M showed increased capacity unrelated to degree of grafting. Resins with capacity more than three times higher than ungrafted Toyopearl DEAE 650M could be obtained. At a certain degree of grafting (19–27%) a sudden increase in capacity was observed which could not be explained simply by an increase in ligand density. This results from the creation of an ionic pore-filling hydrogel. In batch experiments, acceleration of the pore diffusion coefficient was observed but the breakthrough curves showed an increased external mass transfer resistance component.

The following resins were used throughout this work: Toyopearl HW-65M, a hydrophilic methacrylate resin from Tosoh (unmodified resin, not commercially available); Toyopearl DEAE 650M, a weak anion exchange resin from Tosoh; and Toyopearl DEA 650M, a graft-functionalized, commercially unavailable resin prepared by graft polymerization of glycidylmethacrylate onto Toyopearl HW-65M and subsequent reaction with diethylamine.

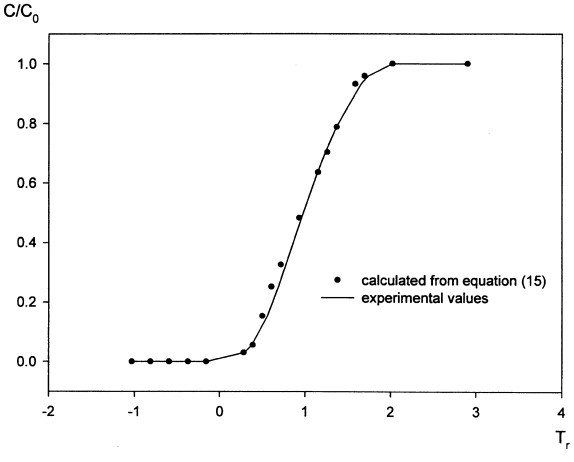

Fig. 14. Breakthrough curve of bovine serum albumin for Toyopearl DEA 650M, graft-functionalized (27% degree of grafting), prediction from Eq. (15).

5. Nomenclature

a	specific surface area (m^{-1})
C	solute concentration in bulk fluid (mg/ml)
RSD	parameter relative standard deviation in non-linear regression expressed as percentage $RSD\% = standard\ error \cdot \frac{100}{parameter\ value}$
C_0	initial concentration (mg/ml)
D_0	surface diffusion coefficient (m^2/s)
D_P	pore diffusion coefficient (m^2/s)
DG	degree of grafting (ml)
k_f	external film mass transfer coefficient (cm/s)
K_{av}	fraction of gel volume which is available for diffusion (%)
L	bed length (cm)
M_P	mass of dried resin after graft polymerization and functionalization (g)
M_S	mass of dried resin before graft polymerization (g)
N_F	film diffusion transfer unit

N_P	pore diffusion transfer unit
Q	particle-average solute concentration (mg/ml)
Q_{max}	particle limiting saturation capacity (mg/ml)
R_P	particle radius (cm)
t	time (s)
T_r	dimensionless throughput
u_S	superficial velocity (cm/min)
V_e	elution volume of proteins (m)
V_f	elution volume of acetone (ml)
V_{FL}	bulk solution volume (ml)
V_G	resin volume (ml)
V_0	elution volume of dextrane (ml)
ε_{ext}	external porosity $= \frac{V_{FL}}{V_{FL}+V_G}$ [28]
ε	bed porosity

Glossary: Ungrafted resin refers to Toyopearl DEAE 650M, Unmodified resin refers to Toyopearl HW-65M

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