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Anion-exchange chromatographic properties of α-lactalbumin eluted from quaternized polyvinylimidazole

Study of the role of the polymer coating

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ABSTRACT

The anion-exchange elution behaviour of α-lactalbumin was studied on cross-linked and quaternized polyvinylimidazole, deposited on various high-performance liquid chromatographic supports (porous silica and diol silica). The influence of the nature and thickness of the coating layer on the retention and band-width properties of the protein elution peak was examined by isocratic elution. The retention properties of α-lactalbumin were studied from the plot of log k' *vs.* log([NaCl]), where k' is the capacity factor and [NaCl] the displacer salt concentration in the aqueous phase. The retention depends on the amount of stationary phase deposited on the support, but an increased hydrophobic effect is found when the polymer films do not coat the chromatographic support uniformly. Band broadening of the elution peaks was studied in terms of plots of plate height *vs.* mobile phase velocity. An important mass-transfer contribution is found, which decreases with increasing k' and increases with the thickness of the coating layer. These effects reveal that the diffusion into the polymer layer is the controlling step of the ion-exchange process with non-uniform polymer layers of large mean thickness.

INTRODUCTION

Anion-exchange chromatography for separations of acidic proteins is a rapidly growing area, especially since the introduction of the adsorbed polymer coating technology applied to high-performance liquid chromatographic (HPLC) supports [1]. The procedure enables a broad range of selectivity to be easily obtained by varying the amount or the nature of the polymer coatings. Excellent selectivities and good protein recoveries were obtained with these stationary phases deposited on silica surfaces.

Alpert and Regnier [2] prepared supports by adsorption and cross-linking of polyethyleneimines on porous silicas. Further studies showed that the retention of proteins can be modulated by controlling the ligand density or the hydrophobicity of the polymer layer [3,4]. Adsorbed polymer technology was used by Sébille *et al.* [5] to

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develop supports for the anion-exchange chromatography of proteins by cross-linking homo- and copolymers of polyvinylimidazole (PVI) adsorbed on porous silica HPLC packings.

Good stability of the cross-linked polymer coatings was generally observed with the solvents commonly used for the HPLC of proteins. The main drawback to the use of silica as a support material is the low stability of the silica matrix at pH values higher than 8. Another limitation is the residual reactivity of the silica support towards the protein after the polymer coating. For instance, some free SiOH groups left after the polymer coating may lead to irreversible adsorption of the proteins. Other support materials were therefore selected for the ion-exchange chromatography of proteins, such as alumina [6], agarose [7], poly(methylmethacrylate) [8,9] and polystyrene–divinylbenzene copolymers [10].

Polymer coating technology involves a complex phenomenon in which various parameters may interfere, such as the nature of the support matrix and the coating procedure [11]. A better understanding of the role of these parameters is necessary for developing new ion-exchange materials based on the polymer coating technology.

In this paper we follow the work of Sébille *et al.* [5] and Boussouira [12] and describe the chromatographic properties of an anion-exchange polymeric stationary phase, quaternized polyvinylimidazole (QPVI), a PVI polymer cross-linked with epichlorohydrin. The main purpose is to understand better the role of the solid support in polymer coating technology by comparing the properties of QPVI coatings on pure porous silica and on diol silica, a matrix that can be considered as fairly inert towards proteins; the retention mechanism of proteins on diol silica is mainly size exclusion.

Although poor efficiencies are found when proteins are eluted in the isocratic mode, only a few studies have examined systematically the factors that influence the broadening of protein elution peaks [13–15]. The main reasons for the large plate heights observed in high-performance affinity chromatography are the slow adsorption–desorption kinetics [13] and the restricted diffusion of the protein into the pores [14]. Hearn *et al.* [15] have shown that the nature of the displacer salt affects the band broadening of proteins eluted isocratically from a Mono Q anion-exchange resin. On the basis of the plate-height theory, we shall examine the role of several factors that influence the performances of silica and diol silica supports coated with an anion-exchange polymer. This approach is useful for understanding better and improving the characteristics of ion-exchange polymer supports used for protein separations.

EXPERIMENTAL

Materials

The porous silica (LiChrosorb Si 100) and porous diol silica (LiChrosorb Diol) supports of particle diameter 10 μm and pore size 100 Å were purchased from Merck (Darmstad, Germany).

The reagents used for stationary phase preparation were N-vinylimidazole (NVIM) (Janssen Chimica, Beerse, Belgium), azobisisobutyronitrile (AIBN) (Eastman Kodak, Rochester, NY, U.S.A.) and epichlorohydrin (Prolabo, Paris, France).

The chemicals used for the preparation of buffers were triethanolamine and Tris (Aldrich-Chemie, Steinheim, Germany).

The protein used for the chromatographic study, calciumdepleted bovine α-lactalbumin, was obtained from Sigma (St. Louis, MO, U.S.A.).

Polymerization of PVI

The homopolymerization of NVIM has been described in detail [16–18]. We adopted the protocol of radical polymerization. PVI was synthesized by polymerization of NVIM with AIBN as a catalyst. The distilled monomer (9 g) was dissolved in 55 ml of methanol and polymerized at 60°C under a nitrogen atmosphere for 48 h with AIBN (180 mg). The polymer was precipitated in dioxane according to Palma and Chapiro [19]. The white powder obtained was collected by filtration and vacuum dried for 24 h. The reticulation and quaternization of PVI were performed in one step during the polymer coating procedure.

Polymer coating procedure

Two methods were used for the coating procedure. The adsorption method at saturation consisted in depositing the maximum amount of polymer that could be adsorbed on the supporting matrix. This method could be applied for the coating on silica, a material that can adsorb large amounts of PVI at saturation in methanol [12].

In this instance the porous support (1 g of LiChrosorb Si 100) was introduced into 10 ml of an 8% (w/v) PVI solution in methanol. It was sonicated under vacuum for 2 min in an ice-bath and then left at room temperature for 24 h. The coated support was washed with methanol and filtered in a nitrogen atmosphere and then dried. The beads were then suspended in 10 ml of methanol containing 1 ml of epichlorohydrin. The mixture was heated at 60°C for 2 h with agitation, then filtered in a nitrogen atmosphere, washed with methanol and dried at 60°C.

The other method used consists in coating the required amount of polymer on the support by complete evaporation of the solvent. This was achieved by introducing the porous support (1 g of LiChrosorb Si 100 or LiChrosorb Diol) in a solution containing the given amount of PVI. After adding 1 ml of epichlorohydrin to the mixture, sonication was applied as above. After leaving the mixture at room temperature for 24 h, the mixture was heated at 60°C for 2 h with agitation to complete the reticulation step. The solvent was then evaporated at this temperature. The coated support was washed with methanol and dried at 60°C. This method has the advantage of better control of the amount of stationary phase deposited on the support.

TABLE I

CHARACTERISTICS OF THE CHROMATOGRAPHIC SUPPORTS COATED WITH QUATERNIZED POLYVINYLIMIDAZOLE

Support	QPVI (%)	Specific surface area (m^2/g)	Exchange capacity (μequiv./g)
Silica	0	280	–
	7.6[a]	200	200
	3.8[b]	235	50
	8.5[b]	190	230
Diol silica	0	245	–
	3.0[b]	200	85
	9.0[b]	165	250

[a] Adsorbed from solution.

[b] Coated by solvent evaporation.

The exact amount of polymer on the support was determined by nitrogen elemental analysis. The characteristics of the supports studied are summarized in Table I. The particles were examined with × 100 magnification using an optical microscope (BHB, Olympus, Tokyo, Japan) linked to a semi-automatic particle analyser (ASM 68K, Ernst-Leitz-Wetzlar, Wetzlar, Germany), thanks to the courtesy of Besins Iscovesco Laboratories. There was no change in the particle diameter after the polymer coating and the thickness of the polymer layer was too low to be evaluated by this method.

The specific surface areas were measured by adsorption of nitrogen at − 196°C using the BET method. The ion-exchange capacity of the support was measured by frontal chromatography using 15 m*M* Tris–HNO_3 buffer (pH 7) as the mobile phase and 15 m*M* Tris–HCl as the displacer eluent. The specific surface areas of the supports decrease with increasing amount of polymer coating.

Chromatographic evaluation

The chromatographic experiments were performed on an HPLC system consisting of a pump (HPLC pump 420; Kontron Instruments, Zurich, Switzerland), a sample injector (Model 7125; Shimadzu, Kyoto, Japan) operating at 280 nm).

The silica or diol silica supports coated with QPVI were slurry packed into 7 × 0.41 cm I.D. columns. The temperature of the column was maintained at 20°C during the experiments using a thermostated water-bath. The eluent was 15 m*M* triethanolamine (pH 7). For the frontal analysis the eluents used were 15 m*M* Tris–HNO_3 buffer (pH 7) and 15 m*M* Tris-HCl buffer (pH 7). The amount of protein injected was chosen to be sufficiently small not to overload the column. The plate heights were determined from measurements of peak width at 0.6 of the peak height.

The capacity factor, k', and the linear velocity, u, were calculated by reference to the retention volume of a tracer; u is related to the interstitial velocity, u_e by $u = u_e/(1 + k_0)$, where k_0 is the column permeation ratio of the protein studied. The selected tracer was a polysaccharide (Shodex Standard kit; Sopares, Gentilly, France) of molecular weight (MW) 20 800 g/mol, close to that of the protein studied. The retention volume of the tracer was determined using a differential refractometer (model R401; Waters Assoc., Milford, MA, U.S.A.).

RESULTS AND DISCUSSION

The chromatographic study of the ion-exchange supports was performed by examining both the retention behaviour of α-lactalbumin and the band broadening of its elution peak. This globular protein (MW = 16 000 g/mol, pI = 4.8, Stokes radius = 1.95 nm) [20] was chosen as the solute sample, as anion exchangers are often used to separate milk proteins. The quaternized homopolymer QPVI was found to have a high anion-exchange capacity and α-lactalbumin was the first acidic protein to elute in a reasonable retention time for performing systematic chromatographic studies.

Evaluation of retention

The retention behaviour of proteins on ion-exchange supports may be evaluated by using the stoichiometric displacement model. This model, which was first used by Regnier and co-workers [21,22] to describe the variation of the retention volume with salt concentration, was recently extended by Melander *et al.* [23] to include electrostatic and the hydrophobic interactions.

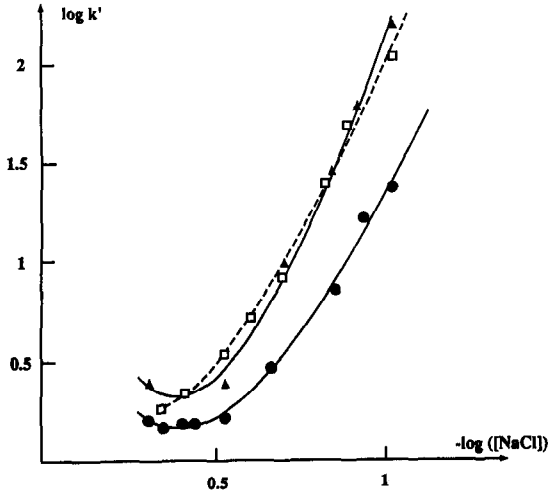

Fig. 1. Dependence of log k' on log (salt concentration). Eluent, 0.015 M triethanolamine buffer with NaCl (pH 7); flow-rate, 1 ml/min; support, silica. Coating method: dashed line, adsorption; solid lines, solvent evaporation. QPVI: □ = 7.6%; ▲ = 8.5%; ● = 3.8%.

A three-parameter equation relates the capacity factor, k', to the concentration of the displacing salt, c_s:

$$\log k' = \alpha - Z \log c_s + \gamma c_s \quad (1)$$

where Z and γ are constants characterizing the electrostatic and hydrophobic interac-

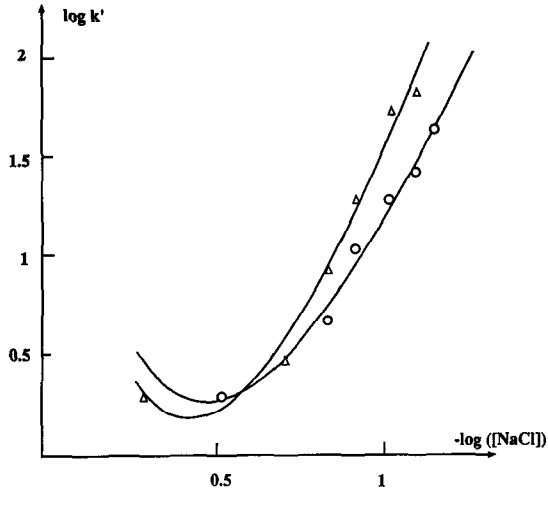

Fig. 2. Dependence of log k' on log (salt concentration). Eluent, 0.015 M triethanolamine buffer with NaCl (pH 7); flow-rate, 1 ml/min; support, diol silica; coating method, solvent evaporation. QPVI: △ = 9%; ○ = 3%.

TABLE II

PARAMETERS OF RETENTION OF α-LACTALBUMIN ON QPVI COATED ON SILICA SUPPORTS

Support	QPVI (%)	α	Z	γ
Silica	7.6[a]	−3.2	4.9	3.9
	8.5[b]	−5.0	6.6	6.8
	3.8[b]	−3.5	4.5	4.8
Diol silica	9.0[b]	−4.7	5.7	6.7
	3.0[b]	−3.7	4.4	5.7

[a] Adsorbed from solution

[b] Coated by solvent evaporation.

tions, respectively; $Z = Z_p/Z_s$, where Z_p is the number of protein charges and Z_s is the valency of the displacing salt.

The variations of k' with the displacing salt concentration are shown in Figs. 1 and 2 for the reticulated PVI stationary phase adsorbed or coated by evaporation on silica or diol silica. Eqn. 1 fits the experimental data well for the plot of log k' *vs.* log c_s, where the hydrophobic effect generates a slight curvature of the lines predicted to be straight from a pure ion-exchange model. The corresponding parameters α, Z and γ are listed in Table II.

Evaluation of band width

The band broadening of the elution peak is characterized from the variation of the plate height, H, with the flow-rate. The plate height is the sum of various increments [24,25]: axial dispersion, eddy diffusion, diffusion into the pores and kinetic mass transfer between the mobile phase and the stationary phase. The last two contributions are proportional to the interstitial velocity, u_e. The plate height increment due to the diffusion into the pores is given by [25]

$$H_i = \frac{d_p^2}{30D_m} \cdot \frac{(k_o + k' + k'k_o)^2}{k_0(1 + k_0)(1 + k')^2} \cdot u \tag{2}$$

where d_p is the particle diameter and D_m is the diffusion coefficient of the solute inside the porous particle.

The plate-height contribution to solute mass-transfer kinetics in the ion-exchange stationary film [24] is given by the sum of two contributions, corresponding to the diffusion into the stationary phase film H_s:

$$H_s = \frac{k'}{(1 + k')^2} \cdot \frac{qd_f^2}{D_s(1 - \phi)} \cdot u \tag{3}$$

and the adsorption–desorption process H_k:

$$K_k = \frac{2k'}{(1 + k')^2} \cdot \frac{\phi}{k_d} \cdot u \tag{4}$$

where D_s is the diffusion coefficient of the protein into the polymer, k_d the desorption rate constant of the ion-exchange process and ϕ is the fraction of retained solute which is adsorbed; the mean depth of the stationary phase units is d_f and the value of the configuration factor q depends on the precise shape of these units. The value of q is 2/3 for a uniform stationary film of thickness d_f and 2/15 for spherical ion-exchange beads, with $d_f = d_p/2$.

At high flow-rates the plate height varies linearly with the velocity u:

$$H = A + Cu \tag{5}$$

In this expression the plate height due to longitudinal diffusion is neglected and the A term roughly approximates the plate height due to eddy diffusion. The term Cu may be considered as approximately equal to the sum $H_i + H_s + H_k$. Evaluating the relative importance of each contribution to the band broadening is difficult. The increment for diffusion into pores may be determined from variations of the particle size [26]. This approach was not considered here because, as shown later from k' variations, the most important cause of efficiency loss was slow mass-transfer kinetics.

The relative importance of the contributions of H_s and H_k to the plate height may be demonstrated from variations of H with the capacity factor. For $k' \gg 1$ the plate height increment H_i due to the diffusion into the pores is almost independent of

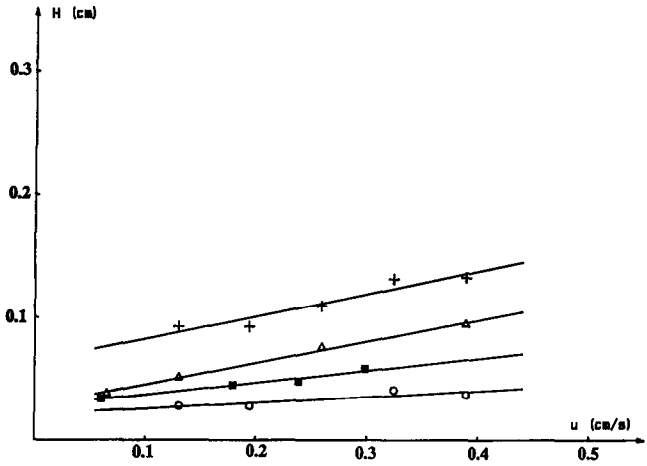

Fig. 3. Influence of capacity factor on plate height for α-lactalbumin eluted from QPVI. H *vs.* u plot [$u = u_e/(1 + k_0)$]. Eluent, 0.015 M triethanolamine buffer with NaCl (pH 7); support, 7.6% QPVI adsorbed on silica.

	k'	[NaCl] (mM)
+	8	210
△	27	150
■	52	125
○	115	100

k' variations, whereas the sum of H_s and H_k is roughly proportional to $1/k'$. This approach is valid if the other constants producing band broadenings are kept constant while the experimental conditions are modified to obtain a retention variation.

In agreement with eqn. 5, the plate height is a linear function of the mobile phase velocity, as shown in Fig. 3 for the column packed with QPVI adsorbed on silica. The large decrease in the column efficiency, together with the larger slopes C observed for lower k' values, show that the solute mass transfer between the mobile and the stationary phase contributes significantly to the plate-height term.

The efficiencies are low, as the reduced plate heights (H/d_p) lie between 20 and 140. The restricted diffusion of α-lactalbumin into the 100-Å pores of the support used can partially explain [14] the poor efficiencies observed for the protein peak. Moreover, this range is commonly found when dealing with the isocratic elution of proteins in ion-exchange chromatography, as shown by Hearn *et al.* [15], who measured the plate heights for several proteins eluted from a Mono Q anion-exchange resin column.

The influence of k' variations on the plate height was used to determine the most important contributions responsible for the large band broadening observed with the α-lactalbumin peak. Testing the plate-height model with the influence of k' is valid if the overall retention mechanism is kept constant. For given k' values, Hearn *et al.* [15] showed that the nature of the displacer salt influences the mechanism of the protein band broadening. In high-performance affinity chromatography, the influence of k' on plate height was obtained by changing the concentration of the competiting inhibitor in the eluent [13].

In this work, the influence of retention on band broadening was obtained by varying the salt concentration in the mobile phase. The mean time τ spent by the solute in the stationary phase will be considered. Its theoretical expression, τ_{th}, can be derived from the previous plate-height expressions:

$$\tau_{th} = \frac{qd_f^2}{2D_s(1 - \phi)} + \frac{\phi}{k_d} \tag{6}$$

It may be determined experimentally from the slope of the H *vs.* u curves if one can correct it for the mass transfer from the mobile phase to the stagnant fluid. For affinity experiments [26,27], the correction for the diffusional contribution in the stagnant fluid is difficult to determine because of the inacurate calculations of the predicted plate height as a function of k'.

If we assume that the main contribution to the C term is due to the diffusion into the polymer stationary phase film (H_s) and to the adsorption–desorption process (H_k), the experimental value of the mean desorption time, τ_{ex}, can be calculated from

$$\tau_{ex} = (1 + k')^2 \, C/2 \, k' \tag{7}$$

The values of τ_{ex} calculated from the slope C of the straight lines in Fig. 2 are listed in Table III. Within experimental error, the τ_{ex} measurements (2.7 s) are independent of k' variations in the range $27 < k' < 115$. This confirms the assumption that for large k' values one can neglect in the C term the contribution of the restricted diffusion into the pores. Moreover, these results show that the mechanism responsible

TABLE III

RESULTS OF MASS-TRANSFER KINETIC MEASUREMENTS

Support	QPVI (%)	[NaCl] (*M*)	k'	C (s)	τ_{ex} (s)
Silica	7.6[a]	100	115	0.05	2.7
		125	52	0.11	3.0
		150	27	0.17	2.5
		210	8	0.20	1.1
	8.5[b]	125	63	0.48	16.0
	3.8[b]	75	62	0.09	2.9
Diol	9.0[b]	100	57	1.6	47.0
silica	3.0[b]	85	42	0.38	8.3

[a] Adsorbed from solution.
[b] Coated by solvent evaporation.

for the band broadening of α-lactalbumin is not modified by varying the salt concentration in the range 0.1–0.15 *M* NaCl. Slow mass transfers between the liquid phase and the stationary phase are therefore mainly responsible of the loss of efficiency observed at higher flow-rates. These effects include diffusion in the polymer film (H_s term) and the adsorption–desorption process on the ion-exchange site (H_k term).

For smaller capacity factors ($k' = 8$), a deviation from the plate-height model predictions was observed since $\tau_{ex} = 1$ s. This effect may be due to the additional retention mechanism observed at higher salt concentrations and caused by an increase in the hydrophobic effect.

Karger and co-workers [28–30] have shown from fluorescence and chromatographic measurements that conformational changes of α-lactalbumin can occur on hydrophobic surfaces from the folded to the unfolded protein state. These effects, which increase with increasing degree of hydrophobicity of the stationary phase and temperature, may lead to additional broadenings when the elution peaks of the conformers overlap. One cannot exclude the occurrence of such a mechanism in the present experiments carried out at 20°C, although the ion-exchange surface is weakly hydrophobic.

To evaluate the effect of film thickness, given amounts of polymer were deposited on the silica support by evaporation of the solvent. A poor coating was obtained by this method, as shown in Fig. 4, where the efficiencies of α-lactalbumin on the supports prepared by polymer adsorption from the solvent or by evaporation of the solvent are compared. For about the same amount of polymer coated on silica and the same k' value, a large decrease in the column efficiency is observed when the polymer is coated by evaporation. No variation in the particle size was observed with silica supports coated with large amounts of polymer and the poor characteristics of the support coated by evaporation are close to those of the support coated by adsorption, as shown from surface area measurements (Table I).

Hence the poor efficiency observed with α-lactalbumin eluted from the support coated by evaporation is due to the sum of the H_s and H_k contributions, as the slope of the straight line (H *vs.* u) is five times larger for the same amount of PVI on silica

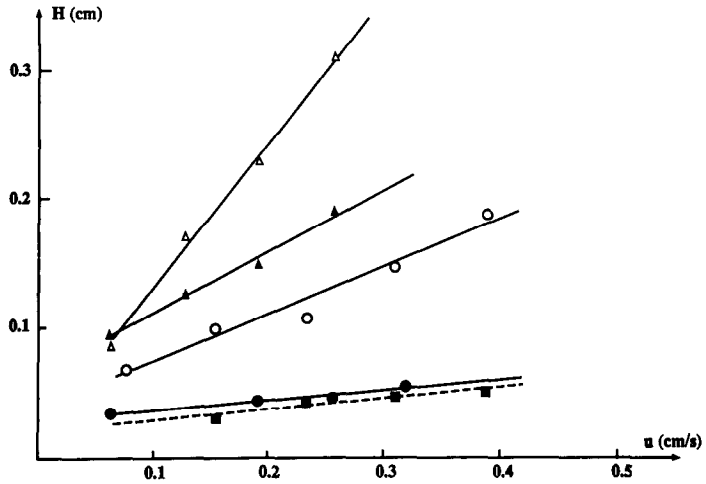

Fig. 4. Comparison of the band broadening of α-lactalbumin eluted from various QPVI coatings. *H vs. u* plot [$u = u_e/(1 + k_0)$]. Eluent, 0.015 *M* triethanolamine buffer with NaCl (pH 7). Coating method: dashed line, adsorption; solid lines, solvent evaporation.

	QPVI (%)	Support	k'	[NaCl] (m*M*)
■	7.6	Silica	63	125
▲	8.5	Silica	52	125
●	3.8	Silica	62	100
△	9.0	Diol silica	56	100
○	3.0	Diol silica	42	85

(Fig. 4). This loss of efficiency observed with the polymer deposited by evaporation is probably due to the formation of several layers taking place in units which are not uniformly distributed on silica.

With a support coated with a smaller amount of polymer by evaporation (3.8% PVI), an improved efficiency was observed (Fig. 4), with a value of τ_{ex} now close to that obtained with the adsorbed polymer (Table III). The 3.8% and 8.5% PVI supports were both obtained by solvent evaporation. The coatings are similar and the ratio of the mean stationary film thickness is probably close to 2.2, leading to an H_s contribution that is 4.8 times greater with the large PVI coating. Therefore, the solute diffusion into the polymer film mainly contributes to the large increase in the plate heights observed at high flow-rates and large percentages of PVI coated by evaporation.

The retention volume was measured as a function of the displacing salt concentration for various amounts of polymer coatings applied on the silica support (Fig. 1). Larger polymer coatings produce higher ion-exchange capacities (Table I) and therefore higher α-lactalbumin capacity factors. For similar experimental conditions, the retention volume on QPVI adsorbed on silica is close to that observed with approximately the same amount of polymer deposited by evaporation. The parameters of eqn. 1 calculated by fitting the model to the plot of log k' *vs.* log [NaCl] are listed in Table II. The parameters Z and γ are larger on the polymeric phase having a poor

coating layer, obtained by solvent evaporation. An increase in the number of protein charges is then observed ($Z_s = 1$ and $Z = Z_p$) with an increase in the degree of hydrophobicity of the stationary phase.

With the adsorbed polymeric coating the hydrophobic properties ($\gamma = 3.9$) are even lower than those observed with a stationary phase having half the amount of polymer but coated by evaporation ($\gamma = 4.8$). These results show that stationary phases with faster mass-transfer exchanges and lower hydrophobic properties are obtained when the polymer film has a smaller thickness and is more uniformly distributed on the support.

The role of the solid support was studied by comparing the chromatographic properties of silica coated with QPVI with those of a diol silica coated with the same polymer. The diol support is fairly inert and does not have high adsorption properties. Therefore, coating the polymer by the adsorption procedure was not possible and the polymer coating was achieved by evaporation of the solvent.

The same trends as were found with porous silica were observed when various amounts of QPVI are deposited on diol silica: slower mass-transfer exchanges and greater hydrophobic character for larger amounts of polymer coated from solvent evaporation (Tables II and III). In Fig. 4 the steeper lines at higher percentage of QPVI on diol silica reveal a slow diffusion effect in the polymer stationary phase.

The efficiency of the column packed with the diol silica support coated with QPVI is lower than that observed with the corresponding silica column having about the same amount of polymer deposited by solvent evaporation (Fig. 4). The results in Table III show that the mass transfer term τ_{ex} is roughly three times larger with a polymer layer deposited on diol silica. The coating of QPVI on diol silica is therefore less uniform with stationary film units of mean depth roughly 1.7 times larger than those on silica. These results show the importance of the role of the solid support for achieving thin films of uniform thickness: the contribution of the slow diffusion process in the stationary phase increases with increasing non-uniformity of the coating and thickness of the polymer layer.

However, one cannot exclude a contribution of the slow adsorption–desorption kinetics, which were found in affinity systems to be mainly responsible for the poor efficiencies for the isocratic elution of proteins [13,14]. It is in fact difficult to differentiate between the H_s and H_k terms as the Z values increases with increasing amount of polymer deposited by evaporation. This result indicates a larger number of contact points of the protein with the support, which may lead to slower desorption kinetics with a larger contribution of the H_k term. Compared with the silica support coated by evaporation, the slope C of the H *vs.* u curve is 3–4 times larger with diol silica (Table III), but similar Z values are observed for comparable amounts of polymer deposited on silica by evaporation (Table II). This shows that the diffusion mechanism dominates with the diol silica support and probably with the silica support coated by evaporation with a large amount of polymer.

CONCLUSIONS

At high flow-rates the most important contribution to the band broadening of the α-lactalbumin elution peak is the slow mass transfer between the mobile phase and the stationary phase. On the basis of a sole band-broadening analysis, it is not

possible to distinguish between the contributions of the diffusion of the protein into the stationary phase and the conformational changes. A fluorescence study of the kinetic mechanism [26] would be helpful for determining the relative importance of this last effect.

However, in ion-exchange chromatography, slow diffusion which may lead to protein conformational changes is a rate-controlling step and partially explains the low efficiencies observed in isocratic elution with non-uniform coatings of large thickness: the H_s increment given by eqn. 3 increases strongly with increase in ϕ, the ion-exchange adsorption fraction which is always close to 1 in ion-exchange chromatography. It is therefore important, as shown experimentally in this work, to achieve uniform polymer coatings of small thickness in order to increase the efficiencies and to reduce the degree of hydrophobicity of the stationary phase.

The role of the supporting material was demonstrated, as the best efficiencies were obtained by adsorbing the polymer on porous silica. The high concentration of SiOH permits an irreversible adsorption of the functional groups of the polymer. This produces a more uniform coating and a thinner film. It is therefore to be expected that selecting an inert or a deactivated matrix will produce a poor polymer coating with a high degree of hydrophobicity that may lead to protein conformational changes.

This paper was limited to the study of the chromatographic properties of α-lactalbumin. However, the same adsorption technology on silica supports is now used with weaker anion exchangers based on PVI copolymers [31] and gives satisfactory elution behaviours with good protein recoveries.

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