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Journal of Chromatography A, 776 (1997) 55–63

JOURNAL OF
CHROMATOGRAPHY A

Modification of polystyrenic matrices for the purification of proteins II. Effect of the degree of glutaraldehyde–poly(vinyl alcohol) crosslinking on various dye ligand chromatography systems

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Abstract

A poly(styrene–divinylbenzene) chromatography matrix, CG1000sd (TosoHaas) has been modified by the adsorption and crosslinking of poly(vinyl alcohol) (PVA) to create a matrix suitable for the attachment of dye ligands for the adsorption of lysozyme. However, it is shown that there was limited recovery and repeated drops in capacity with adsorption of human serum albumin (HSA). The effect of changing the nature of the PVA crosslinking on the HSA binding characteristics was studied, as well as the effect of using differing dye ligands. The total amount of irreversible HSA binding decreased with greater crosslinking and there were large differences in HSA adsorption characteristics between differing dye types.
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Keywords: Poly(vinyl alcohol) coating; Dye ligands; Affinity adsorbents; Stationary phases, LC; Poly(styrene–divinylbenzene) adsorbents; Proteins

1. Introduction

Affinity chromatography has become an established protein purification technique [1]; however, problems associated with the support matrix have prevented the full exploitation of its benefits in preparative purification of proteins. The most popular matrix to date, agarose, is limited by its mechanical rigidity even in heavily crosslinked varieties, thus restricting its application in high-pressure operations and has limitations due to the inability to produce rigid agarose containing very wide pores.

Poly(styrene–divinylbenzene) (PS–DVB) matrices have been used in chromatography for many years and these matrices can be operated under high pressures without compression. However, these matrices cannot directly be used to purify proteins, as the surface is difficult to derivatise and strong hydrophobic interactions between the matrix and the protein results in irreversible adsorption and reduced protein yield.

Recently there has been a trend to modify the surface of PS–DVB matrices with hydrophilic groups. Such modifications ensure a surface which both minimises non-specific hydrophobic protein interactions and provides reactive groups to allow easy derivatisation. These modifications include the introduction of covalently bonded carboxyl groups [4] and the adsorption of various polymers such as polyoxyethylene [5], polyethyleneimine [6] and an epibromohydrin and glycidol copolymer [7–9]. Such coatings are, however, relatively complicated to prepare and have had limited applications.

The adsorption and subsequent crosslinking of poly(vinyl alcohol) (PVA) onto PS–DVB surfaces

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PII S0021-9673(97)00469-X

have recently been performed [10–12]. This surface modification technique creates a rigid PS–DVB matrix with a hydrophilic coating with functional groups which allow derivatisation. The effectiveness of the PVA coating in minimising non-specific interactions is highly dependent on the molecular mass and degree of hydrolysis of the adsorbing PVA [12]. At low crosslinking ratios the PVA layer is more likely to desorb from the PS–DVB surface [10].

Immobilised biomimetic dye ligands have increasingly been used as alternatives to other ligands in affinity chromatography [2]. Their popularity has stemmed from the cheapness of dyes compared with other ligands (downstream processing accounts for between 50 and 80% of total production costs [3]), the ease of coupling dyes to a support matrix, and the ability of dyes to withstand severe cleaning regimes.

It has been found that lysozyme can be recovered from Procion Blue MX-R–PVA–PS–DVB adsorbents with yields approaching 100% [12], whereas albumin recoveries are lower with yields between 70 and 90% [11]. The degree of recovery is thought to depend on the degree of crosslinking of the adsorbed layer, the dye type used and the degree of interaction between the dye ligand and the protein.

This paper investigates further the PVA–PS–DVB modification technique to produce dye-affinity adsorbents suitable for protein adsorption. It will examine the effect of the degree of PVA crosslinking on the HSA adsorption characteristics on dye ligand systems. It will also compare the adsorption characteristics of PVA–PS–DVB matrices with commercially modified PS–DVB matrices (modified using epibromohydrin and glycidol copolymer).

2. Experimental

2.1. Materials

PVA (M_r 13 000–23 000, 87–89% hydrolysed) was purchased from Aldrich (Gillingham, UK), as were sodium hydroxide, iodine, potassium iodide, ethanol, methanol, disodium hydrogenorthophosphate, sodium hydrogenphosphate, tris(hydroxymethyl)aminomethane and sodium carbonate. Boric acid (99.5% purity), glutaraldehyde and HCl were purchased from Sigma (Poole, UK).

Procion Blue MX-R (CI Reactive Blue 4), Procion Red HE-3B (CI Reactive Red 120), Cibacron Blue F3-GA (CI Reactive Blue 2) were purchased from Aldrich and Procion Yellow HE-3G (CI Reactive Yellow 81) was purchased from Sigma. Blue Dextran 2000 was purchased from Pharmacia Biotech (St. Albans, UK).

Chicken egg white lysozyme (M_r 14 300) and human serum albumin (HSA) (M_r 66 000) were obtained from Sigma.

CG1000-sd beads were purchased from TosoHaas (Philadelphia, USA), Blue Sepharose Fast Flow was obtained from Pharmacia Biotech and POROS 20 (OH-activated) beads were purchased from PerSeptive Biosystems (Cambridge, MA, USA).

2.2. Instrumentation

UV–Vis spectrophotometry was carried out using a Shimadzu UV-160A (VA Howe, Oxon, UK). Chromatography was carried out on a Pharmacia FPLC system (fast protein liquid chromatography) categorised as 2×P-6000 pumps, UV-1, Frac-100, Rec-482 obtained from Pharmacia LKB (Uppsala, Sweden). The column used was an HR5/5 of 5×0.5 cm I.D. (volume 1 ml).

2.3. Coating of PS–DVB beads with PVA

The coating procedure was essentially the same as described in a previous paper [12]. Washed and dried PS–DVB beads (approximately 2.5 g) were placed into test tubes (30 ml) and methanol added (20 ml) to ensure that the PS–DVB surface was 'wetted' and that all the air in the bead interior was displaced with liquid. The beads were then washed with water (500 ml) in a number 2 sintered disk to displace excess methanol from the bead interior. After washing, the beads were placed back into a 30-ml test tube and PVA solution (M_r 13 000–23 000, 87–89% hydrolysed, 100 mg/ml, 25 ml) was added. The test tubes were sealed and left on a rotating mixer (Mixer 820, Swelab instrument, Sweden) at 30°C for 1 week. The maximum amount of PVA bound to the PLRP4000s

beads was calculated using methods also previously described [12].

2.4. Crosslinking of the adsorbed PVA layer

Crosslinking was carried out at room temperature using glutaraldehyde. Various crosslinking ratios were used for the CG1000sd beads, the maximum crosslinking ratio being 250 mol glutaraldehyde/mol adsorbed PVA.

Once the PS–DVB beads had been coated with PVA, the beads were washed thoroughly with water in a number 2 sintered glass disc and were then returned to test tubes of appropriate size. One-ml quantities of beads were crosslinked in a 7-ml test tube; 5-ml quantities of beads were crosslinked in a 25-ml McCartney bottle. Glutaraldehyde (10 mg/ml) and water were then added to the test tubes to ensure total liquid volumes for each sample size of 4.9 and 19.6 ml, respectively. The beads were then left for 30 min on a rotary mixer (Mixer 820, Swelab instrument, Sweden) to allow glutaraldehyde to diffuse into the bead interior. Then 5 *M* HCl (0.1 and 0.4 ml, respectively) was added to catalyse the crosslinking reaction. The overall HCl concentration was 0.1 *M*. The sealed tubes were left for 16 h on a rotary mixer at 30°C to allow the reaction to complete.

2.5. Determination of non-specific protein adsorption on PVA–PS–DVB matrices

Non-specific protein adsorption was carried out using lysozyme and HSA. The PVA-modified and cross-linked PS–DVB samples were placed into 20-ml centrifuge tubes and protein solution (lysozyme, 1 mg/ml, 10 ml in 50 m*M* phosphate buffer, pH 5.0; HSA, 1 mg/ml, 10 ml in 25 m*M* phosphate buffer, pH 5.0) was added. The samples were left for 16 h on a rotating mixer at room temperature, after which the protein concentration in solution was determined by measuring the absorbance (A) value in a quartz cuvette at 280 nm. The A_{280} value was used to calculate protein concentration by employing extinction coefficients of 2.64 and 0.53 ml mg^{-1} cm^{-1} for lysozyme and HSA, respectively [16]. The quantity of protein bound non-specifically was calculated from a mass balance on the supernatant.

2.6. Dyeing of modified PS–DVB matrices

The PVA–PS–DVB-modified matrices and the OH-activated POROS 20 beads were both dyed using identical methods. The samples were washed thoroughly with water and 1-ml samples were placed in a test tube (7 ml), and the 5-ml samples were placed in a round bottomed flask (50 ml). Water was then added to give total volumes of 5 and 20 ml, respectively. Dye (200 and 2000 mg, respectively) was added and the dye mixture mixed for 30 min (the 7-ml test tubes on a rotating mixer, and the 50-ml round bottomed flask on a Buchner rotameter). After mixing, 22% (w/v) NaCl (1 and 4 ml, respectively) was added to the dye mixture and left for another 30 min to ensure adequate mixing. Sodium carbonate (50 and 200 mg) was then added and the samples left for 3 days at 60°C to ensure binding of the dye to the hydroxyl groups on the beads. (The dichloro dye, Procion Blue MX-R was heated to 60°C for 16 h only, due to the faster reaction kinetics.) The dyeing procedure was repeated for each sample. After the dyeing processes, the beads were thoroughly washed with an excess of water and methanol, and were washed again a number of times over a period of a week. Determination of dye ligand concentration could not be determined by digestion of the matrices as the PS–DVB was chemically and thermally stable, and so the quantity of dye that had bound to the PS–DVB matrices was determined from a mass balance calculated from the dye added to the beads and from the dye in the wash supernatant. Dye concentrations in solution were determined from absorbances and extinction coefficients (Procion Blue MX-R, extinction coefficient 10 500 l mol^{-1} at 620 nm [13]; Cibacron Blue F3-GA, extinction coefficient 12 600 l mol^{-1} at 617 nm [14]; Procion Red HE-3B, extinction coefficient 40 796 l mol^{-1} at 535 nm [14] and Procion Yellow HE-3G, extinction coefficient 35 600 l mol^{-1} at 400 nm [15]).

2.7. Frontal analysis

Dynamic loading capacities of dyed-modified PS–DVB matrices were determined using frontal analysis. The protein solutions used in these experiments were lysozyme (1 mg/ml, adsorbed in 50 m*M*

phosphate buffer, pH 7.5, eluted in 1 M NaCl in 50 mM phosphate buffer, pH 7.5) and HSA (1 mg/ml, adsorbed in 25 mM phosphate buffer, pH 5.0, eluted in 0.5 M NaSCN in 50 mM phosphate buffer, pH 8.0). Frontal analysis was carried out by pumping the protein solution through an HR5/5 column (5 cm× 0.5 cm I.D.) at 1 ml/min (superficial velocity 306 cm/h).

The system voidage (column and associated tubing) was determined for each protein on the undyed PVA–PS–DVB beads as there were slight differences in the void volume of the column between the different sized proteins. The system voidages were measured to be 0.81 ml for lysozyme and 0.67 ml for HSA.

The dynamic capacity was determined at 5% breakthrough (the outlet absorbance at 280 nm being 5% that of the inlet). The 5% dynamic capacity was calculated by determining the quantity of protein applied to the column at the point at which the outlet stream had a protein concentration 5% that of the feed stream, and deducting from this value the amount of protein contained in the system void volume.

The solutions applied to the column were collected as three separate fractions, as the adsorption, wash and elution stages. Protein concentration in solution was determined by measuring the A_{280} in a quartz cuvette. The A_{280} value was used to calculate protein concentration by employing extinction coefficients of 2.64 and 0.53 ml mg^{-1} cm^{-1} for lysozyme and HSA, respectively [16]. The recovery of protein was determined as the ratio of protein eluted in the elution stage to the amount of protein bound to the column prior to elution. The amount of protein bound prior to elution was calculated from a mass balance by considering the protein applied to the bed, and deducting the amount of protein which had passed through the bed in the adsorption and wash stages.

3. Results and discussion

3.1. Protein recovery from dye–PVA–PS–DVB matrices

Fig. 1 shows the dynamic capacities and recoveries of lysozyme and HSA on dyed Procion Blue MX-R PVA–PS–DVB CG1000sd matrices, on Blue Sepharose Fast Flow and on Procion Blue MX-R dyed POROS 20 beads. The Procion Blue MX-R PVA–PS–DVB bead samples are samples 1–4 shown in previous work [12]. The adsorbed PVA layers were crosslinked at a ratio of 10 mol glutaraldehyde/mol PVA). It can be seen that the lysozyme recovery from the Procion Blue MX-R PVA–PS–DVB matrices approaches 100%, whereas the recovery of HSA is much lower, at about 50%. The commercial Blue Sepharose Fast Flow has a recovery approaching 100% under the experimental conditions, but the dyed OH activated POROS 20 beads (a PS–DVB matrix with a hydrophilic coating consisting of epibromohydrin and glycidol) has a higher recovery than the Procion Blue MX-R PVA–PS–DVB beads, but still only has a recovery of about 90%.

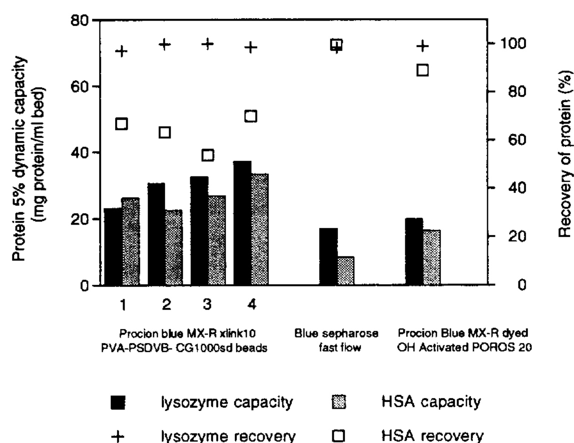

Fig. 1. The 5% dynamic capacities and recoveries of lysozyme and HSA from Procion Blue MX-R–PVA–PS–DVB CG1000sd beads, Procion Blue MX-R dyed OH-activated POROS 20 beads and Blue Sepharose Fast Flow. The adsorbed PVA coating was crosslinked with glutaraldehyde at a ratio of 10 mol glutaraldehyde/mol PVA.

3.2. Non-specific protein adsorption on PVA–PS–DVB matrices

The effect of crosslinking on the non-specific protein adsorption characteristics of PVA–PS–DVB matrices can be seen in Table 1. It can be seen that non-specific interactions between the protein and

Table 1
The effect of crosslinking on the non-specific adsorption of protein on PVA adsorbed and crosslinked PSDVB CG1000sd matrices: operations carried out as described in Section 2

Sample no.	PVA adsorbed (mg/g bead)	Crosslinking ratio	HSA adsorbed (mg/g bead)	Lysozyme adsorbed (mg/g bead)
1	0	—	39.5	66.8
2	250	0	5.9	0.6
3	250	10	0.0	0.0
4	250	50	0.0	0.3
5	250	100	0.0	0.5

surface occurs with no crosslinking, but as soon as slight crosslinking is introduced into the adsorbed coating then the PVA coating shields the hydrophobic PS–DVB surface from non-specifically adsorbing protein. It was noted that the non-crosslinked PVA–PS–DVB beads were stripped of dye and PVA when placed in contact with 1 *M* NaOH or methanol.

3.3. Dye ligand immobilisation

Raising the crosslinking ratio will reduce the number of hydroxyl groups in the PVA layer and this has the undesirable effect of providing fewer moieties on which to immobilise the dye ligand. Fig. 2 shows the amount of Procion Blue MX-R dye ligand that becomes immobilised to the PVA–PS–DVB CG1000sd beads with changes in crosslinking ratio.

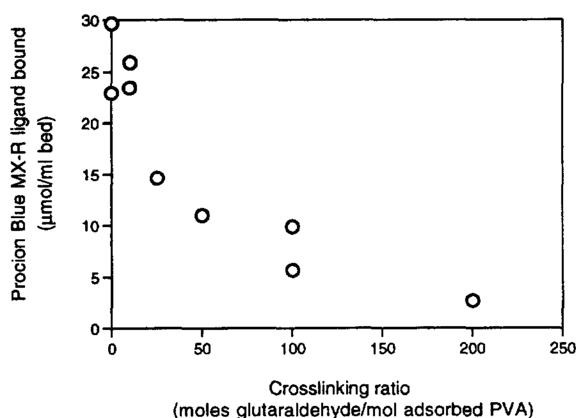

Fig. 2. The effect of the degree of crosslinking of the adsorbed PVA coating on the amount of dye ligand (Procion Blue MX-R) that was immobilised onto PVA–PS–DVB CG1000sd matrices. The CG1000sd beads were coated with PVA and crosslinked with glutaraldehyde as described in Section 2. The PVA–PS–DVB beads were then subsequently dyed with Procion Blue MX-R dye as described in Section 2.

The amount of immobilised ligand dropped as the crosslinking ratio increased, and this was directly due to the decrease in the number of hydroxyl groups available for reaction. The amount of protein that can bind to the dye–PVA–PS–DVB matrices will be related to the amount of immobilised dye, and the amount of protein that can bind generally increases with increased ligand density.

3.4. Initial dynamic capacities and recoveries with Procion Blue MX-R–PVA–PS–DVB matrices

A range of the dynamic capacities and recoveries from PVA samples with varying crosslinking ratios can be seen in Table 2. Each sample had enough PVA adsorbed to ensure no non-specific protein adsorption (except the samples without crosslinking) and all the samples were dyed individually. It can be seen that the capacities of the dyed CG1000sd beads showed large differences in capacities for both lysozyme and HSA adsorption. This was due to the differences in immobilised dye on each sample as it was difficult to immobilise similar amounts of dye. However there were large discrepancies between the recoveries of lysozyme and HSA. When the PVA layer was crosslinked at a ratio of 10 mol glutaraldehyde/mol PVA, in all ten samples the initial lysozyme recovery approached 100%, whereas the recovery of HSA for the first HSA injection fluctuated considerably around 50%. In all the CG1000sd samples, at all crosslinking ratios, the recovery of lysozyme was high, whereas recoveries of HSA from these matrices was lower. The recoveries of HSA tended to increase with an increase in crosslinking ratio, reaching an average recovery of 90% with a crosslinking ratio of 50 mol glutaraldehyde/PVA molecule.

Table 2
5% dynamic capacities and recoveries with the first adsorption run using lysozyme and HSA adsorption onto Procion Blue MX-R dyed PVA coated CG1000sd PS–DVB matrices with various PVA crosslinking ratios: operations carried out as described in Section 2

PVA layer crosslink ratio	Protein adsorbed	No. of samples	5% dynamic capacity		Recovery of protein in eluent	
			Average (mg/ml)	S.D. (mg/ml)	Average (%)	S.D. (%)
0	Lysozyme	1	52.5	—	90.0	—
	HSA	3	25.8	3.5	67.0	10.5
10	Lysoyme	10	35.6	12.0	99.0	1.2
	HSA	10	25.2	6.8	52.9	20.6
25	Lysozyme	0	—	—	—	—
	HSA	2	20.8	3.7	45.5	3.5
50	Lysozyme	3	17.2	0.5	97.7	2.5
	HSA	6	16.5	5.0	90.2	9.6
>100	Lysozyme	2	14.0	0.8	94.0	7.1
	HSA	6	14.9	4.9	83.2	7.9

3.5. HSA dynamic capacities with repeated adsorption cycles

The dynamic capacities of Procion Blue MX-R dyed, crosslinked, PVA–PS–DVB CG1000sd beads are shown in Fig. 3. The HSA dynamic capacities of POROS 20 and Blue Sepharose Fast Flow are also shown. It can be seen that the dynamic capacities of all the systems, with the exception of Blue Sepharose Fast Flow, show decreases in capacity with repeated use. It would be expected with the high-affinity sites which bind protein irreversibly, that eventually the capacities will stabilise with continued adsorption cycles. This result indicates that the polymer coating is not totally satisfactory. Generally, as the cross-linking ratio increased, the dynamic capacities decreased, and this was due to the decreased immobilised dye concentration associated with samples crosslinked to a higher extent.

The dynamic capacity for the Procion Blue MX-R POROS 20 beads fell with initial use but the dynamic capacity stayed constant after the first run. The dynamic capacity of these beads fell from 16.4 to 14.3 mg/ml (a drop of 13%), indicating that

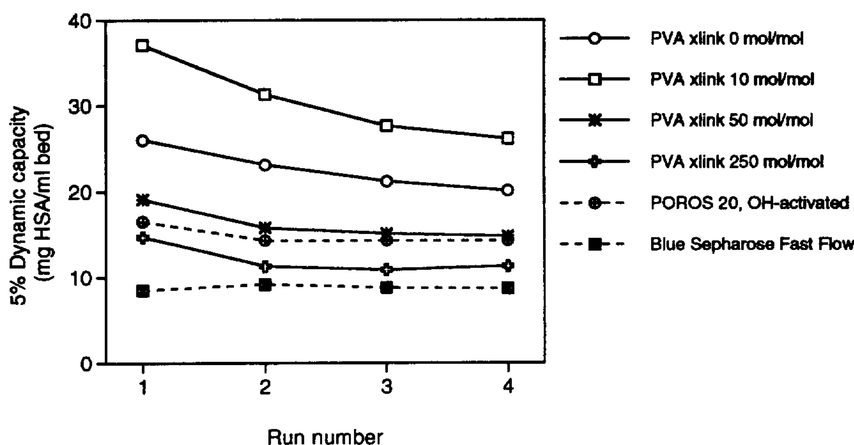

Fig. 3. The effect of crosslinking on repeated HSA 5% dynamic capacities on Procion Blue MX-R–PVA–PS–DVB CG1000sd beads, Procion Blue MX-R dyed POROS 20 beads and Blue Sepharose Fast Flow. PVA adsorption, crosslinking and subsequent dyeing were carried out as described in Section 2. The dynamic capacity was determined at 5% breakthrough, the point at which the absorbance (at 280 nm) of the outlet stream was 5% that of the feed stream. Protein was applied to an HR5/5 column (5×0.5 cm I.D.) at 1 ml/min. HSA (1 mg/ml) was adsorbed in 25 m*M* phosphate buffer, pH 5.0, and eluted with 0.5 *M* NaSCN in 50 m*M* phosphate buffer, pH 8.0. The bed was extensively washed with 25 m*M* phosphate buffer, pH 5.0, between runs.

irreversible adsorption still occurred on a PS–DVB matrix coated by a different technique, but was less than the PVA–PS–DVB matrices. Blue Sepharose Fast Flow, however, showed little irreversible HSA adsorption.

3.6. The effect of dye ligand type on HSA capacities

The characteristics of adsorption of HSA onto dye–PVA–PS–DVB matrices are almost certainly dependent on all the combinations of PS–DVB, the PVA shielding layer and the dye ligand. Experiments were carried out to investigate the effects of immobilising different dye ligands on the modified matrix. PVA–PS–DVB CG1000sd beads were crosslinked at two different crosslinking ratios: 10 and 50 mol glutaraldehyde/mol PVA. The PVA was adsorbed onto the CG1000sd beads in one batch and the beads separated into two batches for crosslinking. The crosslinked PVA–PS–DVB beads were separated subsequent to dyeing. The dynamic capacities of HSA on these modified matrices with different immobilised dye ligands can be seen in Figs. 4 and 5. The HSA adsorption characteristics were substantially different between PVA–PS–DVB matrices with the two crosslinking ratios and immobilised dye type.

Fig. 4 shows the dynamic capacities of the dye–PVA–PS–DVB matrices with the PVA crosslinked at a ratio of 10 mol glutaraldehyde/mol PVA. At this crosslinking ratio, most dyes showed irreversible adsorption of HSA. Procion Red HE-3B and Procion Yellow HE-3G showed such dramatic drops that only two HSA runs were carried out. However Cibacron Blue F3-GA modified dyes, although displaying low capacities, showed approximately the same dynamic capacity for all four runs. At this crosslinking ratio the adsorbed PVA coating offers little shielding between the adsorbed dye and the PSDVB surface.

Fig. 5 shows the dynamic capacities for HSA on dye–PVA–PS–DVB CG1000sd matrices with the PVA crosslinked at a ratio of 50 mol glutaraldehyde/mol PVA. The dynamic capacities of the dye–PVA–PS–DVB matrices were all lower at this crosslinking ratio, as expected due to the fewer dye ligands bound, and the only large decrease in dynamic capacity occurred with Procion Blue MX-R dye. Procion Red HE-3B dye showed a small decrease in dynamic capacity with use, whilst Cibacron Blue F3-GA and Procion Yellow HE-3G showed no deterioration in binding capacity with repeated use.

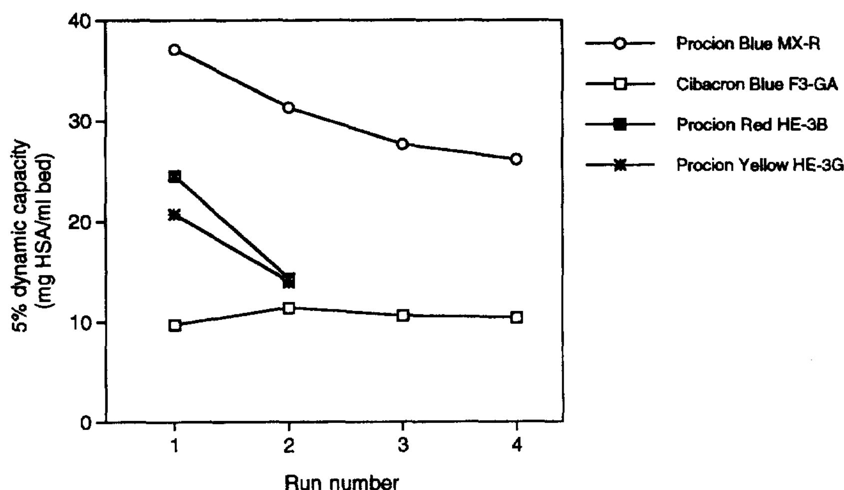

Fig. 4. HSA dynamic capacities on dye–PVA–PS–DVB CG1000sd beads. The adsorbed PVA layer was crosslinked at a ratio of 10 mol glutaraldehyde/mol PVA. Various dye ligands were immobilised (Procion Blue MX-R, Cibacron Blue F3-GA, Procion Red HE-3B and Procion Yellow HE-3G). PVA was adsorbed, crosslinked and dyed as described in Section 2. The dynamic capacity was determined at 5% breakthrough, the point at which the absorbance (at 280 nm) of the outlet stream was 5% that of the feed stream. HSA (1 mg/ml) was applied to an HR5/5 column (5×0.5 cm I.D.) at 1 ml/min in 25 m*M* phosphate buffer, pH 5.0.

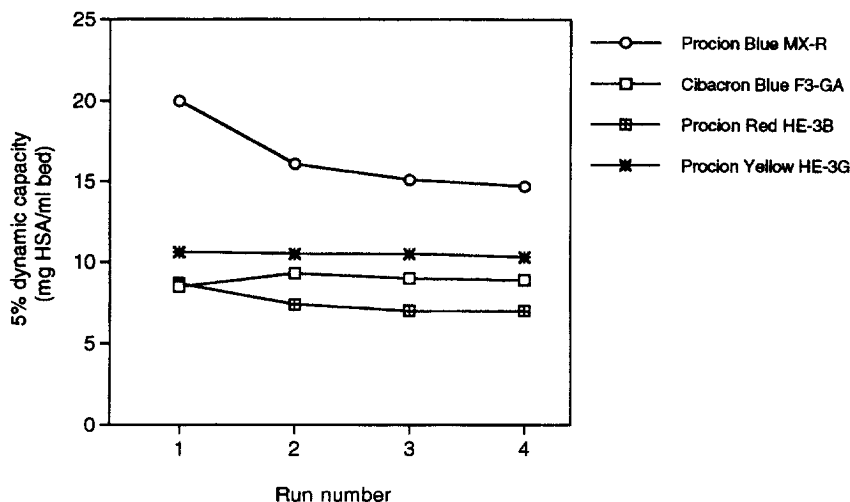

Fig. 5. HSA dynamic capacities on dye–PVA–PS–DVB CG1000sd beads. The adsorbed PVA layer was crosslinked at a ratio of 50 mol glutaraldehyde/mol PVA. Various dye ligands were immobilised (Procion Blue MX-R, Cibacron Blue F3-GA, Procion Red HE-3B and Procion Yellow HE-3G). PVA was adsorbed, crosslinked and dyed as described in Section 2. The dynamic capacity was determined at 5% breakthrough, the point at which the absorbance (at 280 nm) of the outlet stream was 5% that of the feed stream. HSA (1 mg/ml) was applied to an HR5/5 column (5×0.5 cm I.D.) at 1 ml/min in 25 m*M* phosphate buffer, pH 5.0.

At this crosslinking ratio, the PVA coating shields the PS–DVB surface much better than at the lower degree of crosslinking, and for some dye ligand types there was minimal irreversible binding.

3.7. Discussion

There has been substantial work carried out on controlled drug release from hydrogels, and the effect of glutaraldehyde crosslinking on PVA microspheres on the degree of molecular penetration has been studied [19,20]. PVA shrinks and swells under varying solvent conditions, and the shrinking and swelling characteristics of the PVA are heavily dependent on the PVA type and degree of crosslinking. However, studies carried out on protease enzyme release from PVA microspheres have indicated that the amount of enzyme entrapped, by diffusion and adsorption into the hydrogel particles, is dependent on the crosslinking density of the hydrogel [19]. The amount of enzyme entrapped decreases as the crosslinking ratio is increased, but at a crosslinking ratio of 0.2 mol glutaraldehyde/PVA repeating unit (equivalent to a crosslinking ratio of 80 mol glutaraldehyde/mol PVA in this study) substantial quantities of enzyme penetrated into the PVA gel after a contact time of 2 days. A PVA layer was also used for an oil-release study [20] and it was concluded that, as the degree of crosslinking increased, the degree of PVA layer swelling decreased and the polymer membrane became denser. The rate of diffusion of oil through the PVA layer decreased and this was attributed to the reduction of the molecular weight between cross-links and the associated decrease of macromolecular mesh size.

It has been shown that the PVA layer adsorbed onto a PS–DVB surface has a thickness of up to 200 Å [17,18]. Most of the layer thickness is due to tails of PVA molecules positioned away from the PS–DVB surface. These tails make up less than 5% of the total adsorbed mass [17]. The majority of the PVA will bind closely to the PS–DVB surface. It is likely, therefore, that the adsorbed PVA layer on the PS–DVB matrix surface will allow molecular penetration of protein through the layer onto the PS–DVB surface. Once in contact with the hydrophobic PS–DVB surface the protein is likely to bind irreversibly.

HSA did not adsorb to undyed crosslinked PVA–PS–DVB matrices, nor did it adsorb when eluent buffer (0.5 *M* thiocyanate) was applied to the Procion Blue MX-R–PVA–PS–DVB matrix [12].

These results indicate that irreversible binding only occurred once HSA had bound to the dye ligand and had thus come into close contact with the matrix surface. If the protein binds onto the matrix surface then it is more likely to penetrate the PVA layer than if the protein has no strong interaction with the surface.

There was minimal irreversible adsorption of lysozyme. HSA does, however, bind more tightly to the dye ligands tested than lysozyme, and so is more likely to get involved in further surface interactions.

4. Conclusions

The crosslinking of PVA plays an important role in determining the adsorption characteristics of PVA–PS–DVB matrices. Non-specific protein adsorption on PVA–PS–DVB matrices is minimised with even slight crosslinking. However, with low amounts of crosslinking and with certain protein–dye combinations, such as Procion Blue MX-R dye adsorption of HSA, there is a high and variable degree of irreversible binding and a drop in capacities with continued use. A higher degree of crosslinking decreases the amount of dye that can be immobilised, and increases the recovery of protein. Upon immobilisation of Procion Yellow HE-3G or Cibacron Blue F3-GA ligands, at a high crosslinking ratio (50 mol glutaraldehyde/mol PVA), there is minimal irreversible adsorption of HSA. There was also irreversible binding of HSA on Procion Blue MX-R dyed POROS 20 beads, indicating that Procion Blue MX-R modified PS–DVB matrices can bind HSA irreversibly even with other shielding techniques. However, the amount of HSA irreversibly bound was lower using this coating technique.

PVA coating and crosslinking on PS–DVB matrices creates a useful chromatography matrix that allows immobilisation of dye ligands. However this coating technique is not completely satisfactory as irreversible adsorption of HSA occurs on some dye–PVA–PS–DVB matrices, even if the PVA is crosslinked to a high degree.

Acknowledgments

The authors acknowledge support for this work from the BBSRC (UK).

References

[1] J. Bonnerjea, S. Oh, M. Hoare, P. Dunhill, Biotechnology 4 (1986) 954.
[2] C.R. Lowe, in: M.A. Vijayalakshmi, O. Bertrand (Eds.), Protein–Dye Interactions: Developments and Applications, Ch. 1, Elsevier Applied Science, 1989.
[3] S. Narayanan, J. Chromatogr. A 658 (1994) 237.
[4] Y.B. Yang, F. Nevejans, M. Verzele, Chromatographia 20 (1985) 735.
[5] C. Desilets, M. Rounds, F.E. Regnier, J. Chromatogr. 631 (1993) 107.
[6] L. Lloyd, J. Chromatogr. 544 (1991) 201.
[7] L. Varady, Y.B. Yang, S.E. Cook, F.E. Regnier, US Pat., 5 030 352, 1991.
[8] L. Varady, N. Mu, Y.B. Yang, S.E. Cook, N. Afeyan, F.E. Regnier, J. Chromatogr. 631 (1993) 107.
[9] Y.B. Yang, K. Harrison, J. Kindsvater, J. Chromatogr. A 723 (1996) 1.
[10] M. Leonard, C. Fournier, E. Dellacherie, J. Chromatogr. A 664 (1995) 39.
[11] A. Tuncel, A. Denzili, D. Purvis, C.R. Lowe, E. Piskin, J. Chromatogr. A 634 (1993) 161.
[12] D.C. Nash, G.E. McCreath, H.A. Chase, J. Chromatogr. A 758 (1997) 53.
[13] Y.D. Clonis, K. Jones, C.R. Lowe, J. Chromatogr. 363 (1986) 31.
[14] W.K. Alderton, C.R. Lowe, D.R. Thatcher, J. Chromatogr. A 677 (1994) 289.
[15] G. Johansson, M. Joelsson, J. Chromatogr. 393 (1987) 195.
[16] H.A. Sober (Ed.), CRC Handbook of Biochemistry: Selected Data for Molecular Biology, 2nd ed., CRC Press, Cleveland, OH, 1973.
[17] J.A. De Witt, T.G.M. van de Ven, Langmuir 8 (1992) 788.
[18] M.J. Garvey, Th.F. Tadros, B. Vincent, J. Colloid Interface Sci. 49(1) (1974) 57.
[19] A.R. Bachtsi, C. Kiparissides, J. Microencapsulation 12(1) (1995) 23.
[20] A.R. Bachtsi, C. Kiparissides, J. Control. Release 38 (1996) 49.