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Journal of Chromatography A, 755 (1996) 1–10

JOURNAL OF CHROMATOGRAPHY A

Dextran-coated silica and its behavior in high-performance size-exclusion chromatography

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Received 17 April 1996; revised 21 June 1996; accepted 21 June 1996

Abstract

Aminopropyltriethoxysilane-treated silica was coated with *p*-nitrophenyl chloroformate-activated dextran. The remaining active carbonates on the dextran layer were hydrolysed in aqueous environment. Hence, the silica surface is covered with a hydrophilic dextran layer free of residual active groups. The performance of this covalent dextran-coated silica was evaluated for its use in size-exclusion chromatography of biopolymers. From the obtained data, one can conclude that the dextrans form a good neutralizing barrier between acidic silanols and proteins. The protein adsorption was dependent on the ionic strengh of the eluent and the thickness of the dextran layer, which was controlled by the molecular mass of the applied dextran.

Keywords: Proteins; Dextran-coated silica; Coating; Stationary phases, LC

1. Introduction

Matrices based on cross-linked polysaccharides (e.g. dextrans) have been applied for many years in aqueous size-exclusion chromatography [1–3]. Unfortunatly, these soft gels cannot be used in high-performance chromatography due to their low pressure stability. In contrast, silica is mechanically stable but contains acidic silanol groups that cause strong and often irreversible non-specific adsorption of proteins in aqueous media [4,5]. By coating silica beads with polysaccharides, it is possible to combine the advantages of the traditional soft gels with the mechanical properties of silica supports.

The preparation of dextran-coated silica supports was already reported by Tayot and Tardy [6,7], and later by Muller and Jozefonvicz [8,9]. In these studies the silica beads were covered with a monolayer of diethylaminoethyl dextran (DEAE-dextran) at pH 11.5, followed by a crosslinking reaction with 1,4-butanediol diglycidyl ether. The DEAE-dextran-coated silica supports possess high hydrophilicity, good porosity, resistance to alkali and have no longer non-specific adsorption properties. They present minimal cation-exchange capacity, since it is difficult to obtain an exact balance between the ion-exchange capacities of the DEAE-dextran and the native silica. The total neutralization of the silanol functions requires an excess of positive charges and leads to a residual anion-exchange capacity in the coverlayer.

In view of the above, Muller and Jozefonvicz have prepared a double-coated silica support [10]. After a

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0021-9673/96/$15.00
PII S0021-9673(96)00592-4

preliminary coating with DEAE-dextran, the silica beads were recoated with native dextran or agarose in order to overlay the positive charges of the DEAE groups on the surface.

In this work, dextran-coated silica is prepared in an alternative way. Aminoalkyltriethoxysilane-treated silica was coated with chloroformate-activated dextran. The advantage of this method is the possibility to hydrolyse the remaining active carbonates on the dextran layer. Hence, the dextran coating is free of charges or other residual active groups. The performance of this covalent dextran-coated silica is evaluated for their use in size-exclusion chromatography of biopolymers.

2. Experimental

2.1. Reagents

The spherical silica, Bio-Sil (mean pore diameter: 11.6 nm, surface: 150 m^2/g, size: 5 μm) was kindly supplied by Bio-Rad (Eke, Belgium). Nucleosil 25.300 (mean pore diameter: 30 nm, surface: 100 m^2/g, size: 25–40 μm) and Nucleosil 25.500 (mean pore diameter: 50 nm, surface: 35 m^2/g, size: 25–40 μm) were purchased from Macherey–Nagel (Düren, Germany). 4-N,N-Dimethylaminopyridine and 3-aminopropyltriethoxysilane were obtained from Aldrich (Bornem, Belgium). *p*-Nitrophenyl chloroformate was from Merck (Darmstadt, Germany).

2.2. Preparation of dextran-coated silica

A 4-g amount of P_2O_5-dried dextran was dissolved in 200 ml of dimethyl sulfoxide–pyridine (1:1). Dimethylaminopyridine (0.2 g) and 1.5 g *p*-nitrophenyl chloroformate were added at 0°C. After 4 h, the activated dextran was precipitated in dry ethanol–diethyl ether (4:1). The suspension was filtered and washed several times with ethanol–diethyl ether (4:1) till the wash solvent was free of *p*-nitrophenol. *p*-Nitrophenol gives a yellow colour in an alkalic solution.

The aminopropylsilica was prepared in dry toluene as previously reported [11]. The conditions were chosen to obtain maximum coverage in each case. Only dry solvents were used, and moisture was excluded. A 5-g amount of aminopropylsilica was suspended in 60 ml dimethyl sulfoxide–pyridine (1:1) and degassed by ultrason vibration. While the suspension was gently stirred, 3.5 g chloroformate activated dextran, dissolved in 60 ml dimethyl sulfoxide, was added dropwise over 1 h. After 24 h of stirring, the silica beads were filtered and washed three times with 20 ml dimethyl sulfoxide, ten times with 100 ml water and three times with 20 ml ethanol–diethyl ether (1:1). The dextran-coated silica beads were dried for 24 h at 65°C.

2.3. Assay of the dextran content

The anthrone solution was prepared as reported by Black [12]. A 20-mg amount of dextran-coated silica was suspended in 1.5 ml 60% sulfuric acid. After 4 h of agitation, 0.9 ml of the upperlayer was withdrawn and added to 0.6 ml anthrone solution. This solution was placed in a boiling bath for exactly 15 min and immediately cooled in an ice bath. The content of dextran was determined by measuring the UV absorbance at 625 nm against a calibration curve, prepared with native dextran. The obtained results were in good agreement with thermogravimetric analysis.

2.4. Assay of the (residual) aminopropyl content

A published assay [13] based on the picric acid ion-pairing capacity was used to measure this property.

2.5. Chromatographic system

The high-performance size-exclusion chromatography (HPSEC) experiments were performed using a Kontron 420 HPLC pump (Kontron, Switzerland) fitted with a 150×4 mm I.D. column. The columns were of precision bore stainless steel with stainless steel frits at the ends. Samples were applied with a Valco C6W injector (Chenkon, Switzerland) equipped with a 25-μl loop. The samples were monitored with a LKB 2151 variable-wavelength monitor (Bromma, Sweden) or a Sicon Analytical LCD210 refractive index (RI) detector (Berlin, Germany). The recorder was a Kipp and Zn BD112 (Ankersmit, Belgium). The dextran-coated silica was

introduced into the column by a slurry packing method with isopropanol and a packing pressure of 400 bar. The colums were eluted at a flow-rate of 0.3 ml/min with a potassium dihydrogenphosphate–disodium hydrogenphosphate buffer (pH 7.00 and (ionic strength) I=0.2 or 0.088 M), prepared by a published procedure [14]. The eluent was prepared with Milli-Q water and analytical grade reagents. The solutions were filtered through a 0.45-μm Millipore membrane and degassed. The concentration of the solutes was 1 mg/ml and they were detected as follows: arginin (UV 220 nm), benzylalcohol (UV 250 nm), proteins (UV 280 nm and RI), water (RI), citric acid (RI) and the dextran standards (RI).

3. Results and discussion

3.1. Characterization of the dextran-coated silicas

Previous attempts to couple native dextran via reductive amination of the reducing end group [15] were not succesfull. Only oligodextran of molecular mass 1000 led to a significant coverage after a very long reaction time. In our opinion, this can be attributed to a conformation transition of dextran. Dextran of molecular mass below 2000 has a stretched uncoiled conformation. At higher molecular mass a randomly coiled structure is formed [16] which makes the end groups less accessible. Hence, in order to attach dextrans of various molecular masses onto aminopropylsilica, the dextran was modified with pendent reactive carbonate groups. The p-nitrophenyl chloroformate activation [17,18] was selected because of the high degree of activation that can be achieved. It was shown that up to 25% of the anhydroglucoside units can be substituted with an aromatic carbonate. As reported before [19] cyclic carbonates can be formed as well during this activation. However, both types of carbonate esters react smoothly with aliphatic primary amines.

In a first stage, the influence of the chloroformate activation on the coating properties was investigated. Several coatings were made using dextrans with a varying amount of reactive carbonates. The amount of coupled dextran was determined via the anthrone test and by thermogravimetric analysis.

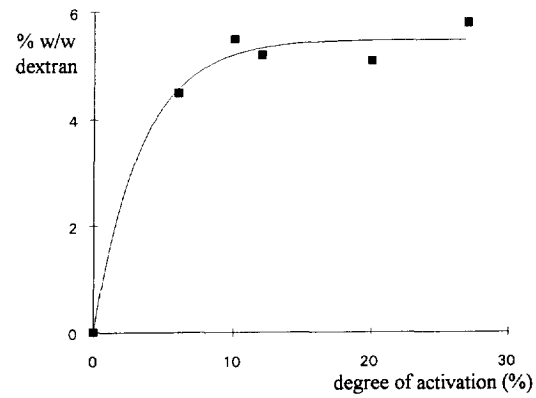

Fig. 1. Amount of M_r $10 \cdot 10^3$ dextran coated on Nucleosil 25.300 in relation to the activation degree of dextran.

From the results, given in Fig. 1, it was concluded that the amount of coated dextran does no longer increase as the activation degree is higher than 10% (10 carbonates per 100 glucosides). The evaluation of the different modified supports was limited to one hundred runs. During that time we did not observe a change in the behaviour of the reports. The dextran coating was found to be stable at room temperature at pH 8.0. No loss of organic material was observed after 3 months incubation.

In order to investigate the influence of the molecular mass of the dextrans, activated dextrans (10%) with different molecular mass were selected to cover the silica surface. The amount of coated dextran in the different modified silica beads, prepared in a similar way, is given in Table 1. The mass percent of dextran per square meter is also expressed to correlate for the different specific surfaces of the silicas used.

These results indicate that the molecular mass M_r of the dextrans had a minor influence on the surface coverage. Only M_r 1000 coated Bio-Sil 5.116 showed a significant lower coverage. This can be explained by the low degree of activation of the dextran (10%). A dextran polymer of molecular mass 1000 consists of only 6 to 7 glucose units. Hence the average degree of activation is less than one carbonate per dextran chain.

In addition to the dextran content, the number of aminopropylsilyl groups can influence the chromatographic properties of the support. Hence the amine content of the different modified supports was mea-

Table 1
Effect of molecular mass of dextran on the dextran content in modified silica

Silica	M_r of dextran $\times 10^3$	% Dextran coverage	μg Dextran/m^2
Bio-Sil 5.116	1	3.9	260
	5	11.2	746
	10	10.5	700
Nucleosil 25.300	10	5.5	550
	70	5.7	570
Nucleosil 25.500	10	2.4	685
	70	2.5	714

The determinations were carried out by anthrone reagents and thermogravimetric analysis. The quantity dextran per square meter was calculated by applying the surface data provided by the manufactures.

sured by the picric acid counter ion-capacity test. The obtained results, summarized in Table 2, clearly indicate that the number of residual propylamines increases with increasing molecular mass of the dextran coatings. The exception observed for the M_r 1000 dextran-coated Bio-Sil 5.116 can be explained by its inadequate coating (cf. Table 1).

The properties of the different modified silicas were evaluated as support for HPSEC analyses. This characterisation consisted of two parts. In a first instance, low-molecular-mass probes were used in order to identify the conditions under which non-size-exclusion effects occurred. A similar characterisation has been described by Regnier et al. [20] for a number of commercial HPSEC columns. Hence, it was possible to compare the results of the different dextran-coated silicas with that data. Subsequently, an investigation was performed to mark protein-support adsorption effects.

3.2. Evaluation of dextran-coated silica using low-molecular-mass probes

It was anticipated that, if no specific interactions occur, all species would have a distribution-coefficient K_D of 1, according to Eq. (1):

$$V_E = V_o + K_D V_i \quad (1)$$

where V_E is the elution volume of a product, K_D the corresponding distribution coefficient, V_o the void volume and V_i the pore volume of the column. Deviations from Eq. (1) indicates non-size-exclusion partitioning. It was reasoned that column adsorption

Table 2
Initial and residual propylamine content of the different dextran-coated silica materials

Kind of modified silica	Type of dextran	Amine content	
		μmol NH_2/g	μmol NH_2/m^2
Bio-Sil 5.116	NH_2 [a]	608	4.05
	M_r $1 \cdot 10^3$	420	2.80
	M_r $5 \cdot 10^3$	266	1.77
	M_r $10 \cdot 10^3$	353	2.35
Nucleosil 25.300	NH_2 [a]	215	2.15
	M_r $10 \cdot 10^3$	44	0.44
	M_r $70 \cdot 10^3$	140	1.40
Nucleosil 25.500	NH_2 [a]	108	3.08
	M_r $10 \cdot 10^3$	31	0.88
	M_r $70 \cdot 10^3$	49	1.40

The determination is based on the picric acid counter ion capacity in CH_2Cl_2.
[a] Unmodified aminopropylsilicas.

effects of an anion-exchange, cation-exchange and hydrophobic nature could be studied by treating the columns as either ion-exchange or reversed-phase materials. Under these conditions low ionic strength favors ion exchange, while high-ionic-strength mobile phases would produce solvophobic effects. In this study, we used phosphate buffer pH 7.0 ($I=0.2$), a buffer frequently used in protein analysis. With this ionic strength Regnier [20] marked ionic as well as solvophobic interactions. The cation- and anion-exchange partitioning was investigated using arginine, resp. citric acid. Benzyl alcohol a neutral, aromatic and water-soluble molecule served as probe for solvophobic effects. Dextran T-2000 and [^{2}H]-water were used as probes to estimate the accurate void volume (V_o), respectively the total liquid volume (V_T). As the total liquid volume, $V_T=V_o+V_i$, substituting for V_i, in Eq. (1) gives $K_D=(V_e-V_o)/(V_T-V_o)$. The K_D values for the columns packed with modified silicas are given in Table 3.

The low but significant retardation observed for arginine and benzyl alcohol suggests that these columns behaved under the given elution conditions as weak cation exchangers with some hydrophobic effects. That is surprising since an anion-exchange effect was expected due to remaining primary amines. At pH 7.0, these primary amines should act as positively charged ionic groups. However, it can be expected that in addition to residual propylamines, some free silanols were still present. These could result in a net negative charge of the surface. It is worth noting that similar interactions have been reported for glucosylated silica [21]. These supports are prepared via covalent coupling of glucose onto aminopropylsilica by reductive amination. Comparison of our results with the data reported by Regnier [20] demonstrates that the ion-exchange effect of our material is in the same range as observed for the commercial silica-based supports. However, the solvophobic retentions are lower for our silica materials. In the literature, similar cation-exchange [22] and hydrophobic interactions [23,24] were also reported for Sephacryl and Sephadex gels, which are cross-linked dextran matrixes. The authors attributed the cation-exchange effects to the presence of carboxyl groups on the dextran matrix, while the hydrophobic interactions were attributed to the hydrophobic nature of the cross-linking agent.

3.3. Evaluation using high-molecular-mass probes

The performance of the different dextran-coated silicas as SEC support for biopolymers was investigated by calibration with dextran standards and globular proteins. The molecular parameters of the dextran standards and the proteins used in this study are summarized in Table 4 and Table 5.

The separation of dextrans by size exclusion is easy since interaction with stationary phases is rare to occur. For that reason, dextrans have been recommended for the calibration of SEC columns [38,39]. Unlike the dextrans, the elution of proteins, which are polyelectrolytes, show interaction with most stationary phases, depending on the nature of the bonded phase and the presence of unreacted silanols. To suppress these interactions, it is convenient to use mobile phases of a high ionic strength and/or modifiers [40]. In this work, the mobile phase

Table 3
K_D values of arginine, citric acid and benzyl alcohol for the silicas coated with dextran of different molecular mass

Support	M_r of dextran ($\times 10^3$)	K_D value		
		Arginine	Citric acid	Benzyl alcohol
Bio-Sil 5.116	1	1.68	0.97	1.27
	5	1.21	1.00	1.24
	10	1.08	1.00	1.24
Nucleosil 25.300	10	1.03	1.00	1.16
	70	1.04	1.00	1.08
Nucleosil 25.500	10	1.09	1.00	1.13
	70	1.11	n.d.	1.11

Column: 15×0.4 cm I.D.; flow-rate: 0.3 ml/min; buffer: phosphate pH 7.0, $I=0.2$.

Table 4
Characterisation of the dextrans used in this study

	$M_{r,peak}$ [a]	$[\eta]$ (ml/g) [b]	$\log M_r[\eta]$	R_h [d] (nm)
Pharmacosmos dextran 670	401 300	75.2	7.48	16.7
410	276 000	62.6	7.24	13.9
270	196 000	52.8	7.01	11.7
150	123 600	42.1	6.71	9.31
80	66 600	31.0	6.31	6.84
50	43 500	25.2	6.04	5.53
25	21 400	17.7	5.57	3.89
12	9890	12.1	5.08	2.65
5	4440	8.17	4.56	1.78
1	1080	4.07[c]	3.64	0.88

[a] From manufacturer (Pharmacosmos, Viby Sj., Denmark).
[b] Calculated from column 2, using the following equation $[\eta]=0.13M_r^{0.493}$ ml g^{-1} [25].
[c] Only applicable for dextran of molecular mass above 2000 [26].
[d] This viscosity radius is calculated from $R_\eta=0.0271M_r^{0.498}$ nm [27].

was the same as in the evaluation with low molecular probes. This allowed us to detect non-size-exclusion properties of the support.

Using the concept of universal calibration, proposed by Benoit et al. [41] and assuming that dextrans show an 'ideal' size-exclusion behavior, it was possible to create an 'ideal' calibration plot for the proteins. The elution volumes of the proteins had to fit with this curve. Deviation is an indication for interaction with the support. The intrinsic viscosity $[\eta]$ of the dextran standards, given in Table 4, were calculated using Eq. (2) [42]:

$$[\eta] = 0.13\, M_r^{0.493} \quad (ml/g) \tag{2}$$

For the proteins for which the intrinsic viscosity has not been reported, viscosity numbers were calculated from the published viscosity radius R using Eq. (3) [43,44]:

$$[\eta] = \frac{\pi N_A R_\eta^3}{30 M_r} \tag{3}$$

with $[\eta]$ the intrinsic viscosity, R the viscosity radius, M_r the molecular mass and N_A the Avogadro number. In order to allow comparison of the results for the proteins with those obtained for the dextrans, the viscosity numbers had to be determined in the same conditions. However, this is not true for all the data listed. Nevertheless, within experimental errors, the calculated values seem to be valid, because the intrinsic viscosity of proteins is insensitive to the

Table 5
Characterisation of the proteins used in this study

Kind	Source	[a]	M_r	pI	$[\eta]$[b] (ml/g)	$R_{[\eta]}$ (nm)	$\log M_r[\eta]$	Ref.
Thyroglobulin	Bovine	P	660 000	5.1	4.7	7.9	6.49	[28]
Ferritin	Horse spleen	P	440 000	5.0	3.8	6.1	6.22	[29]
Catalase	Bovine liver	P	232 000	5.4	3.8	5.2	5.95	[30]
Aldolase	Rabbit	P	158 000	–	3.9	4.6	5.79	[31]
Albumin	Bovine serum	S	67 000	5.5	3.8	3.5	5.41	[32]
Ovalbumin	Chicken egg	S	43 700	4.7	3.2	2.8	5.14	[33]
Chymotrypsin	Beef	S	21 400	9	4.1	2.1	4.99	[34]
Lysozyme	Chicken egg	S	14 400	11	2.8	1.9	4.60	[35]
Cytochrome *c*	Horse heart muscle	J	12 600	10.5	2.5	1.7[c]	4.49	[36]

[a] Supplier: P = Pharmacia, S = Sigma and J = Janssen Chemica.
[b] Calculated from column 7, via Eq. (2).
[c] This value is calculated from the diffusion coefficient. These size estimates are nearly equivalent for the native, globular proteins [37].

temperature [45] and the solvent system has no significant effect on the Stokes radius of globular proteins near pH 7 [46]. On the other hand, the intrinsic viscosity of dextrans is not sensitive to small changes in the salt concentration of buffer solutions [42]. The universal calibration graphs for the different supports are expressed in Fig. 2, Fig. 3, and Fig. 4.

Fig. 2 clearly demonstrates that the protein–support interactions decreased when higher-molecular-mass coatings were applied. Moreover, for the M_r 1000 dextran coating, these interactions were so large that all the proteins eluted after the solvent peak. These observations confirm the significant non-size-exclusion effects of the low-molecular-mass probes (cf. Table 3) and the inadequate amount in the dextran coating (cf. Table 1). The adsorption of the positive ($pI>7$), as well as the negative ($pI<7$) charged proteins demonstrate that the elution was also influenced by other interactions than Coulombic ones, probably hydrophobic interactions. Nevertheless, the remarkable decrease of non-size-exclusion effects by applying higher molecular mass dextrans is promising. Unfortunately, in spite of the promising trend, the performance of the supports decreased for the higher-molecular-mass dextran coatings. This is explained by the fact that the thickness of the dextran layer increased when higher-molecular-mass dextrans were applied. Subsequently, the pore volume and the permeability of the column decreased. The decrease in the pore size is demonstrated by the position of the dextran calibration curves in Fig. 2. The K_D value expresses the fraction of the pore volume which is effectively accessible for a particular solute. It is clear that the accessible pore volume of the M_r $10 \cdot 10^3$ dextran coating was almost

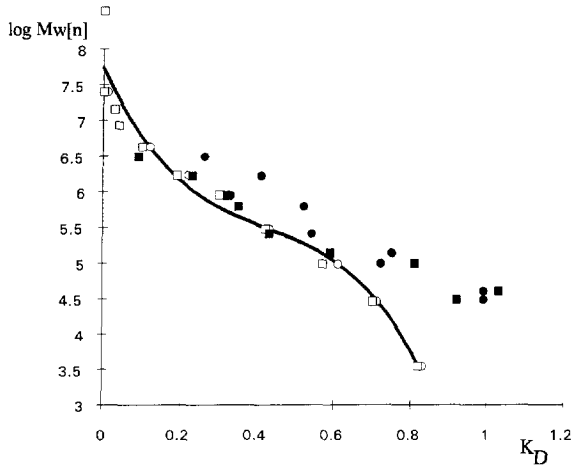

Fig. 3. SEC calibration curves of dextran standards and proteins on dextran-coated silica 25.300. The results for different molecular mass coatings are symbolized as follows: M_r $10 \cdot 10^3$: ○ and ● and M_r $70 \cdot 10^3$: □ and ■. The open and filled symbols represent the dextran standards, respectively the proteins.

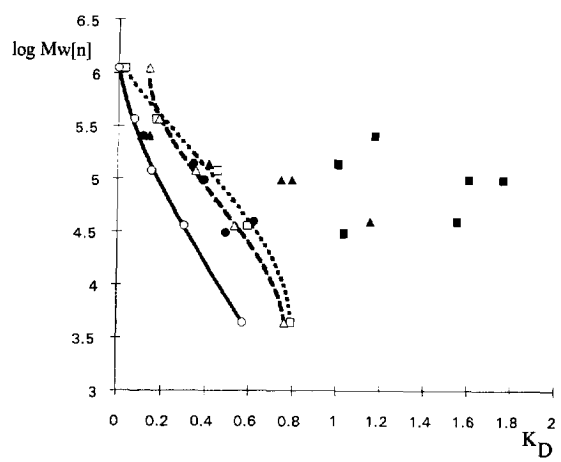

Fig. 2. SEC calibration curves of dextran standards and proteins on dextran-coated silica 5.116. The results for different molecular mass coatings are symbolized as follows: M_r $1 \cdot 10^3$: □ and ■; M_r $5 \cdot 10^3$: △ and ▲; M_r $10 \cdot 10^3$: ○ and ●. The open and filled symbols represent the dextran standards, respectively the proteins.

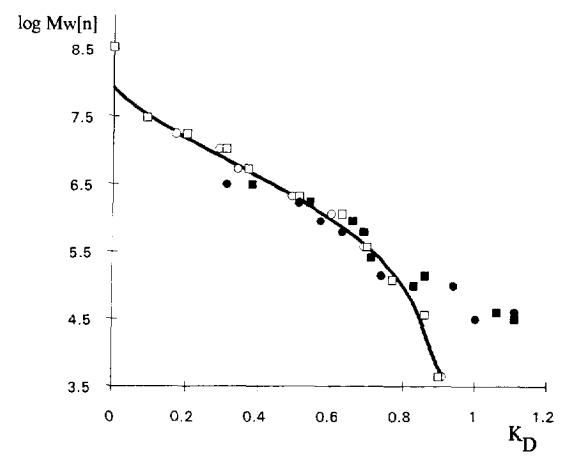

Fig. 4. SEC calibration curves of dextran standards and proteins on dextran-coated silica 25.500. The results for different molecular mass coatings are symbolized as follows: M_r $10 \cdot 10^3$: ○ and ● and M_r $70 \cdot 10^3$: □ and ■. The open and filled symbols represent the dextran standards, respectively the proteins.

20% smaller than the M_r $5 \cdot 10^3$ coating. This relative large decrease of the pore volume between the M_r $10 \cdot 10^3$ and the M_r $5 \cdot 10^3$ dextran-silica was not expected by the results given in Table 1, where an equal amount of bounded dextran was found. An acceptable explanation is that high-molecular-mass coatings result in dextran layers of lower density, due to the swelling of the dextran coil. Indeed, the density of solubilized dextran decreases in relation to the molecular mass, as is expressed by the Mark–Houwink equation [42]. Atomic force microscopy (AFM) [47], surface plasmon resonance (SPR), ellipsometry and angle-resolved XPS experiments [48] were carried out to complement this observation. From the obtained results, the trend of increasing thickness respectively decreasing density of the dextran layer with increasing molecular mass can be confirmed. However, when comparing the M_r $1 \cdot 10^3$ and the M_r $5 \cdot 10^3$ dextran coating, the decrease of the pore volume was not significant, although the dextran content of the last one was even larger. This is difficult to explain at the moment.

Given the fact that higher-molecular-mass coatings on Bio-Sil 5.116 would result in a drastic loss of pore volume and that higher-molecular-mass dextran coatings were necessary to suppress the protein support interactions, higher-molecular-mass coatings were investigated on 30 nm and 50 nm pore size material. The results are expressed in Figs. 3 and 4. There was no remarkable decrease of the pore size when comparing the M_r $70 \cdot 10^3$ dextran and the M_r $10 \cdot 10^3$ coated materials. For this reason, the plotted calibration curve is representative for the M_r $10 \cdot 10^3$, as well as the M_r $70 \cdot 10^3$ dextran coating. Apparently, the difference of the thickness is too small in comparison to the mean pore diameter of the support. The lower adsorption effects observed for these larger pore size material can be attributed to the lower specific surface. Nevertheless, the M_r $10 \cdot 10^3$ dextran coating on the 30 nm pore size silica showed still a significant protein adsorption, whereas the M_r $70 \cdot 10^3$ dextran coating suppressed the protein adsorption almost completely. Hence, this confirms our earlier observation for Bio-Sil 5.116 that higher-molecular-mass coatings resulted in an improved suppression of the protein–support interactions.

In spite of the fact that the M_r $70 \cdot 10^3$ dextran-coated supports did not show non-size-exclusion behavior for the higher-molecular-mass proteins, the retardations for the smaller ones were still remarkable. Moreover, the retardations of these smaller proteins were not decreased in comparison with the M_r $10 \cdot 10^3$ dextran coatings. It is quite reasonable that smaller molecules could penetrate the dextran barrier to interact with the surface silanol groups, while larger molecules would have more difficulty because of steric hindrance. Nevertheless, it remains difficult to distinguish if the observed retardations of the smaller proteins can be attributed to imperfections of the coating or whether they are inherent to our model, the comparison of elution times of dextrans and proteins of equal size. Moreover, literature data demonstrate that these proteins interact with a lot of commercial SEC columns [49,50], while other authors indicate a disparity between stokes radii of these proteins and dextrans as determined by retention volume in SEC [51,52].

The following observation for higher-molecular-mass dextran coatings support the conclusion that the thickness of the bounded layer is dominant to its density. There is a reduction of the protein adsorption effects, while the amount of dextran bounded on the surface is equal to the lower-molecular-mass analogues. Hence, one can conclude that as the thickness of the dextran layer exceeds the counter-ion double layer thickness of the residual ionic groups on the silica surface, electrostatic interactions with the proteins are masked. This hypothesis was confirmed by repeating the above experiments with eluents of various ionic strengh. For higher ionic strengh a complete masking for a M_r $10 \cdot 10^3$ dextran coating was observed, while lower ionic strengh resulted in adsorption effects on the M_r $70 \cdot 10^3$ dextran-derivatised silica.

4. Conclusion

The present study demonstrates that the surface of aminopropyltriethoxysilane-treated silica beads can be masked efficiently by coating with chloroformate activated dextran. The supports were evaluated using low- and high-molecular-mass probes under mediate ion strengh conditions ($I = 0.2$). The collected data made us conclude that M_r $70 \cdot 10^3$ dextran coatings on supports of a pore size of at least 30 nm provide

good size-exclusion properties for biopolymers. Lower-molecular-mass coatings lead to increased protein–support interactions, while smaller pore size supports lead to a remarkable loss of the pore volume and consequently a decreased resolving power of the columns. Since the dextran-coated support possesses still residual propylamines under their dextran layer, the adsorption is expected to be dependent on the ionic strengh of the eluent. Hence, the performance of the modified supports will be dependent on the elution conditions.

Acknowledgments

G.M. is indebted to the I.W.O.N.L. (Belgian Institute for Encouragement of Research in Industry and Agriculture) for a research fellowship. This work was also supported by the Belgian Government, Dienst voor Programmatie van het Wetenschapsbeleid, PAI-III-040. We want to thank Dr. C. Dewaele of Bio-Rad RSL N.V. for his useful comments during the preparation of this manuscript.

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