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Synthesis and chromatographic characterization of dextran-coated zirconia high-performance liquid chromatographic stationary phases

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Abstract

Porous zirconia particles made by the oil emulsion (OE) method and the polymerization-induced colloid aggregation (PICA) method have been coated with a small, carboxymethylated (~5%) dextran polymer and crosslinked in place. The parameters of the coating process (dextran concentration, adsorption time and crosslinker concentration) have all been examined and an optimum value for each determined. The coated and uncoated materials were characterized by nitrogen sorptometry and size-exclusion chromatography (SEC) using solutes (polystyrenes and dextrans) of well-defined molecular masses. Nitrogen sorptometry results show that the PICA material has a much lower pore volume and smaller pore diameter than do the OE materials. Despite this, the elution volumes of the SEC probes change very little upon polymer coating the PICA material while the OE material shows a very large change upon coating.

Keywords: Stationary phases, LC; Zirconia; Dextran-coated zirconia; Coating; Butanediglycidyl ether

1. Introduction

Our laboratory has been developing porous zirconia as a stationary phase support for liquid chromatography [1–16]. Zirconia, while chemically and mechanically stable, cannot be covalently modified like silica due to the instability of Zr-C and Zr-O-Si bonds in water [10]. The surface chemistry of zirconia is also complex. Strong Lewis acid sites on the surface adsorb any available hard Lewis base, such as phosphate, fluoride and carboxylic acids [10]. To achieve different retention mechanisms and block surface interactions, the surface of the zirconia can be coated with a polymer. We have used polyethyleneimine [8] and polybutadiene [15] to make an anion-exchange and reversed-phase material, respectively.

Many other polymer phases have been investigated recently using silica as the supporting substrate ([17] and references therein). We are particularly interested in polysaccharides because of their hydrophilicity and the ease with which they can be further derivatized. Cellulose has been immobilized on the surface of silica and then derivatized for use as an affinity chromatographic phase [18]. Another group has synthesized chiral stationary phases based on derivatized cellulose immobilized on silica [19–22]. Others have adsorbed a slightly functionalized (diethylaminoethyl groups) dextran molecule on silica and then crosslinked it in place to make a size exclusion chromatographic phase [23–25]. Dextran-coated silica particles have also been derivatized to

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make an affinity chromatographic phase for proteins [26,27]. We are interested in using dextran to coat zirconia particles to make a stationary phase that combines the positive aspects of dextran (hydrophilicity and easy modification) with those of zirconia (pH and mechanical stability).

Dextran is a polymer of glucose connected by 1→6 β linkages with some branching as the 1→4 β link (see Fig. 1). The branching occurs on about 5% of the glucose units. The large fraction of pendant alcohol groups of the dextran make it very hydrophilic. The alcohols are also easily derivatized by simple chemistry to a variety of useful groups, including anion-exchange groups (diethylaminoethyl) [23] and affinity groups (triazine dyes) [27]. The chemistry of the alcohol groups also allows simple crosslinking using diepoxides such as 1,4-butanediol diglycidyl ether (BUDGE) [24].

We were interested in the effect polymer coating would have on the pore space of the zirconia materials. This could cause a decrease in pore volume and surface area and slow diffusion of solutes in the stationary phase, leading to poor chromatography [28]. Nitrogen sorptometry may not provide a useful analysis of the dextran-coated material because the surface must be completely dehydrated to make the measurement. Under actual chromatographic conditions in an aqueous mobile phase, the dextran will certainly be highly swollen and fill more space than under the vacuum conditions of the sorptometry measurements. Mercury porosimetry has also been used extensively for pore size characterization. We have shown that nitrogen sorptometry and mercury porosimetry are in good agreement for our materials [49] and thus we chose not to do this experiment. We chose to use inverse

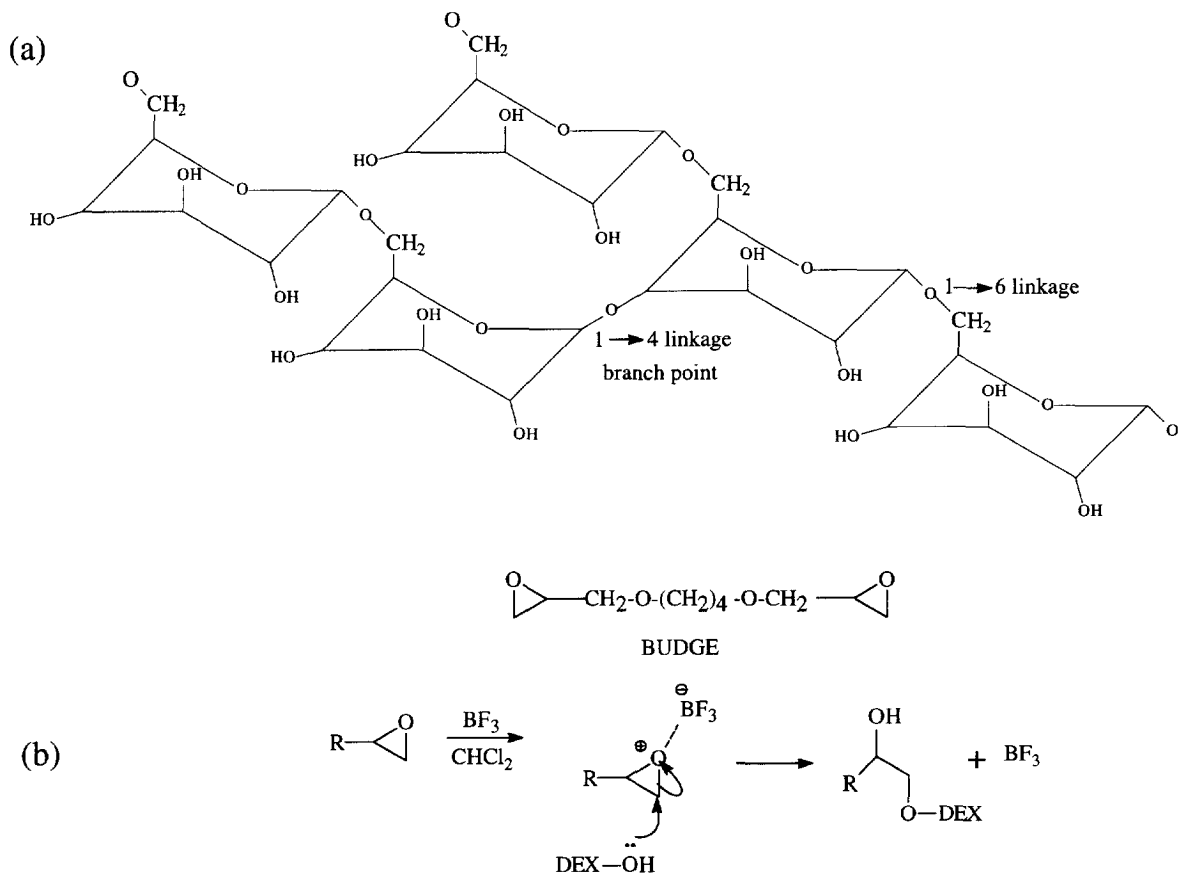

Fig. 1. Structures of the coating material. (a) Dextran. (b) 1,4-Butanediglycidyl ether (BUDGE) and the reaction mechanism of the crosslinking reaction.

size-exclusion chromatography (SEC), which has also been called "macromolecular porosimetry" [29], to investigate the pore space of the coated materials [29–37]. Inverse SEC uses probes of well-defined molecular mass (and thus size) to probe the pore space of the chromatographic material. Although this method has been used to determine pore size distributions [29,34], we will use it in a much more qualitative way. We will monitor the SEC behavior of the probe solutes to determine if the coating significantly changes the accessible pore space of the material. We previously examined the nitrogen sorptometry and SEC behavior of the bare zirconia materials [38]. We found that the nitrogen sorptometry results and the size-exclusion results did not agree in terms of the pore volumes. Thus, both methods should be run to gain a more complete understanding of the effect of polymer coating on the pore space.

In this report, we investigate several parameters in the zirconia coating process. Since this is an adsorptive coating procedure, we were concerned about the possibly slow kinetics of adsorption. We also wanted to maximize the loading of dextran on the zirconia particles to block the Lewis acid sites without blocking access to the pore space. The crosslinking step with BUDGE is also of interest. After an optimized coating procedure was developed, the effect dextran coating has on the accessible pore space, as measured by SEC and nitrogen sorptometry, was investigated. The differences between zirconia particles prepared by two different aggregation methods were also investigated. Particles were prepared by the oil emulsion method (OE) [39] and by the polymerization-induced colloidal aggregation (PICA) method [40]. Our secondary interest in this study is to determine if the dextran coating behaves differently on the two materials.

2. Experimental

2.1. Materials used

Zirconia colloid (1000 Å in nitric acid, nominal pH 2, 20% solids by mass) was obtained from Nyacol (Ashland, MA, USA). The dextran (M_r 9300), fluorescein isothiocyanate (FITC)-labeled dextrans, piperazine-N,N′-bis-[2-ethanesulfonic acid] (PIPES) and iodoacetic acid were obtained from Sigma (St. Louis, MO, USA). Polystyrene probes were purchased from Polysciences (Warrington, PA, USA). Dibasic potassium phosphate (reagent grade), sodium chloride (reagent grade) and methanol (HPLC grade) were obtained from Mallinckrodt (Paris, KY, USA). Concentrated sodium hydroxide solution was obtained from Fisher Chemicals (Fair Lawn, NJ, USA). Concentrated hydrochloric acid and HPLC-grade tetrahydrofuran (THF) were purchased from EM Science (Gibbstown, NJ, USA). All water was deionized and then passed through Barnstead (Boston, MA, USA) ion-exchange and organic-free cartridges followed by a 0.45-μm filter. All water was also boiled for 15 min to remove dissolved carbon dioxide.

2.2. Preparation of zirconia particles

Zirconia particles for chromatography were prepared from colloidal zirconia by two methods. The first is an OE process that produces particles of about 20–30 μm [39]. The second method is PICA [40]. It can be tuned to produce monodisperse particles in the range of 2–8 μm [40]. Particles were made by both processes from the same batch of colloid. The colloid had been centrifuged to remove fines and then resuspended in a 1% nitric acid solution. All batches of particles were sintered at 750°C for 6 h and then at 900°C for 3 h in a muffle furnace [40]. The particles were then pre-treated in a series of chemical steps [38] and dried under vacuum at 110°C. The batch designations for the materials were OM-10 (hereon OE) for the oil emulsion material and Coac-15 (hereon PICA) for the PICA material. The surface area and pore volume of the particles were measured by the nitrogen adsorption isotherm using a Micromeritics ASAP 2000 porosimeter. Surface areas were calculated by the BET method [41]. Pore diameters were estimated using the BJH equation, which assumes cylindrical pores [42]. The physical characteristics of both types of particles are shown in Table 1.

2.3. Preparation of carboxymethylated dextran

A 10-g amount of dextran (M_r 9300) was dissolved in 40 ml of water and cooled to 0–4°C using an ice bath. A 40-ml volume of freshly made 12.5 *M*

Table 1
Physical characteristics of the porous zirconia materials

Material	OE	CMD–OE	PICA	CMD–PICA
Synthesis method	Oil emulsion	Oil emulsion	PICA	PICA
Particle size (μm)[a]	25	25	6	6
Surface area (m^2/g)[b]	29	22	28	21
Pore volume (ml/g)[c]	0.25	0.21	0.14	0.12
Average pore body diameter (Å)[d]	350	390	200	200
Average pore neck diameter (Å)[e]	270	280	170	160

[a] Average from scanning electron micrographs.
[b] From nitrogen sorptometry data using the BET model [41].
[c] From nitrogen sorptometry data.
[d] Median pore body diameter from nitrogen sorptometry (adsorption mode) using the BJH model [42].
[e] Median pore throat diameter from nitrogen sorptometry (desorption mode) using the BJH model [42].

sodium hydroxide, prepared from a concentrated solution, was added and the solution was stirred at 4°C for 30 min. Iodoacetic acid (10.5 g) was added gradually over 10 min. After all of the iodoacetic acid had been added, the solution was stirred for 10 min at 4°C. The temperature was increased to 60°C and the solution was stirred for 30 min. The solution gradually became a darker yellow color as the reaction proceeded. The solution was then cooled in an ice bath and the pH was reduced to nine with concentrated hydrochloric acid. Methanol was gradually added to the solution while stirring, to precipitate the carboxymethyl dextran (CMD). The supernatant was decanted and the precipitate redissolved in water and reprecipitated using methanol. The twice precipitated CMD had a slight yellow color, probably due to residual iodine. The substitution of carboxylic groups was determined by using the assay of Horikawa and Tanimura [43]. Acetic acid was used for the calibration curves. The average percent substitution was 5.1%, or about three carboxymethyl groups per chain of 57 glucose monomers.

2.4. Isotherm measurements

Nine samples containing 0.50 g each of zirconia particles (OE) were placed in 15-ml vials. Different concentrations of CMD were prepared by dilution from a common stock solution. All solutions were made in 100 m*M* PIPES, a non-interacting buffer, at pH 6.5. A 10.0-ml aliquot of each of these solutions, an unbuffered water sample and a blank PIPES sample were added to the vials containing the zirconia particles. These samples were then capped and placed on a shaker bath for three days, occasionally being removed and shaken vigorously to suspend the zirconia. Each coated sample was filtered individually and the supernatant was collected for later testing. The zirconia particles were collected and dried at 60°C under vacuum for 12 h. The adsorbed dextran was removed from the particles by the following procedure. The dried, coated particles were weighed and placed in Erlenmeyer flasks containing 15.0 ml of 2 *M* sulfuric acid. After one week, the samples were filtered and the supernatant was collected. The zirconia particles were then washed with a 6.0-ml aliquot of 10 *M* sodium hydroxide and collected in the same flask as the acidic supernatant. The pH after this step was six, as measured using pH paper. The samples of supernatant from the adsorption step and from solutions containing the stripped dextran that had high concentrations were diluted 1:10 by volume, then assayed for dextran using the phenol–sulfuric acid assay [44].

2.5. Kinetics of the adsorption experiment

A 20.0-ml aliquot of a 5.0 g/l solution of CMD in 100 m*M* PIPES was added to 1.00 g of OE zirconia and placed on a shaker bath at room temperature. Approximately once every 24 h, the particles were allowed to settle for 30 min and a 1.0-ml volume of the solution was withdrawn. After one week (seven

samples total), the collected samples were analyzed for dextran by the phenol–sulfuric acid assay.

2.6. Measurement of optimum BUDGE concentration

A 27.00-g sample of zirconia (OE) was placed in 270 ml of a 5.00 g/l solution of CMD in 100 m*M* PIPES (pH 6.5) and rocked for two days at room temperature. The supernatant was decanted and the particles were washed with 100 ml of ethanol, 100 ml of ethanol–chloroform (50:50, v/v) and 100 ml of chloroform, successively. The coated particles were dried under a slight vacuum. Samples (4.00 g each) were then placed in 30-ml flasks with septa caps and 10.0 ml of chloroform were added. Each sample had BUDGE added to make different concentrations (0.235, 0.144, 0.083, 0.033 and 0.015 m*M*) followed by 0.50 ml of a 20% (v/v) solution of boron trifluoride etherate in chloroform. The reaction was allowed to proceed for 1 h with occasional swirling. The particles were washed with chloroform followed by ethanol and then dried overnight at ambient temperature.

A 0.50-g sample was taken from each of these vials and suspended in 8.0 ml of water. The slurry was then poured into a plastic chromatography column terminated with a silica frit (Bio-Rad, Richmond, CA, USA). After the water level reached the top of the particle bed, a 5.0-ml volume of 0.100 *M* sodium hydroxide was added to each tube. The supernatant was collected, neutralized with hydrochloric acid, and assayed for dextran using the phenol–sulfuric acid assay.

2.7. Preparation of carboxymethyl dextran-coated zirconia

Our method for the preparation of CMD was adapted from the method of Santarelli et al. [24]. CMD (0.10 g) was dissolved in 50.0 ml of 100 m*M* PIPES, pH 6.5, to make a 5.0 g/l solution of CMD. A 4.00-g amount of zirconia particles were added to 40.0 ml of this solution and sonicated under vacuum for 5 min, capped, and placed on a shaker bath for two days, with periodic manual shaking to resuspend the particles. The supernatant was decanted and 40 ml of ethanol were added. The slurry was shaken for 10 min, the particles allowed to settle for 30 min, and the ethanol was decanted. This procedure was repeated with an ethanol–chloroform (50:50, v/v) solution and chloroform after which the particles were then allowed to dry at room temperature.

We used a modification of the method of Hjerten and Liao [45] to crosslink the dextran. The coated particles, along with 10.0 ml of chloroform, were placed in a 30-ml septum-capped flask and sonicated for 5 min. A 17-μl volume of BUDGE was added and the flask was capped as nitrogen was blown over the top. In a separate septum flask, 7.0 ml of chloroform was added and capped. A 0.5-ml aliquot of boron trifluoride etherate was added. A 0.5-ml volume of this solution was added to the flask containing the coated particles and BUDGE solution. The particle suspension was swirled and allowed to sit for 30–40 min. After this time, the solution was removed and the particles were rinsed with ethanol and dried. Carbon analysis was performed on the particles by MHW Laboratories (Phoenix, AZ, USA). The results are shown in Table 2. Nitrogen sorptometry measurements were made as on the bare materials and are shown in Table 1.

2.8. Chromatography

All materials were packed in 5.0×0.46 cm stainless steel columns by the stirred upwards slurry technique [46], using HPLC-grade isopropanol. Chromatography was done on either a Hewlett-Packard (HP) (Rocklin, CA, USA) 1090 liquid chromatograph with a filter photometric detector with a HP 3393 integrator (System I) or a HP 1090L liquid chromatograph with diode array detection and a Chemstation data handling system (System II). Solutes used in the aqueous mobile phase experiments were FITC-labeled dextrans of different molecular masses (See Fig. 2 and Table 3) and *p*-nitrophenylglucose (pnp-glucose). Experiments in the non-aqueous mobile phase used polystyrene probes of different molecular masses. The structures of the probes are shown in Fig. 2 and the relevant properties are in Table 4. All other parameters are discussed in the figures. Retention volumes were calculated from the retention times as measured by the integrator or integration program (i.e. the peak maximum) and the flow-rate. All retention volumes

Table 2
Carbon analysis results for CMD–ZrO_2 materials

Material	%C	Surface area (m^2/g)	μmol glucose/m^2 [c]
CMD PICA	0.87	28[a]	4.3
CMD OE	1.16	29[a]	5.6
Dextran–silica [25]	2–6.4	78[b]	3.6–12
Dextran–silica [24]	3.8–4.7	25[b]	21–27
Dextran–silica [24]	2–4.8	125[b]	2.2–5.6
Cellulose–silica [18]	22–31[d]	190[b]	9–14

[a] Calculated from the nitrogen sorptometry results using the BET equation [42].
[b] As reported by authors.
[c] Calculated assuming carbon from the crosslinker is negligible.
[d] Celluose (%, w/w).

shown in the plots are averages of two or more separate injections.

3. Results and discussion

3.1. Zirconia coating optimization

The optimum concentration of CMD in solution was found by measuring the adsorption isotherm of CMD on zirconia (Fig. 3). The isotherm appears to level off somewhere between 1 and 2 g/l of CMD remaining in solution. This corresponds to an initial CMD concentration of about 6–8 g/l. We wanted to be at a concentration that is on the plateau region of the isotherm so that there is as much dextran on the surface as possible. There is a plateau at high concentrations in the isotherm, implying that multiple layers of dextran are not adsorbed. Thus, under the conditions described above, any initial concentration of CMD greater than 6 g/ml can be used to coat the zirconia.

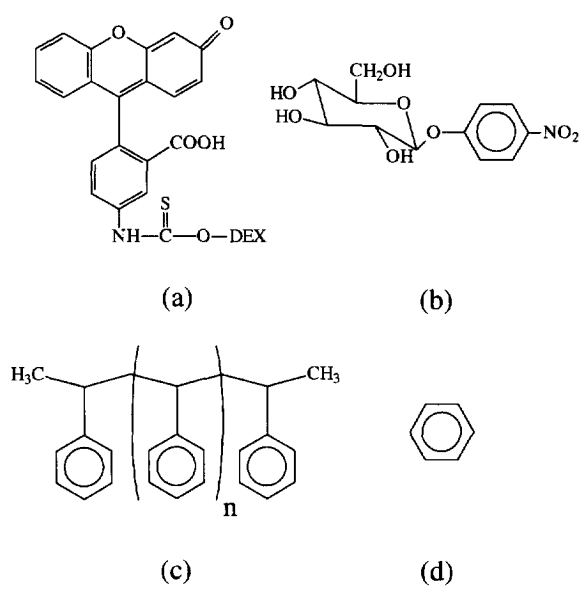

Fig. 2. Structure of SEC probes. (a) Fluorescein isothiocyanate-labeled dextran (FITC dextran). (b) *para*-Nitrophenyl glucopyranoside (pnp-glucose). (c) Polystyrene. (d) Benzene.

Fig. 4 shows a plot of the solution concentration of CMD versus time. As can be seen from the plot, the solution concentration decreases rapidly over about two days, after which it levels off. It is known that adsorption of polymers on to porous substrates can be rather slow [47]. In the present situation, we believe that initially a small amount of polymer is adsorbed in trains on the surface, but as an anchor group desorbs and exposes a Lewis acid site, another molecule can adsorb at that site. The polydispersity of the CMD dextran is also a factor, as the smaller molecules can diffuse more rapidly into the pore space, followed by the larger CMD molecules, which, due to the larger number of carboxymethyl groups, should eventually displace the smaller molecules. Thus, the long time required to reach equilibrium in this system is not surprising. Two days of equilibration was the time chosen for all further experiments.

Once the CMD is adsorbed, it is stabilized by crosslinking with BUDGE. This crosslinker is a bifunctional epoxide and, as such, can self-polymerize in solution if a suitable catalyst (such as boron

Table 3
Physical characteristics of FITC–dextran probes

M_r solute	Number of glucose units	Substitution[a]	Number of FITC/chain	R_g (Å)[b]
303[c]	1	0	0	–
4300	27	0.006	0.159	20
9300	57	0.012	0.689	30
$1.96 \cdot 10^4$	120	0.01	1.21	50
$3.89 \cdot 10^4$	240	0.007	1.68	60
$7.12 \cdot 10^4$	440	0.005	2.20	80
$1.48 \cdot 10^5$	910	0.004	3.64	110
$4.85 \cdot 10^5$	3000	0.004	12.0	180
$2.00 \cdot 10^6$	12 000	0.008	99.0	340

[a] Degree of FITC substitution.
[b] Radius of gyration calculated from equation in [50] from light scattering data.
[c] *p*-Nitrophenylglucopyranoside.

trifluoride) is present. We have observed in preliminary experiments that if the concentration of BUDGE is high enough (0.23 m*M*) the solution will gel. Because of this possibility, our goal was to find the lowest concentration of BUDGE that will still impart the desired stability to the CMD coating. Fig. 5 shows a plot of the amount of dextran stripped by a 0.1 *M* sodium hydroxide solution versus the amount of BUDGE used with respect to the mass of zirconia. There is a plateau in stability when the amount of BUDGE exceeds 82 μM. We set the BUDGE concentration at this concentration for all subsequent experiments.

Table 4
Physical characteristics of polystyrene probes

Molecular mass	M_w/M_n [a]	R_g [b]
80[c]	1	–
300	1.2	20
4000	1.04	83
7600	1.05	120
$1.96 \cdot 10^4$	1.05	210
$3.00 \cdot 10^4$	1.06	270
$4.70 \cdot 10^4$	1.07	350
$1.15 \cdot 10^5$	1.05	600
$1.98 \cdot 10^5$	1.04	830
$4.90 \cdot 10^5$	1.10	1410
$2.43 \cdot 10^6$	1.06	3630
$6.00 \cdot 10^6$	1.20	6190
$2.00 \cdot 10^7$	1.20	12 600

[a] Polydispersity index from manufacturer.
[b] Radius of gyration (R_g) calculated from the equation $R_g = 0.62(MW)^{0.59}$ [32].
[c] Benzene.

3.2. Optimized procedure

Given the results just discussed, our optimized procedure is as follows; a 6-g/l solution of CMD is allowed to be in contact with the zirconia for two days after which it is crosslinked with 82 μM BUDGE. As seen in Table 2, a dextran coating is successfully prepared on the surface, as measured by carbon analysis. The relative difference in the amount coated on the two different types of zirconia is not large. Thus, we believe that the coating procedure is equally valid on both OE and PICA

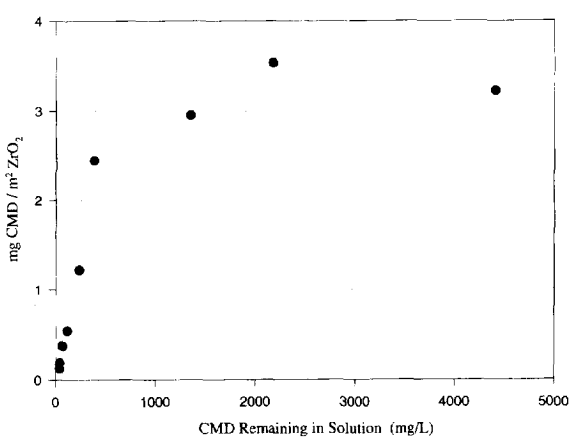

Fig. 3. CMD adsorption isotherm on porous zirconia particles. All solutions were made in 100 m*M* PIPES at pH 6.5. All samples were allowed to equilibrate at 25°C for three days. Adsorbed CMD was hydrolyzed with 2 *M* H_2SO_4 and then stripped from the zirconia with 10 *M* NaOH. All concentrations were measured by the phenol–sulfuric acid assay [44].

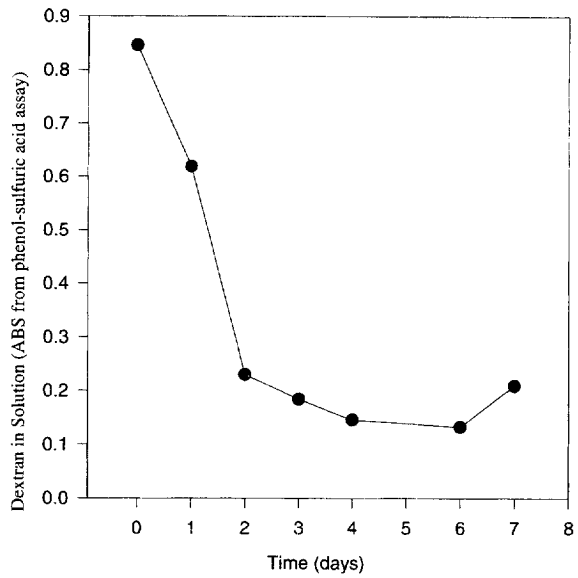

Fig. 4. Amount of CMD in solution in the presence of zirconia as a function of time. Solution was 2.0 g/l CMD in 100 m*M* PIPES at pH 6.5. Absorbance measurements refer to the signal generated by the phenol–sulfuric acid assay.

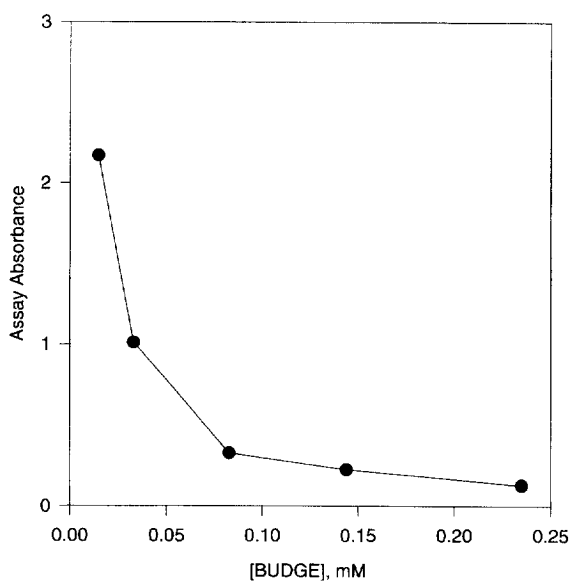

Fig. 5. Stability of a CMD coating as a function of BUDGE concentration. Coating was prepared from a 5.0-g/l CMD solution in 100 m*M* PIPES at pH 6.5. Absorbance measurements refer to the signal generated from a solution collected from a column and then assayed by the phenol–sulfuric acid method.

materials. We have also calculated this number based on other coating procedures using silica as a support material (Table 2). Our numbers are comparable to those found in the literature.

3.3. Comparison of different zirconia supports coated with CMD

We used two different methods for characterizing the pore space of CMD-coated zirconia materials; nitrogen sorptometry and inverse SEC. These two methods were used on both the coated OE and PICA materials and the results compared to the respective bare zirconia materials in order to understand the effects of coating on pore structure.

The results from nitrogen sorptometry on all materials are shown in Table 1. The OE materials (bare and coated) have a higher pore volume than do the corresponding PICA materials. This is expected and is the result of the different synthesis procedures [38]. Both OE and PICA materials have lower pore volumes after being coated with CMD. This indicates that the polymer is occupying space in the pores and is not just coating the outside of the particle. We also see that nitrogen sorptometry reports an increase in the average pore diameter of the OE material. This indicates that the polymer is preventing the nitrogen probes from entering the smaller pores, leaving the larger pores, resulting in a larger average pore diameter. Davankov et al. [48] have reported similar results when coating silica with polystyrene. In contrast to the OE material, the average pore diameter of the PICA material decreases upon coating. We infer that this loss is due to partial filling of the pores by CMD, but the smaller pores are not blocked completely by the coating. These results imply that in both cases, we should see a decrease in the totally included volume (measured by SEC) when either material is coated with CMD.

SEC characteristics of both OE and PICA materials were tested with and without the dextran coating using FITC-labeled dextrans as probes (Fig. 6). The large decrease in elution volume of the intermediate-size SEC probes upon coating the OE particles (Fig. 6a) shows that the CMD coating significantly decreases the pore space available to the probes. In contrast, the PICA particles (Fig. 6b) show very little change in the elution volume after coating. This is

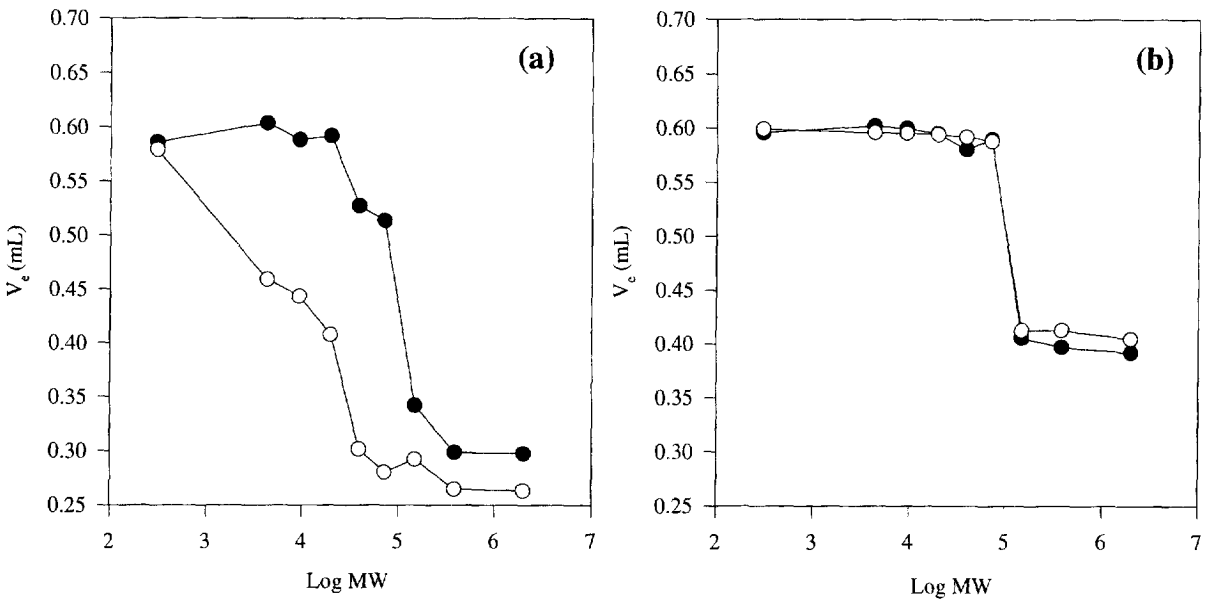

Fig. 6. Size-exclusion chromatography on CMD-coated (optimized) and bare zirconia materials. FITC–dextran probes were used in a mobile phase of 100 m*M* K_2HPO_4 (pH 7) at a flow-rate of 0.5 ml/min. Temperature was 40°C. (a) OE materials. (b) PICA materials. (○) Bare zirconias; (●) CMD-coated zirconias. System II was used.

not surprising, as it has been shown by SEC that the pores of the OE materials are less accessible than those of the PICA material, even when uncoated [38]. Water self-diffusion measurements by NMR have shown that the PICA material has an effective diffusion coefficient inside the pore structure that is similar to that in the OE material [49]. This is despite the nitrogen sorptometry results that indicate larger pores in the OE material. This result implies that the PICA material has a better connected pore space, allowing probes to access more of the pore space [49]. The NMR results also indicate a mean pore diameter that is similar for both materials; this being the case despite the large differences reported by both nitrogen sorptometry and mercury porosimetry. Pore shape may also play a role in the blockage of pores. If the pores have narrow necks in front of wide pores, the necks may become blocked. The desorption branch of the nitrogen sorptometric curve allows estimation of the diameters of these pore necks [42]. The results of this analysis show that the necks in the OE material (270 Å) are larger than those in the PICA material (170 Å).

3.4. Adsorption of FITC–dextran probes

We have shown previously that the FITC–dextran probes adsorb through the carboxylic acid group (see Fig. 2) to the surface Lewis acid sites [38]. If probe adsorption occurs, the SEC curve takes on a characteristic shape; a constant elution volume at low molecular masses followed by a sharp drop in elution volume to another plateau at high molecular masses. Both coated and uncoated PICA materials exhibit this shape, implying that the dextran coating is not impeding probe access to the surface Lewis acid sites. In contrast, the SEC curve changes dramatically when the OE material is coated with CMD. The characteristic SEC curve indicating probe adsorption changes to one that is more typical of SEC. We can explain this behavior in one of two ways; either the dextran coating is more homogeneous on the OE material than on the PICA material and thus attenuates the surface chemistry more efficiently, or pore blockage is more severe in the OE material, reducing the accessible surface area, resulting in a reduction in the elution volume. In order to de-

termine which of these cases is correct, we used a set of non-interacting probes for SEC.

3.5. SEC using polystyrene probes

Polystyrenes of different molecular masses were used as probes with a mobile phase of 100% THF, which is a good solvent for polystyrenes [32]. There should be very little, if any, adsorption of the probes in this system. Fig. 7 shows the elution behavior of the bare and coated PICA and OE materials. As in the aqueous mobile phases, there is very little difference between the coated and uncoated PICA materials (Fig. 7b). This again indicates that there is very little pore blockage when the PICA material is coated with polymer. However, the shape of the SEC curve has changed radically to one more typical of SEC, suggesting that there is no probe adsorption here. When we examine the OE material (Fig. 7a), the polystyrene molecules are found to have a smaller elution volume on the coated materials than on the uncoated materials. Even the smallest probe's elution volume is changed upon coating. This confirms that the OE material's pore space is blocked by the polymer. We speculate that in THF, the dextran is compressed into a denser layer in the pore necks, preventing the smallest probe from entering the pore space. In the aqueous phase, the dextran coating is swollen with water, allowing the smallest probe in this system (pnp-glucose) to move through the polymer layer and access the pore space behind the pore necks. The PICA materials, which have been shown to have a more accessible pore structure [38], do not have this problem, and show very little change in the accessible pore space upon coating.

The apparent lack of probe adsorption in an aqueous mobile phase on the coated OE material can be attributed to the inability of the probe to access the same pore space and thus the same surface area as in the uncoated case. It therefore appears that in order to block zirconia–solute interactions by polymer coating, a significant loss in pore space is inevitable. Adsorption of solutes is not an insurmountable problem, as Lewis acid sites can be blocked by using mobile phases containing Lewis bases, such as phosphate or fluoride solutions. The loss of pore space in the OE materials is a bigger problem, as a loss in pore space results in a loss of surface area and thus sorptive (binding) capacity. The polystyrene probes indicate a 13–14% loss in

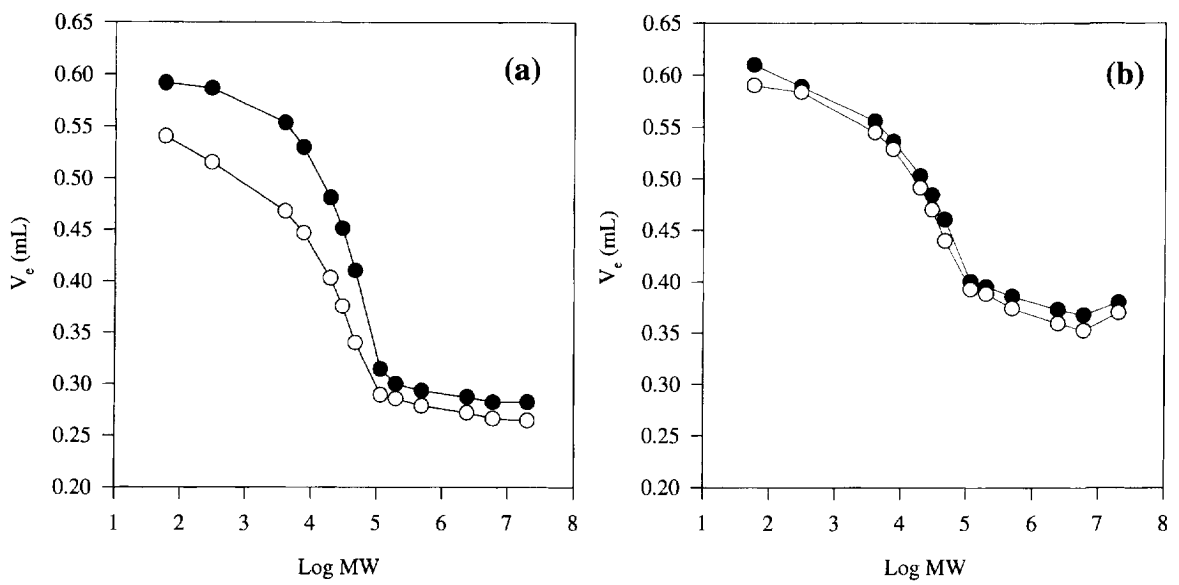

Fig. 7. Size-exclusion chromatography on CMD-coated and bare zirconias. Polystyrene probes were used in a mobile phase of 100% THF at a flow-rate of 0.5 ml/min. Temperature was 60°C. (a) OE materials, (b) PICA materials. (○) Bare. (●) CMD-coated. System I was used.

accessible pore volume when the OE material is coated with dextran. Thus, it appears that the PICA materials are better candidates for use in further studies, especially for larger molecules, such as proteins.

4. Conclusions

Zirconia particles have been successfully coated with a small, slightly carboxymethylated dextran and then crosslinked. The coating procedure was optimized in terms of the dextran concentration in the adsorbing solution, the length of time required for the adsorption to reach equilibrium and the amount of crosslinker needed to stabilize the coating.

The coating procedure was applied to coating zirconia materials prepared by two different methods; the oil emulsion (OE) and the polymerization-induced colloid aggregation (PICA) method. These two materials were then examined by inverse SEC to determine the effect of polymer coating on their accessible pore space. It was found that coating the PICA materials has very little effect on the accessible pore space, as measured by both polystyrene and FITC-labeled dextran probes. The OE material, in contrast, loses between 13 and 14% of its accessible pore space when coated. This loss is attributed to the pore structure in the OE material that allows pore space to be blocked easily. The PICA materials thus appear to be better candidates for use in studies of functionalized dextrans for use in chromatography.

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