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HIGH-PERFORMANCE EXCLUSION CHROMATOGRAPHY OF WATER-SOLUBLE POLYMERS WITH CHEMICALLY BONDED STATIONARY PHASES

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SUMMARY

Chemically bonded stationary phases with 14 different functional groups have been evaluated for use in the exclusion chromatography of water-soluble polymers. They were characterized by their interaction with different polyethylene glycol standards. A bonded glycinamide and a diamine phase showed no interaction with polyethylene glycols and such phases bonded to silicas having different pore diameter distribution are suitable for exclusion chromatography.

A bonded amide phase proved to be more suitable for exclusion chromatography of polyvinyl pyrrolidones, dextrans and gelatine but partially retained the polyethylene glycols.

For the separation of polymers in the molecular-weight range 100–70,000 the average pore diameter of the silica matrix should be at least 200 Å; for those in the molecular-weight range 80,000–500,000 the pore diameter should be *ca.* 500 Å.

INTRODUCTION

The chromatographic fractionation of macromolecules according to their size has been described for polymers in aqueous media[1] as well as for polymers in organic eluents[2], where xerogels[3], *e.g.*, cross-linked dextrans in aqueous media and cross-linked polystyrenes in organic media, have been used as the stationary phase. Classical xerogels swell dramatically in the chromatographic eluent to form networks of solvated polymer chains. The pore structure, which is required for separation, depends on the degree of cross-linking and, of course, on the amount of swelling in the given eluent. Therefore, a given polymer matrix will only form a xerogel in a limited range of eluents, in which the individual polymer chains forming the matrix are at least partially soluble. A change of eluent will result in a change in the degree of swelling and hence in the bed volume of the gel in the chromatographic column. Because of this most columns can only be packed and used with an eluent in which the xerogel has been pre-swollen. The soft gels used at first are not stable even at moderately high flow-rates. Some of the more recently described xerogels[4–6] can be used at the moderately high flow-rates usual in high-performance liquid chromato-

graphy (HPLC) whilst others[7] are available with a small particle size. Nevertheless, a solvent change still alters the bedstructure. Because of this it may be difficult to determine exactly the parameters necessary to define a separation based on exclusion chromatography.

These problems do not occur if rigid, preformed matrices of xerogels[3] such as porous silica are used. Such packing materials do not swell or change their pore structure when the eluent is changed. The packing structure is stable even at relatively high flow-rates, *i.e.* the permeability of the chromatographic column is independent of the pressure applied. The pore size distribution of rigid particles can be determined by independent physical methods such as mercury porosimetry[8,9] and/or nitrogen adsorption[10,11]. The pore size distribution so determined can easily be correlated with exclusion chromatographic experiments[12,13].

Silica and similar materials, such as porous glass, have narrow and well-defined pore size distribution curves[13,14], and are available with average pore diameters ranging from 40 to 25,000 Å. Up to now, only a few of these materials have been commercially available with the average particle diameters around 5 μm or 10 μm required for HPLC. For exclusion chromatography the same relationships between particle diameter and column efficiency are valid as in HPLC[15]. However, in comparing the efficiencies of different columns only the height equivalent to a theoretical plate (h) of a totally excluded sample, or, preferably, that of a low-molecular-weight sample totally penetrating the pores should be used.

The main problem with inorganic packings is the presence of active surface sites, which may adsorb the polymer onto the stationary phase. However, for organic soluble polymers this adsorption can be eliminated by using solvents from the eluotropic series[16,17], which are more strongly adsorbed onto the surface than is the polymer. For instance, on a silica surface polystyrenes are not adsorbed when dichloromethane, chloroform, tetrahydrofuran or dimethylformamide is used as eluent.

In aqueous systems the ionic properties of the surface groups may also cause irreversible adsorption and/or decomposition of water soluble polymers, especially of proteins. Therefore, the use of these materials has been restricted to a few polymers[13–20]. Better results have been obtained by deactivating (heating) the silica surface (removal of silanol groups)[21]. However, in aqueous systems such a surface slowly rehydrates. Differences in the surface structure may be responsible for the irreversible adsorption of dextrans on silica, whereas on porous glass they are not retarded, allowing them to be separated by an exclusion mechanism[22].

The chemical modification of the surface silanol groups of silica with suitable organosilanes yields stationary phases suitable for the exclusion chromatography of water-soluble polymers[23]. The properties of these phases as well as others prepared in our laboratories have been compared, and these phases have been evaluated for their use in the exclusion chromatography of synthetic water-soluble polymers. The influence of the average pore diameter on the exclusion chromatography of these polymers has also been evaluated.

Nomenclature of exclusion chromatography

The elution volume (V_e) of a polymeric sample should depend solely on the size of the molecule in solution (usually a random coil related to its molecular

weight) and on its relationship to the pore size distribution of the stationary phase. The volume of the mobile phase (V_m) in the column can be determined, as is usual in chromatography, from the elution volume of an eluent molecule or another small, non-retarded molecule (sometimes also called the dead volume V° of the column). This includes the volume (V_z) between the particles (interstitial volume) plus the pore volume (V_p) of the stationary phase in the column. V_z can be measured with a totally excluded high-molecular-weight sample, and is identical for all molecules. Depending on the pore size distribution of the stationary phase and on the size of the molecule which is proportional to the molecular weight only part of the pore volume may be accessible for penetration. The sample is always eluted at $V_e = V_z + K \cdot V_p$, where K is the fraction of the pore volume accessible for the component, and is commonly called the Wheaton–Baumann distribution coefficient[24], which by definition in exclusion chromatography never exceeds unity ($0 \leqslant K \leqslant 1$). Therefore, the elution volume V_e is always smaller than V_m, and the end of the separation can be predicted. In LC, however, where the samples may be retained by interaction with the surface of the stationary phase (which is, of course, to a large extent also the surface of the pores), the elution (retention) volume is always larger than V_m.

In comparing different columns, it may be necessary to normalize for the different volumes V_K of these columns. It is recommended that V_K be determined volumetrically before the column is packed. The fraction of the column volume filled with eluent (between the particles and within the pores) is called the total porosity $\varepsilon_T = V_m/V_K$; the interstitial porosity $\varepsilon_z = V_z/V_K$ is that volume fraction of the column between the particles, where the transport occurs along the axis of the column. Whereas $\varepsilon_p = V_p/V_K$ is the fraction of the eluent in the column held within the pores of the stationary phase. It may also be convenient to standardize the elution volume of a compound for the column volume: $\varepsilon_l = V_e/V_K$. Only if $\varepsilon_l < 0.85$ (for silica columns) the separation is based on exclusion chromatography[17].

Preparation and properties of the stationary phases

Some of the triethoxysilanes were obtained from Dynamit Nobel AG, Troisdorf, G.F.R. (amine, diamine, triamine, imidazoline, glycol), Petrarch Systems, Levittown, Pa., U.S.A. (urea, carbamate, nitrile), Ega-Chemie, Steinheim, G.F.R. (mercaptane), the remainder were prepared by us. Before use, the silanes were purified either by distillation or preparative-scale HPLC on a reversed-phase C_{18} column with methanol as eluent. In order to exclude water completely a closed system[25] was used, and the eluent dried and recycled over freshly activated molecular sieve 3 Å (Merck, Darmstadt, G.F.R.). The silanes were characterized by infrared and nuclear magnetic resonance spectroscopy.

The reaction with silica was carried out as previously described[23]. The conditions were chosen to obtain maximum coverage in each case. Only dry solvents were used, and moisture was excluded. Using the same batch of silica, the same silane and the same reaction conditions, identical amount of organic moeity was bonded. Owing to differences in the history of silica[17], different coverages were sometimes obtained with different batches of silica. Differences in the chromatographic properties of these phases in exclusion chromatography were barely noticeable.

In Table I the properties of stationary phases prepared from LiChrosorb SI

100 (Merck) are summarized. This silica has an average pore diameter of 100 Å, a specific pore volume of 1 cm^3/g, and a specific surface area of around 350 m^2/g.

The surface concentrations shown in Table I have been calculated as previously described[26,27], but using the values of the heteroatoms when possible. This was because on comparing the C, H, N, or S atom ratios calculated from the formulae of the silanes with those calculated from the elemental analysis, it was obvious that the analytical H and C values were too high, if the N and/or S values were taken as a reference. (During the preparation and purification no N- or S-containing solvents were used.) The high H values probably arise from the loss of structural water during the combustion of the samples. The elevated C values can be attributed to alkoxy (ethoxy) groups still present, since for experimental raesons only trialkoxysilanes have been used. Strongly adsorbed solvent molecules hardly can contribute to these values, because before CH analysis the samples were dried at elevated temperatures *in vacuo*. It is known[28–30] that when multifunctional silanes are treated with silica surfaces on average only 1.5 alkoxy groups react with surface silanols. Unreacted alkoxy groups may, of course, be hydrolysed to yield new silanol groups. Sometimes the presence of such silanol groups was noticeable.

For some of these phases a relatively high (>4 $\mu mol/m^2$) surface concentration of organic molecules was calculated. With small molecules usually higher coverage was found than with long-chain ones[31]. On the other hand, because of the use of trialkoxysilanes some cross-linking may also have taken place. However, as water was excluded totally no polymerization to bonded siloxanes should occur. The mass transfer term of the Van Deemter plot for these bonded phases was

TABLE I

POLAR BONDED STATIONARY PHASES ON SILICA SI 100

Stationary phases		*Atom-ratio C:H:N:(S)*		*Surface concentration ($\mu mol/m^2$) calculated from N or S value*	*Average place requirement ($Å^2$)*
Name	*Structure*	*Theoretical*	*Found*		
	$\equiv Si–CH_2–CH_2–CH_2–$				
Amine	$–NH_2$	3:8:1	3.3:9:1	5.3	31
Diamine	$–NH–(CH_2)_2–NH_2$	5:13:2	5.3:15:2	2.5	66
Triamine	$–(NHCH_2CH_2)_2–NH_2$	7:18:3	7.6:22:3	1.7	97
Imidazoline		6:11:2	6.2:15.6:2	2.2	75
Urea	$–NH–CO–NH_2$	4:9:2	7.1:17:2	3.5	47
Amide	$–NH–CO–CH_3$	5:10:1	6:13.3:1	4.4	37
Trifluoroacetyl amide	$–NH–CO–CF_3$	5:7:1	5.6:9.6:1	4.4	37
Sulfonamide	$–NH–SO_2–CH_3$	4:10:1:1	5.2:13.4:1:1	5.3	31
Glycinamide	$–NH–CO–CH_2–NH–CO–CH_3$	7:13:2	8:17:2	3.2	52
Nitrile	$–C\equiv N$	4:6:1	4.7:12.6:1	3.2	52
Mercaptane	$–SH$	3:7:1	4:12:1	2.3	72
Diol	$–O–CH_2–CHOH–CH_2OM$	6:13	6:14	2.5*	66*
Carbamate	$–O–CO–NH–C_2H_5$	6:12:1	7:16:1	2.8	59
RP-C_6	$–CH_2–CH_2–CH_3$	6:13	6:14.5	3.4*	48*

* Calculated from C content.

identical with that of the naked silica. Whereas with polymeric bonded phases a higher C term is usually obtained, owing to the slower mass transfer in the bonded polymeric phases[32].

The stability of these phases was good. Some columns were continuously used for more than six months with different buffer and salt solutions in the pH range 2.5–7.5, without apparent change in performance and selectivity. It is preferable not to store the columns over long periods in contact with aqueous buffer and salt solutions but in an organic solvent such as methanol.

Besides SI 100 some phases were also prepared with narrower and wider pore silicas, *i.e.* with LiChrosorb SI 60 (average pore diameter 65 Å, specific pore volume 0.75 cm^3/g, specific surface area 450 m^2/g), Fractosil SI 200 (average pore diameter 175 Å, specific pore volume 0.75 cm^3/g, specific surface area 170 m^2/g), LiChrosorb SI 500 (average pore diameter 480 Å, specific pore volume 0.75 cm^3/g, specific surface area 100 m^2/g) and with the spherical silica LiChrospher SI 300 (with pore diameters ranging from 150 to 450 Å, specific pore volume of 2 cm^3/g and a specific surface area of 250 m^2/g) (Merck). The surface concentration of the bonded silanes was hardly affected by the pore size distribution of the silica, except for the silica with the smallest pore diameter (60 Å). Here the achievable surface concentration was *ca.* 10% smaller than with the other wider pore materials.

EXPERIMENTAL

The liquid chromatograph consisted of a M6000 solvent delivery system (Waters Assoc., Milford, Mass., U.S.A.), a syringe loadable sample loop (Nr. 7120; Rheodyne, Berkeley, Calif., U.S A.), and a variable-wavelength detector (SF 770, Schoeffel, Trappenkamp, G.F.R.) in series with a differential refractometer (R 401, Waters Assoc.). The drilled[33] stainless-steel columns, with an I.D. of 4.2–4.6 mm, were 30 cm long. The exact volumes of the empty columns were determined volumetrically. For slurry packing of the 10-μm particles, *ca.* 2.5 g of the stationary phase was suspended in a mixture of 15 ml of cyclohexanol and 30 ml of isopropanol. The final packing pressure was 480 bar. The displacement liquid was *n*-heptane. The columns were measured with dichloromethane as eluent and afterwards brought via methanol to aqueous eluents. Some characteristic values of the columns are summarized in Table II. The interstitial porosity ε_z was calculated using the elution volume of a polystyrene standard ($\bar{M}_w = 2.6 \cdot 10^6$) in dichloromethane and compared with that of the dextran standard $\bar{M}_w = 2 \cdot 10^6$ in water as eluent. The total porosity

TABLE II

CHARACTERISTIC VALUES OF SOME COLUMNS

Column length, 30 cm.

Phase	*Pore diameter (Å)*	V_K *(ml)*	ε_T	ε_p	ε_z
Diamine	100	4.34	0.79	0.33	0.46
Glycinamide	100	4.46	0.79	0.33	0.46
Amide	60	5.13	0.71	0.25	0.46
Amide	100	4.48	0.79	0.33	0.46
Amide	200	4.33	0.73	0.28	0.45
Amide	500	4.83	0.77	0.31	0.46
Amide	*ca.* 300	4.48	0.88	0.54	0.34

ε_T was determined from the elution volume of $C^2H_2Cl_2$ in dichloromethane and compared with that of ^2H_2O in water as eluent. Within the error of measurements the porosities with CH_2Cl_2 and H_2O eluents were identical.

Deionized water and analytical grade reagents were used to prepare buffer and salt solutions. Commercially available polyethylene glycols (Hüls, Marl, G.F.R.), dextrans (Pharmacia, Frankfurt/Main, G.F.R.) and polyvinylpyrrolidones (Fluka, Flawil, Switzerland) were used as polymer standards. The polystyrene standards were purchased from Waters Assoc.

RESULTS AND DISCUSSION

Characterization of the stationary phases

A crude measure of the suitability of these stationary phases for aqueous exclusion chromatography is their wettability by water[23]. However, this cannot indicate the absence of specific interaction of water-soluble polymers with the stationary phases. Therefore, the stationary phases were compared for their possible interactions with polymers using polyethylene glycols (PEG) as models.

The stationary phases can be divided into three groups. The first group contains all those phases that interact strongly with even the small oligomers of the PEG, such as ethylene glycol, di-, tri- and tetraethylene glycol. PEG standards with molecular weights (MW) of 1000 or higher are not eluted from these columns in a reasonable time (capacity factor, $k' > 10$) using water as eluent. In Fig. 1 the elution diagrams of the oligomers of ethylene glycol on six of these "non polar" phases are shown in order of decreasing interaction with the oligomers. The volume of the mobile phase in the column was determined using ^2H_2O as inert sample. Its elution volume was identical with that of benzene and of $C^2H_2Cl_2$ in dichloromethane on the same column. The differences in the V_0 values in Fig. 1 arise from differences in the inner diameters of the columns (different empty column volumes).

It is not surprising that the retentions were highest with the reversed phase column owing to hydrophobic interaction, which is the reason for the retention of the samples on the mercapto, the trifluoroacetamide and the nitrile phase. However, the retention behaviour of the oligomers on silica and on the diol phase was unexpected. With the diol phase a hydrophobic interaction may be possible, whereas hydrogen-bonding to silanols and the hydroxyl groups respectively may be the reason for the retention of the oligomers. It should be noted that this diol phase proved valuable in the exclusion chromatography of proteins[34,35], which usually exhibit strong hydrophobic interactions.

The second group, shown in Fig. 2, contains the stationary phases having a "medium" polarity. In water, oligomers of ethylene glycol are not retained, *i.e.* they are eluted with ^2H_2O. The PEG standard with MW 1000 is slightly retained, that with MW 40,000 is strongly retarded ($k' > 10$) by these phases with water as eluent. It should also be mentioned here that the amide phase has been used successfully in the exclusion chromatography of proteins[36].

The third group of stationary phases, shown in Fig. 3, does not interact with small PEG molecules, including PEG 1000, using water as eluent. Ethylene glycol, which elutes together with ^2H_2O, was used here as a t_0 marker. It can be seen in all chromatograms as a sharp peak. PEG 40,000 is the other sample injected, whose

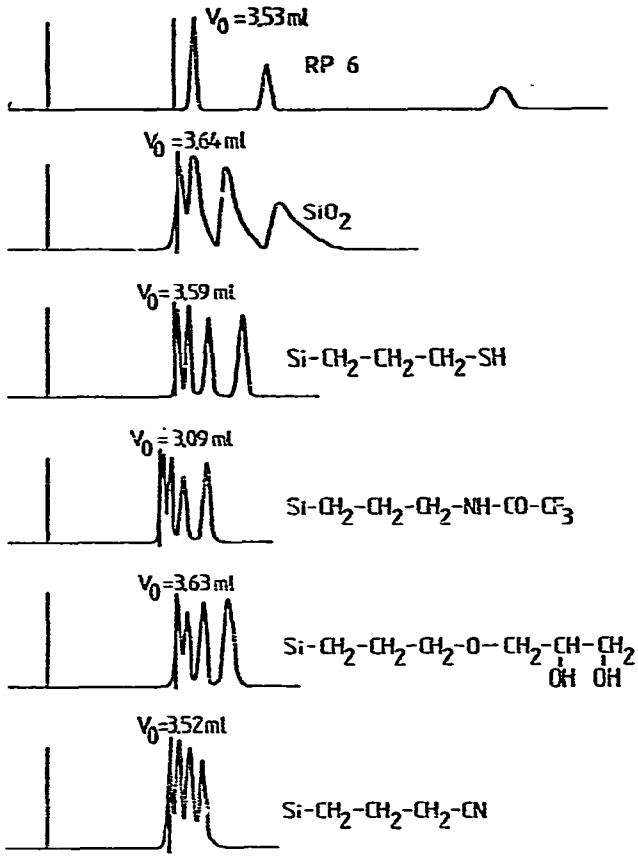

Fig. 1. Selectivity of "non-polar" phases. Columns: stationary phase as indicated, bonded to SI 100; length, 30 cm Eluent: water; flow-rate, 3 ml/min Samples: Mono- to tetra-ethyleneglycol (the elution order was always identical). V_0 determined with ^2H_2O.

interaction with the stationary phase decreases from the upper left (amine phase) to the lower right (diamine phase). The skewed peaks of the PEG 40,000 standard on some of the phases can be explained by a mixture of exclusion and sorption mechanisms. PEG 40,000 has access to only a very small portion of the pores and hence to the surface of the stationary phase. (The geometrical (outer) surface of the particles is less than 1% of the total surface.) Consequently, the accessible surface is totally overloaded by the amount of sample applied (usually 10^{-5}–10^{-4} g), resulting in severe tailing of the peak. As the strength of the interaction with the surface decreases, the peak becomes sharper and elutes finally unretarded and excluded as with the glycineamide and the diamine phases. Only in these two cases is a linear relationship observed between the height of the excluded peak and the sample size (10^{-6}–10^{-3} g). In those cases where noticeable sorption takes place the height of the excluded part of the peak did not show a linear relationship with sample size. The glycineamide phase may still exhibit a very weak interaction with the PEG, because the elution volumes of identical PEG standards are slightly greater with water as

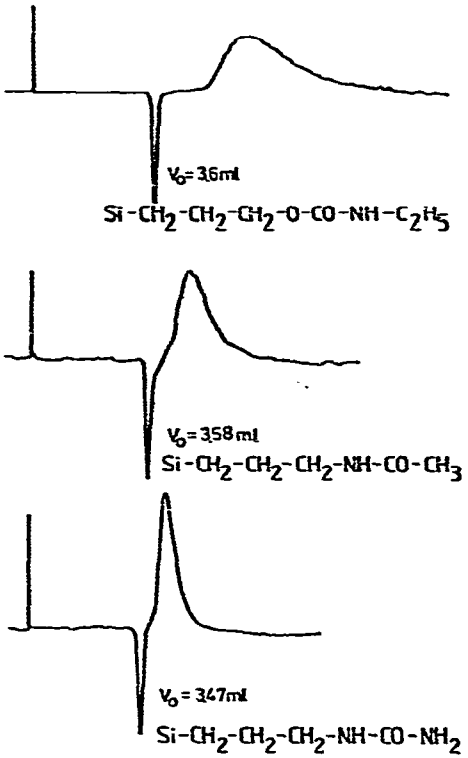

Fig. 2. Selectivity of "medium polar" phases. Columns: stationary phases as indicated, bonded to SI 100; length, 30 cm. Eluent: water; flow-rate, 3 ml/min. Samples: ^2H_2O and PEG 1000.

eluent than with dimethylformamide under otherwise identical conditions[23]. However, differences in the size of the random coil in both eluents may also be the reason for those variations.

The three stationary phases with the same functional group at the end of the silane (amine, diamine, and triamine) differ significantly in their interaction with PEG. This is not surprising, because besides the differences in the structure of the silanes, they differ distinctly in their surface coverage (*cf.* Table I). Reversed phases only show identical selectivity if their surface coverage is identical[30]. Therefore, it is only possible to attribute a certain selectivity to a functional group of a bonded silane, if the surface coverages of the phases to be compared are identical[17].

Stationary phases with free amino groups may be unstable in water because they may raise the pH enough to cause hydrolysis of the silica. For short term use (2–3 weeks) this is not a problem, however, it is recommended to use buffered solutions instead of pure water with such phases. The elution behaviour should not be influenced through the addition of salts or soaps if the separation mechanism is solely exclusion. On the other hand, these problems do not occur with the neutral glycineamide phase. The possible slight retention of the PEG due to hydrophobic interaction can be eliminated by adding a few percent of methanol, glycol, etc., to the eluent.

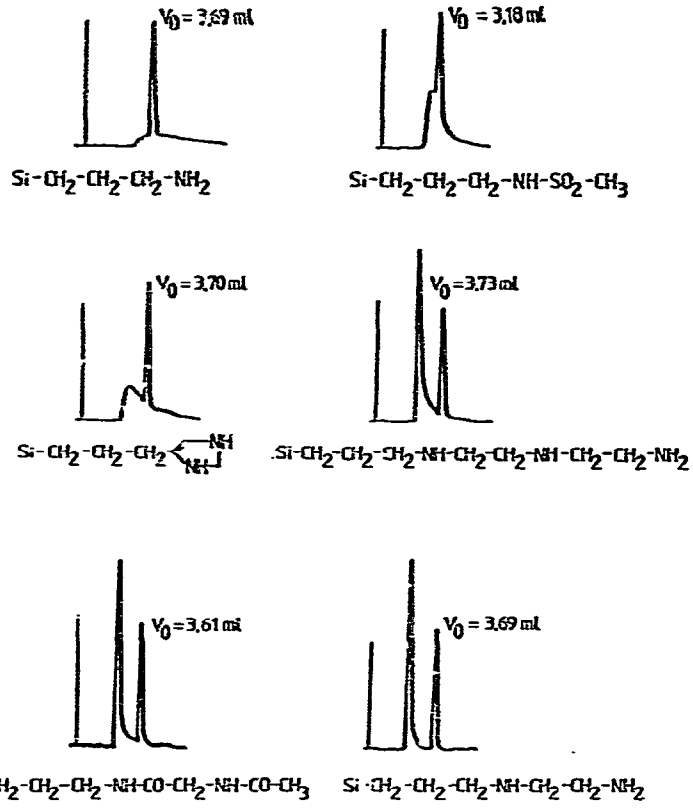

Fig. 3. Selectivity of "polar" phases. Columns: stationary phases as indicated, bonded to SI 100; length, 30 cm. Eluent: water; flow-rate, 3 ml/min. Samples: PEG 40,000 and ethylene glycol (V_0).

Exclusion chromatography of polyethylene glycols

The separation range of silica particles with an average pore diameter of 100 Å which have been modified with a diamine phase is demonstrated for PEG in Fig. 4. The exclusion chromatographic calibration curve for this silica with PEG is shown in Fig. 5, and compared with that for polystyrene standards run on the same column but with dichloromethane as eluent. To enable comparison with other columns the percentage of the pore volume accessible for a given standard was used as abscissa. The PEG 10,000 has access to *ca.* 30% of the pore volume, the PEG 20,000 to *ca.* 15%. For the separation of PEG with a MW of 20,000 and higher, a silica with a larger pore diameter, preferably 200 Å, is required.

With these data the relationship between weight-average molecular weight, $\bar{M}_w$, and the pore diameter $\varnothing$ at which the molecule is excluded can be established[13], by using a modified form of the Mark–Houwink equation[37]:

$$\varnothing = 0.57 \cdot \bar{M}_w^{1/1.63}$$

The constants in this equation are very similar to those obtained for polystyrene in dichloromethane (0.62 and 1/1.7, respectively[13], indicating similar molecular weight–

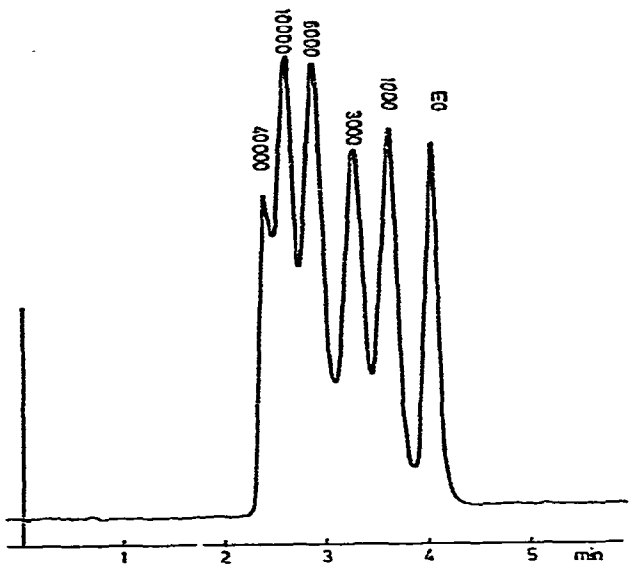

Fig. 4. Separation of PEG standards on diamine on SI 100. Column: 30 cm × 4.3 mm I.D.; particle diameter, 10 μm. Eluent: water; flow-rate, 0.9 ml/min; flow-velocity, 1.2 mm/sec; pressure drop, 24 bar. Samples: PEG 40,000 to PEG 1000, ethylene glycol.

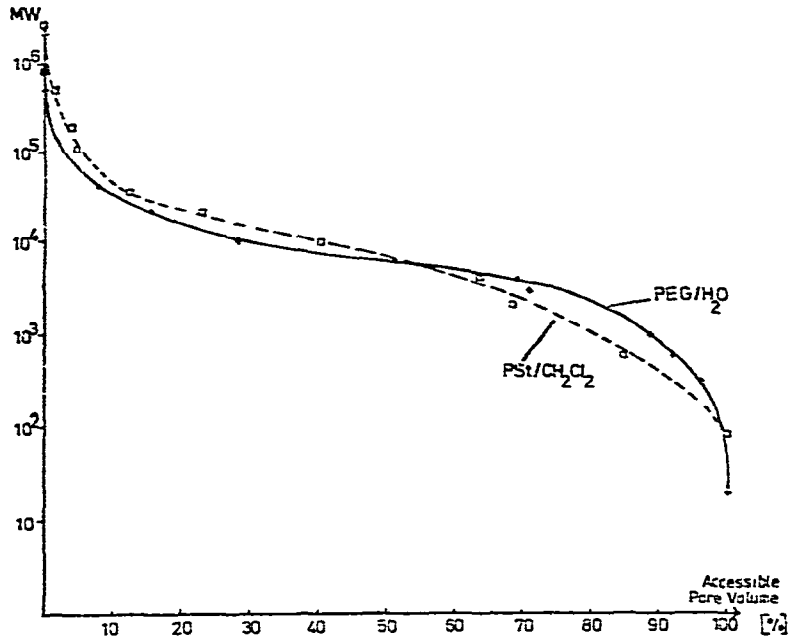

Fig. 5. Calibration curves for diamine on SI 100. Column and stationary phase as in Fig. 4. Continuous line: PEG; eluent, water; flow-velocity, 4.7 mm/sec; flow-rate, 3.3 ml/min; pressure, 89 bar; inert, ^2H_2O. Dashed line: polystyrenes; eluent, CH_2Cl_2; flow-velocity, 4.5 mm/sec; flow-rate, 3.2 ml/min; pressure drop, 36 bar; inert, $C^2H_2Cl_2$.

random coil size relationships for PEG in water and polystyrenes in dichloromethane —each being a "good" solvent for that polymer. According to ref.13, the size of the molecule is smaller by a factor of *ca.* 2.5 than the pore diameter at which it is excluded.

Exclusion chromatography of dextrans

The separation of dextrans by exclusion chromatography is easier because interaction occurs with only a few phases. They show interaction with only "naked" silica, reversed-phase and chemically bonded stationary phase with basic functional groups (*i.e.* amine, etc.). All the other stationary phases prepared so far showed no interaction with dextrans. Because the amide phase turned out to be the phase where the other water-soluble polymers and the proteins showed no retention, exclusion chromatography of dextrans was also carried out using this phase. The dextrans tend to form high-molecular-weight ($MW > 2 \cdot 10^6$) association products in water. This can be minimized by the addition of inorganic salts. Therefore, a 1% solution of sodium azide was used as eluent, the azide ion then prevents the fermentative degradation of the dextrans in solution.

Fig. 6 shows the calibration curves for dextran standards with the amide phase, bonded to different silicas. Silica with an average pore diameter of 60 Å is not suitable for exclusion chromatography of dextrans, because the smallest standard (MW 10,000) is already more than 90% excluded from the pore volume. Even silica with a pore diameter *ca.* 100 Å seems to be too small for exclusion chromatography

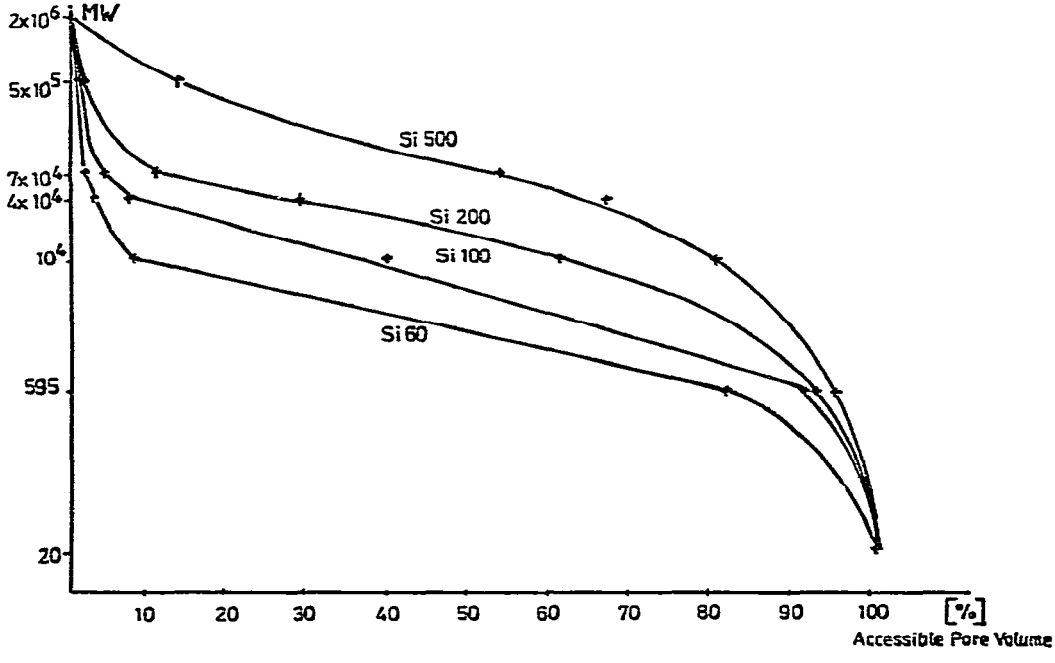

Fig. 6. Calibration curves for dextrans on amide bonded on different silicas. Column length, 30 cm; eluent, 1% (w/w) NaN_3 in water; flow-rate, 1.5 ml/min. For additional data see Table II. Samples: dextran standards MW 500,000 to 10,000, raffinose (MW 595). Inert: ^2H_2O.

of dextrans, because the standard with a MW of 40,000 is also totally excluded from the pores. Silicas with an average pore diameter of 200 Å are optimal for exclusion chromatography of dextrans with MW up to *ca.* 80,000. Those with an average pore diameter of 500 Å are optimal for high-molecular-weight dextrans (MW ≈ 500,000).

The separation of dextran standards shown in Fig. 7 also demonstrates the application range of the amide-bonded phase with silica supports with average pore diameter of *ca.* 200 Å and *ca.* 500 Å.

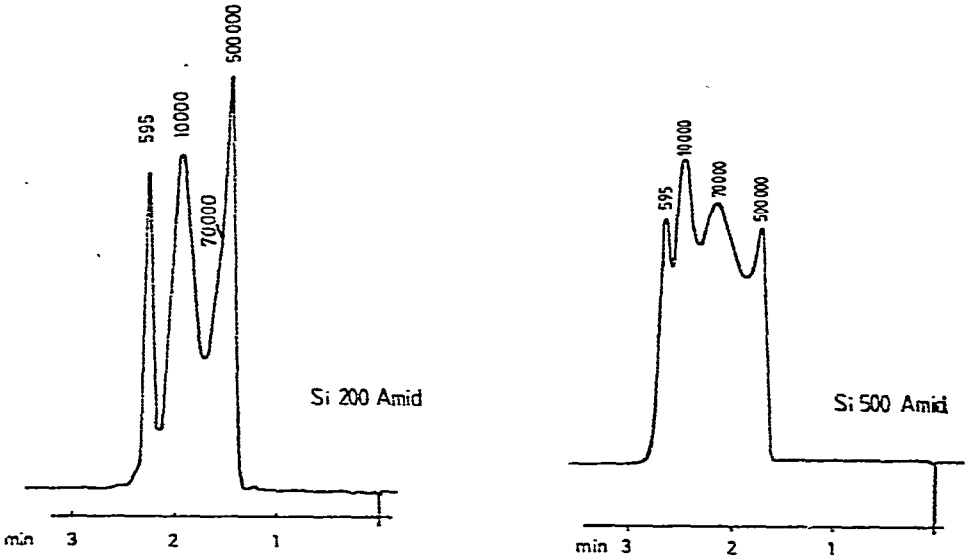

Fig. 7. Separation of dextran standards on amide phase. For columns, eluent and samples see Fig. 6 and Table II. Particle diameter, 10 μm; flow-rate, 1.3 ml/min; flow-velocity, 2 mm/sec; pressure drop, 30 bar.

The approach described for polystyrenes[13] and discussed above for PEG for correlating MW and the pore diameter ⌀ at which a molecule is excluded, did not show a similar relationship for low- and high-molecular-weight dextrans. For small dextrans (MW < 50,000) only the exclusion pore diameter could be related by a factor of 2.5 (as found for PEG and polystyrenes) to the Stokes diameter given in literature[38]. For the higher-molecular-weight dextrans pore diameters *ca.* 10% smaller than expected are calculated by this approach. An explanation for this may be that the dextrans, usually poly(α-1,6)glucose molecules, contain to a certain extent short-chain branches resulting from a α-1,4 condensation reaction. This may be the reason why the molecules appear in solution to be smaller than predicted by the Mark–Houwink equation, which is valid only for the calculation of the size of random coils of linear molecules.

Other applications

Polyvinylpyrrolidones (PVP) were adsorbed from water or buffer solutions by all stationary phases. The hydrophobic interaction was weakest, but still noticeable, with the amide phase. However, by the addition of 10% (v/v) ethylene glycol to the

eluent this interaction could be eliminated. A 0.1 *m* Tris–HCl buffer, pH 8.0, whose ionic strength was adjusted to 0.5 by adding Li_2SO_4, was the eluent.

Fig. 8 shows the elution curves of five PVP standards with MW between 10,000 and 360,000. For these chromatograms an amide-bonded phase (silica LiChrospher SI 300) with a large specific pore volume was found to be optimal. The PVP 360,000 and the PVP 160,000 are totally excluded from this stationary phase. The 160,000 standard contains some low-molecular-weight material which penetrated into the pores. The samples with MW between 10,000 and 40,000 can be characterized well with this stationary phase. With increasing MW the peak maximum moves to smaller elution volumes, and the fraction of excluded polymer increases. The impurities in the PVP 24,000 and PVP 40,000 standard, which elute after t_0, *i.e.* retarded, are certainly of low MW, because they could be removed from these standards by dialysis against water. Because of the relatively broad MW distribution of the availble standards no calibration curve was constructed.

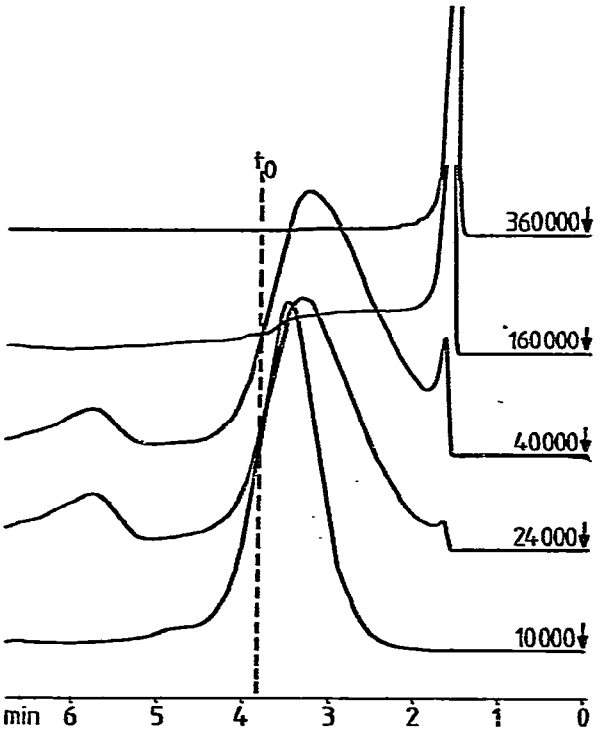

Fig. 8. Characterization of polyvinylpyrrolidones. Stationary phase: amide on LiChrospher SI 300; particle diameter, 10 μm. Column: 30 cm × 4.2 mm I.D. Eluent: 0.1 *m* Tris–HCl-buffer, pH 8.0, with Li_2SO_4 to ionic strength 0.5 + 10% (v/v) glycol; flow-velocity, 1.2 mm/sec; pressure drop, 30 bar. Samples: PVP standards MW 10,000 to 360,000. Inert determined with ^2H_2O.

Gelatine samples could also be characterized by exclusion chromatography with this stationary phase. In Fig. 9 the elution diagram of two different commercially available gelatine samples are shown. They differ in their MW distribution as well as in the elution volume of the peak maximum. The MW indicated (7000 and

11,000) are those of polystyrene standards eluted with identical elution volumes in dichloromethane. The low-MW sample has also a narrower MW distribution, whereas part of the other sample is already excluded. The exclusion limit of this stationary phase for polystyrenes lies at a MW > 200,000.

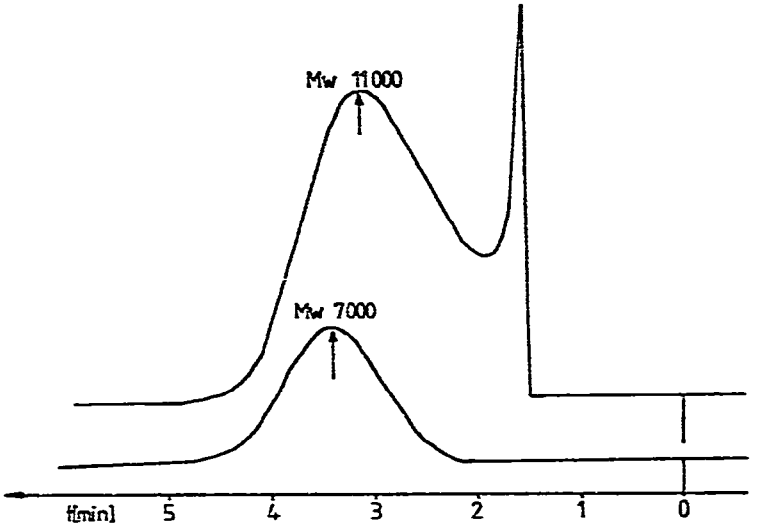

Fig. 9. Characterization of gelatine. Conditions as in Fig. 8. Samples: gelatine (MW correspond to those of polystyrenes eluted at the same time).

CONCLUSIONS

The surface of silica can be modified by chemical reaction to eliminate the interactions of polymeric samples with the surface, so that the separations depend only on exclusion of the molecules from the pores. These materials preserve the advantages of xerogels: they are stable against pressure and/or flow, and their bed and pore volume as well as their pore size distribution are independent of the eluent. This permits the use of the same column with organic eluents as well as with water or salt solution. The different calibration curves can easily be correlated.

From the 14 different bonded phases evaluated three are applicable to the exclusion chromatography of water-soluble polymers. For PEG separation by exclusion chromatography a bonded glycineamide or a diamine phase can be used. The bonded amide phase has less interactions with dextrans, PVP and gelatine. It can also be used for protein separations, which will be discussed in a forthcoming paper.

The optimal pore size diameter for exclusion chromatography of low to medium-molecular-weight polymers (3000 < MW < 100,000) seems to be *ca.* 200 Å. For polymers with MW up to 500,000 a silica matrix with pore diameters *ca.* 500 Å is required. The silicas most commonly used in HPLC have pore diameters of *ca.* 60–100 Å and already totally exclude polymers with MW 10,000 and 20,000 respectively from penetrating the pores. In order to obtain highly efficient columns the particle diameter of these packings should be *ca.* 5 μm or 10 μm.

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