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Review

Polymer-coated reversed-phase packings in high-performance liquid chromatography

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ABSTRACT

The synthesis and properties of polymer-coated RP stationary phases are reviewed. The sorbents are classified according to the method of synthesis. More flexibility in the tailoring of polymer-coated packings is noted. The impact of the polymer coating on the porosity of the oxides to be modified and their chromatographic properties is discussed.

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1. INTRODUCTION

Reversed-phase (RP) packings are the most often used type of stationary phases in HPLC. Most of these products are made from microparticulate porous silicas by means of silanization with appropriate alkylsilanes. The packings demonstrate excellent chromatographic performance and selectivity in the separation of different types of analytes, but they suffer from limited chemical and pH stability. In order to overcome this inherent disadvantage, silica and alumina have been coated with lipophilic polymers or, as an alternative, totally porous, cross-linked hydrophobic polymers have been used. For composite materials the major focus was to deposit a thin polymeric layer onto the surface of a support which shields the latter from interactions with aggressive eluents, while maintaining the well defined pore structure of silica or alumina. Despite the progress made in this field, the introduction of hydrophobic polymer-based or polymer-coated packings into the market appears to be a slow process. As with silanized silicas, the user has a choice between different types of polymer-coated oxides, which feature different retention patterns and selectivity. In this context, it is essential to obtain reliable answers to the following questions: (i) what are the significant structural and physico-chemical differences between silanized and polymer-coated materials?; and (ii) how do polymer-coated packings differ from silanized packings in terms of retention and selectivity?

The aim of the paper is to review briefly the synthetic procedures for polymer-coated packings and to elucidate the retention mechanisms on these columns for a variety of analytes.

2. METHODS OF SYNTHESIS OF POLYMER-COATED RP PACKINGS

Depending on the starting material in the synthesis and the type of immobilization of the polymer layer onto the surface (physisorption or chemisorption), one can distinguish the following synthetic procedures and polymer coatings: polymerization or polycondensation of physisorbed monomers onto the surface without chemical bonding of the polymer layer to the support (type I); polymerization or polycondensation of physisorbed monomers onto the surface with chemical bonding of the polymer layer to the support (type II); immobilization of physisorbed prepolymers without binding of the polymer layer to the support (type III); and chemisorption of presynthesized polymers onto the surface of the support (type IV).

Type I packings were introduced by Horváth and Lipsky [1] in 1966. A mixture of styrene and divinylbenzene was polymerized on the surface of non-porous ceramic spheres of mean diameter 30–40 μm and resulted in a completely insoluble cross-linked polymer layer with a thickness of 1–2 μm. Microparticulate porous silica modified by the same procedure shows a decrease in the specific pore volume and the specific surface area [2,3], with a tendency to form completely non-porous particles or even agglomerates. The reduction of the specific surface area causes a decrease in the loading capacity of the packing. Further, the bulk polymer in the pores leads to significantly slower mass-transfer kinetics, *i.e.*, it impairs the column efficiency. The procedure was improved by Suzuky *et al.* [4], who polymerized a mixture of chloromethylstyrene and divinylbenzene. The monomers were adsorbed on the surface of porous silica from a solution in N,N-dimethylformamide. After equilibration, the excess of monomers was removed by filtration. Following this procedure, a relatively large decrease in the specific surface area and the specific pore volume compared with the parent silica was still observed. Also, it was difficult to avoid the formation of bulk polymer in the pores [3,4].

The main feature of type II packings is the chemical binding of the polymer layer to the native surface. In 1973, Bubkin and Tzetlin [5] suggested the chemical binding of the polymeric layer to the surface of the support under the action of irradiation [5]. In this case it is assumed that the surface radicals $SiO^{\cdot}$ and $Si^{\cdot}$ are responsible for the chemical grafting of polymers to the surface. During this reaction large amounts of non-bonded polymers are formed in the system and have to be removed from the packing afterwards.

Another variant of the synthesis of the type II packings includes the pretreatment of the sup-

port with an unsaturated reagent, *e.g.*, vinyltrichlorosilane, as a first step and subsequent polymerization of the monomer on the modified surface [6,7]. First, this pretreatment can make the surface of oxides more favourable for the adsorption of monomers and polymers. Second, unsaturated groups of the surface also take part in the polymerization and are introduced into the polymer chains. In such a way the polymer layer is chemically bound to the surface. The unreacted groups of the surface have to be deactivated after the synthesis and the non-bonded polymer which was formed during the polymerization has to be removed by extraction. The extraction of the non-bonded polymer is strongly dependent on its molecular mass. In radical polymerization, for instance, a polymer of broad molecular mass distribution is obtained. High-molecular-mass fractions need a very long time to be extracted from the pores. Even after thorough extraction a large amount will remain and thus block the pores or cause so-called 'bleeding' during the chromatographic application. Generally, the *in situ* polymerization (or polycondensation) of monomers on the surface of porous supports is mainly used for the modification of wide-pore packings or for systems with insoluble polymers, *e.g.*, for the synthesis of perfluoroethylene-coated silica [8].

The synthesis of the packings discussed above can probably be improved by using prepolymers (type III packings). In 1971, Hiatt *et al.* [9] applied polyethylene oxide for the dynamic coating of porous glass in order to prevent the adsorption of viruses during the separation. RP packings with physically adsorbed polymer layers have been developed by Schomburg since 1984 [10,18]. The procedure is very simple and consists of the fixation of the polymer layer by evaporation of the solvent from a polymer solution in the presence of the support. Subsequently, the polymer is cross-linked by thermal treatment. The method can be applied to any type of inorganic oxide in combination with any type of cross-linkable polymer. Excellent chromatographic separations have been demonstrated on this type of packing [10,11], despite the irregular distribution of the polymer [12].

The type IV packings are synthesized by chemisorption of prepolymers with reactive groups on the surface of native or premodified mineral oxides. RP packings synthesized by this method were described in 1983 by Kurganov *et al.* [13], who applied a copolymer of styrene and vinylmethyldiethoxysilane for the synthesis of polystyrene-coated silicas. The coating procedure is very similar to those used for the synthesis of conventional monomeric RP packings and one can expect that each copolymer chain forms several covalent links to the surface silanol groups. Since the (co)polymer has to react with the surface, some kind of chemical compatibility between them is required. Polymer chemistry supplies numerous types of (co)polymers with desirable properties and makes this restriction insignificant.

TABLE 1

RP PACKINGS GROUPED ACCORDING TO THE DIFFERENT POLYMER COATING PROCEDURES

Coating polymer	Type of coating procedure	Ref.
Polysiloxane	III	10, 11, 24
Polysiloxane	II	28, 29
Polybutadiene	III	10–12, 18, 21
Polystyrene	II	6, 7, 40
Polystyrene	IV	13, 17
Polystyrene	I	1–5
Polymethacrylate	III	19, 20
Polytetrafluoroethylene	I	8

Table 1 gives selected examples of RP packings synthesized by different methods of polymer coating on supports. In the following, we discuss the chromatographic properties of mainly type III and IV packings.

3. SEPARATIONS ON POLYMER-COATED RP STATIONARY PHASES

The generally accepted mechanism for the retention in RP-HPLC assumes a hydrophobic association of the solute and the stationary phase with the involvement of solvation processes both in the mobile and the stationary phase [14–16]. Later, Martire and Boehm [41] and Dorsey and Dill [42] proposed a more refined mechanism in RP-HPLC. The question arises of whether the retention mechanism on polymer-coated RP

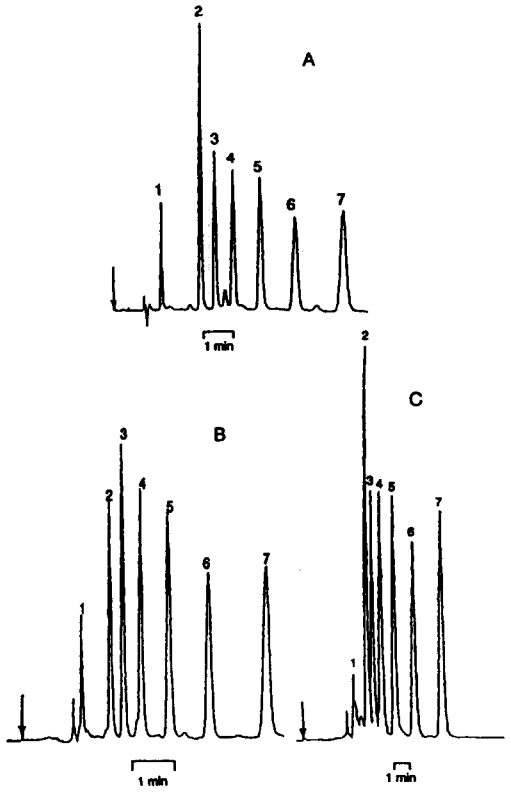

Fig. 1. Separation of a mixture of alkylbenzenes on polystyrene-coated Zorbax PSM-60 (A), PSM-500 (B) and PSM-1000 (C), all of $d_p = 5$ μm (DuPont, Wilmington, DE, USA). Eluent, water–acetontrile (40:60, v/v); flow-rate, 1 ml/min; column, 150 × 4.6 mm I.D. Solutes: 1 = acetone; 2 = benzene; 3 = toluene; 4 = ethylbenzene; 5 = propylbenzene; 6 = butylbenzene; 7 = pentylbenzene.

packings differs from that observed on silanized silicas. For "pure" hydrophobic polymer coatings such as polystyrene [17] or polybutadiene [18] the same retention sequence of lipophilic analytes was observed as for silanized packings. Fig. 1 shows as an example the separation of alkylbenzenes. The retention of the solutes on polystyrene-modified silica is proportional to the content of polystyrene per unit volume of the modified packing, which depends on the specific surface area of the silica to be modified for the chemisorption method of modification. The highest retention was observed for the packing based on silica PSM-60, which exhibits the highest value of specific surface area and the highest content of polystyrene (Table 2). The least retention was observed for the wide-pore packing PSM-1000 with the smallest value of specific surface area and carbon content among the silicas studied.

Polymer layers with structural fragments other than hydrophobic moieties, *e.g.*, polar groups in polymethacrylates, give rise to additional possible interactions that contribute to the retention of analytes. By changing the monomer composition for co-polymethacrylates one can gradually regulate the properties of the hydrophobic stationary phase [19,20].

The variation of the polymer loading on a given support is an additional means of varying the retention of analytes. This dependence was

TABLE 2

CHARACTERISTICS OF POLYSTYRENE-COATED SILICAS [17]

(a) Native silica; (B) coated silica.

Silica	Specific surface area (m^2/g)		Specific pore volume (ml/g)		Mean pore diameter (nm)	Carbon content (%, w/w)	Coating density (μmol/m^2)
	(A)	(B)	(A)	(B)	(A)	(B)	(B)
Zorbax PSM-60	384	334	0.70	0.41	6	13.4	3.6
Zorbax PSM-500	29	28	0.31	0.26	50	2.4	9.6
Zorbax PSM-1000	17	17	0.24	0.23	100	1.3	8.4

TABLE 3

POLYBUTADIENE LOADING ON MONOSPHER [21]

PDB employed (%, w/w)	Loading of PDB (%, w/w)	d^a (nm)	Coating density[b] (mmol/m^2)
0.2	0.16	0.8	1.65
0.5	0.39	1.8	4.01
1.0	0.93	4.1	9.57
2.0	1.79	7.9	18.42
3.0	2.72	12.0	27.98
5.0	4.25	18.8	43.72
10.0	8.31	36.9	85.49

[a] d = Calculated thickness of coating.
[b] Coating density calculated from the data in ref. 21.

investigated for the non-porous silica Monospher (Merck, Darmstadt, Germany), coated with polybutadiene according to procedure III (Table 3) [21]. The capacity factors, k', of a homologous series of *n*-alkylbenzenes increased proportionally with increasing polybutadiene loading up to 4% (w/w) and showed an upward swing to higher retention at much higher loads (Fig. 2). This increase in retention was explained by the formation of bulky polymer clusters, which were identified by scanning electron microscopy (Fig. 3) on highly loaded Monospher [21] and on LiChrosphere Si 300 porous silica [43].

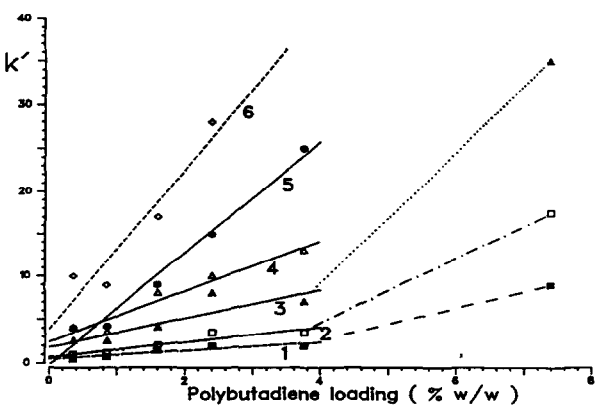

Fig. 2. Dependence of the capacity factor, k', of alkylbenzenes on the polybutadiene loading on Monospher, d_p = 1.7 μm (Merck). Solutes: 1 = ethylbenzene; 2 = *n*-propylbenzene; 3 = *n*-butylbenzene; 4 = isopentylbenzene; 5 = *n*-pentylbenzene; 6 = *n*-hexylbenzene [21].

Another interesting observation was made for inorganic supports with chemisorbed polymers. For polystyrene the coating density calculated in micromoles of monomer units per square metre of specific surface area showed a lower value for the 60 Å pore-size silica than for two larger pore-size packings (PSM-500 and -1000) (Table 2). The same was observed for other types of polymer-coated packings synthesized by the chemisorption method [22,23]. One can assume that the actual concentration of monomer units per square metre of silica is approximately the same for all supports, but for small pore-size packings a certain part of the surface is inaccessible to the modification and the mean calculated value is lower for these packings.

A dense polymer layer should shield the native surface from interactions with analytes sensitive to silanol groups. Excellent separations of mixtures of basic compounds have already been reported for many different polymer-coated packings (Fig. 4). However, the silanol groups of the silica surface still have an impact on the separation and end-capping or presilanization of the silica surface can improve the chromatographic performance of the polymer-coated packings [24]. As a demonstration, Fig. 5 depicts the dependence of the retention of the basic 4-*n*-octylpyridine on the polybutadiene content for the coated non-porous silica Monospher [21]. The plot suggests that the retention declines to a minimum for a load of 1% (w/w) polybutadiene and then increases proportionally for higher

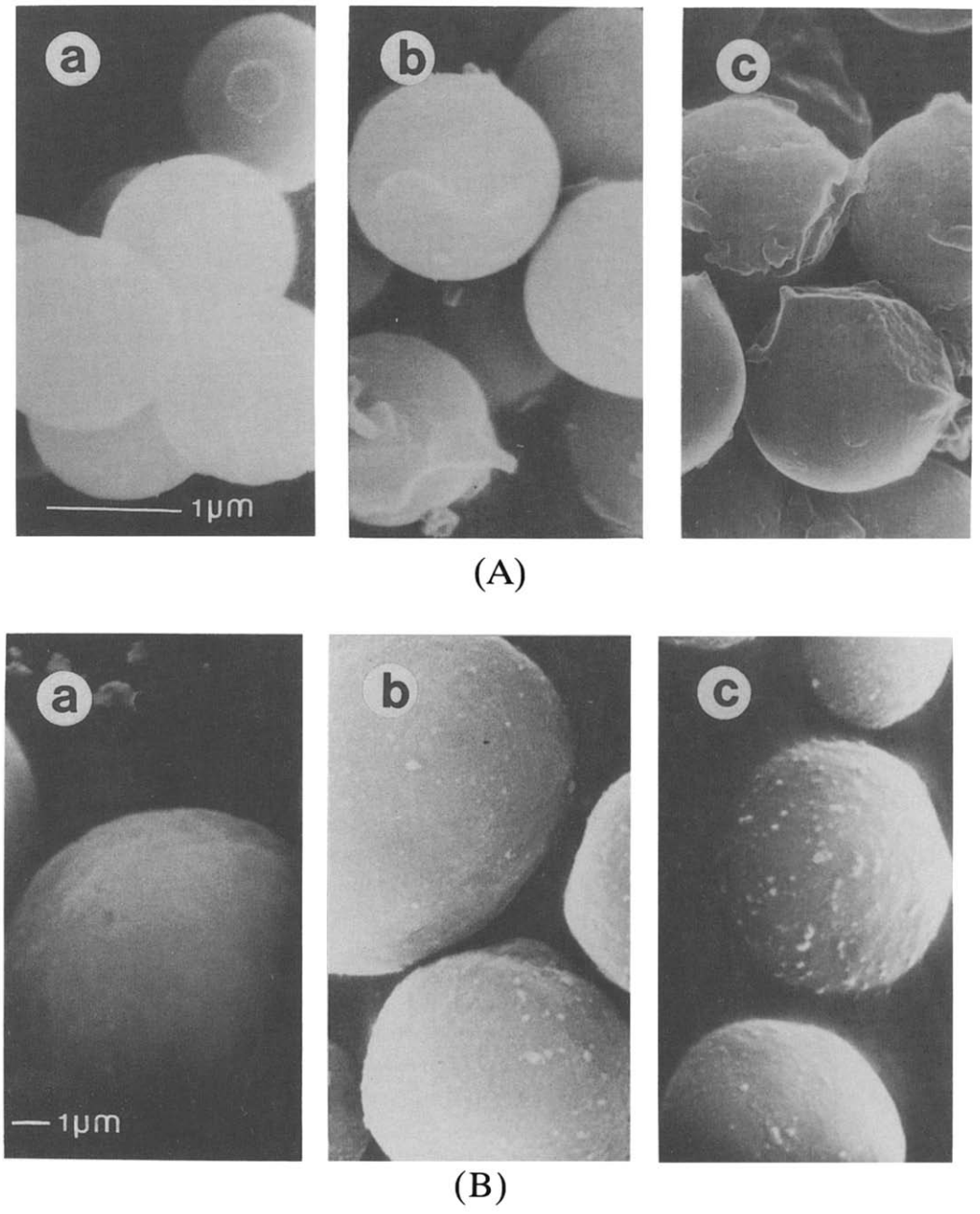

Fig. 3. Scanning electron micrographs of (A) Monospher and (B) LiChrospher Si 300 (both from Merck) coated with different loading of polybutadiene: (A) (a) 1.0%, (b) 5.0% and (c) 10.0% and (B) (a) 0%, (b) 5% and (c) 40% polybutadiene [43].

loadings. The increase in retention for higher loadings is analogous to those described for alkylbenzenes and indicates that the lipophilic interactions dominate at high loadings. The initial part of the curve (Fig. 5) suggests a significant contribution from silanophilic interactions, which influences the selectivity of the separation in the same manner as for monomeric RP packings. This dependence is shown in Fig. 6, where the selectivity of the separation of two

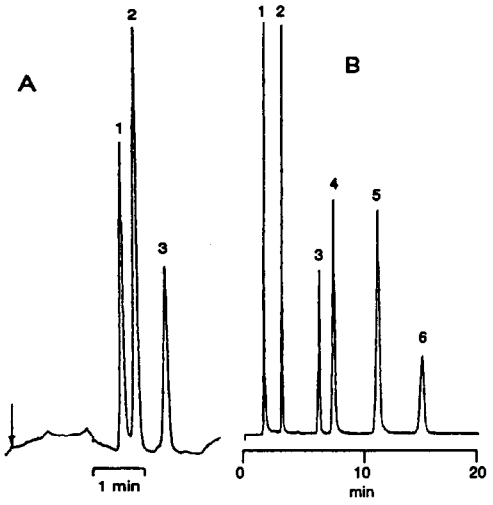

Fig. 4. Separation of basic test solutes with polymer-coated RP packings. (A) Polystyrene-coated Zorbax PSM 500 (DuPont). Column, 150 × 4.6 mm I.D.; eluent, water–acetonitrile (40:60, v/v); flow-rate, 1 ml/min. Solutes: 1 = 2,6-dimethylpyridine; 2 = 1-naphthylamine; 3 = benzylamine [17]. (B) C_{18}-polymethylsiloxane-coated Nucleosil 100-5 (Macherey–Nagel, Düren, Germany). Eluent, methanol–water (70:30, v/v); flow-rate, 1 ml/min; column, 125 × 4.5 mm I.D. Solutes: 1 = α-picoline; 2 = N,N-dimethylaniline; 3 = ethylbenzene; 4 = 2-*n*-octylpyridine; 5 = 2-*n*-nonylpyridine; 6 = *n*-butylbenzene [11].

pairs of solutes, namely *n*-octylpyridine–benzyl benzoate and benzyl benzoate–benzophenone, is plotted against the polybutadiene loading. For the polar and neutral analytes benzyl benzoate

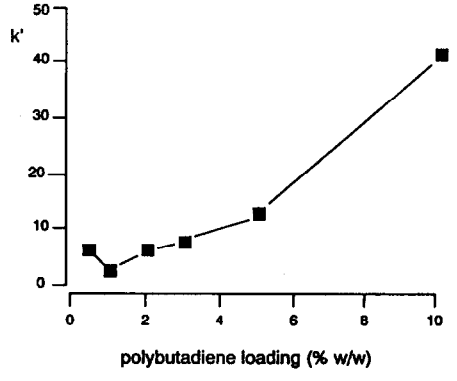

Fig. 5. Plot of the capacity factor, *k'*, of *n*-octylpyridine *versus* the polybutadiene loading on Monospher (Merck) [21].

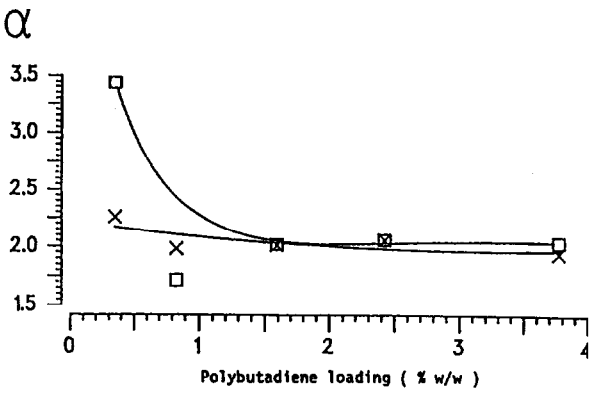

Fig. 6. Plot of the selectivity coefficients, α, of two pairs of solutes *versus* the polybutadiene loading on Monospher (Merck) [21].

and benzophenone, the selectivity of the separation remains constant with increasing polymer loading. Therefore, the same retention mechanism for both compounds is proposed. For the pair *n*-octylpyridine–benzyl benzoate the selectivity decreases at an early stage of the loading and remains constant with further loading. This behaviour confirms the contribution of silanol groups of the silica to the retention of the basic pyridine derivative. The contribution of this interaction becomes negligible for non-porous beads with a polymer concentration higher than 1% (w/w), where hydrophobic interactions of the analyte with the modifying polymer dominate for both analytes.

In spite of the great similarity of the retention mechanism on monomeric and polymeric stationary phases, polymers can provide some specific features. One of these particular features is the contribution of the micropores possibly contained in the polymeric layer to the separation. Small pores can appear during the process of cross-linking of bulk polymers but also occur in polymer layers [25] and manifest themselves under certain conditions of chromatography. These pores can have a mean diameter of less than 2 nm and can contribute very little to the total specific pore volume (*ca.* 0.1 ml/g). The presence of these micropores, however, causes slower mass-transfer kinetics and might change the selectivity due to the steric exclusion of analytes. The assessment of small pores, in

particular micropores, in any kind of packing, especially their size distribution, is not a trivial procedure. Mercury porosimetry (MP) measures macropores in rigid materials down to mesopores of about 4 nm pore diameter, depending on the maximum pressure. Thus, MP is a method insensitive to micropores. Low-temperature nitrogen adsorption (NA) enables one to prove the existence of micropores by means of the so-called t or a_s plot, which is the plot of the amount adsorbed *vs.* the statistical thickness of the film (t) or the normalized adsorption (a_s). The t-values are obtained from the adsorption on a reference sample which is non-porous [26]. For microporous materials having the same bulk composition as the porous material, a positive intercept is observed that is equivalent to the micropore volume. One should emphasize that in nitrogen sorption at 77 K the sample is outgassed at elevated temperature under high vacuum prior to measurement. Hence the sample is not measured with the solvent used as the mobile phase in HPLC. A method for detecting micropores in a packing under *in situ* conditions is inverse size-exclusion chromatography (ISEC). If micropores are present, the calibration graph (logarithm of molecular mass *versus* elution volume) of the column should show fractionation in the low-molecular-mass range of about 2000 [25,27].

Further, the pore structure in the micropore range of a polymer or polymeric layer might give rise to a specific shape selectivity [28–30]. Such an effect was observed for polyaromatic hydrocarbons (PAHs) on RP packings synthesized by polycondensation of octadecyltrichlorosilane. A so-called "slot mechanism" for the separation of PAHs on these packings has been proposed [31,32]. It assumes the existence of microcavities in the polymer coating on the surface, which are well suited for the penetration of planar PAHs, but not for PAHs distorted from planarity.

Changes in the pore-size distribution of the parent silica during coating procedure IV with polystyrene have been studied [17]. Three different methods were applied to elucidate these changes: MP, NA and ISEC. Typical results of these investigations are shown in Fig. 7 and Table 2. MP measurements (Fig. 7A and B) do not show any significant changes in the pore structure of native silica with a pore diameter of 100 nm (Zorbax PSM-1000), as could be expected for the method of synthesis applied. The specific surface area and the specific pore volume of this packing remained unchanged after the modification. Only silica with a pore diameter of 6 nm (Zorbax PSM-60) shows a large decrease in the specific surface area after the modification. For such supports we can expect blockage of small pores with the polymer. These changes are of the same order of magnitude as those observed during the synthesis of monomeric RP silicas. More pronounced changes in the porosity of packings were detected in studies by means of ISEC (Fig. 7C and D) in a solvent causing swelling of the bonded polymer. Here a shift of the pore diameters to smaller values was observed even for wide-pore materials. Swelling of the polymer layer, lifting up the loose ends and loops of polymeric chains from the solid support into the mobile phase under chromatographic conditions, diminishes the mean pore diameter determined by ISEC. At the same time, the specific pore volume determined by ISEC for modified wide-pore silicas does not differ much from the values for the parent silicas. One can conclude that not only the pores of the silica but also those of the polymer layer are still accessible for the penetration of low-molecular-mass com-

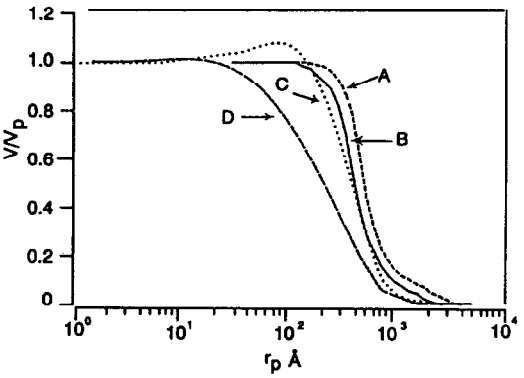

Fig. 7. Cumulative pore-size distribution of the parent and polystyrene-coated Zorbax PSM-1000 (DuPont). Mercury porosimetry (A) before and (B) after polymer modification. Inverse size-exclusion chromatography (C) before and (D) after polymer modification [17].

pounds. Nevertheless, we did not observe the appearance of a second plateau in the low-molecular-mass range of the SEC calibration graphs due to micropores. The absence of microporosity in composite materials can be explained by the small thickness of the polymer film.

Using a different method of immobilizing polymeric chains, the properties of the final packing can differ strongly from those discussed above. Lecourtier *et al.* [33] prepared polystyrene-coated silicas by binding the ends of living polystyrene chains to the silica surface and reported a significant decrease in the specific pore volume after modification. Unexpectedly, the modified sorbent showed a better separation ability in SEC of high-molecular-mass analytes than the initial silica. From these and other data it was concluded that long polymer chains were mainly bound to the outer surface of the particles, almost totally blocking the pore entrances. The large pores then arose in the bulk polystyrene at the outer surface and led to the separation of high-molecular-mass polymers.

Peptides and proteins are relatively high-molecular-mass analytes that can be used for testing the mass-transfer kinetic properties of polymer-coated RP stationary phases. The separation of these solutes by means of RP-HPLC has been reviewed elsewhere [34,35]. According to many experimental findings, the length of the alkyl chains of brush-type monomeric RP packings exerts no significant effect on the retention of proteins, because large protein molecules cannot penetrate the hydrocarbonaceous layer. For polymer-coated packings the structure of the hydrophobic layer, the porosity of the packing and the residual surface silanol groups can all contribute to the retention. The typical dependence of k' of proteins on the eluent composition is a U-shaped curve with a strong increase in the retention at lower and higher contents of organic modifier in the eluent [36]. Fig. 8 shows the dependence of the retention of peptides and proteins on the acetonitrile content in the mobile phase [0.1% trifluoroacetic acid (TFA) in water] on polystyrene-coated silicas of different pore sizes [37]. For proteins a sharp decrease in the retention with the increase of the fraction of organic solvent, φ, up to 0.5 can be observed. At

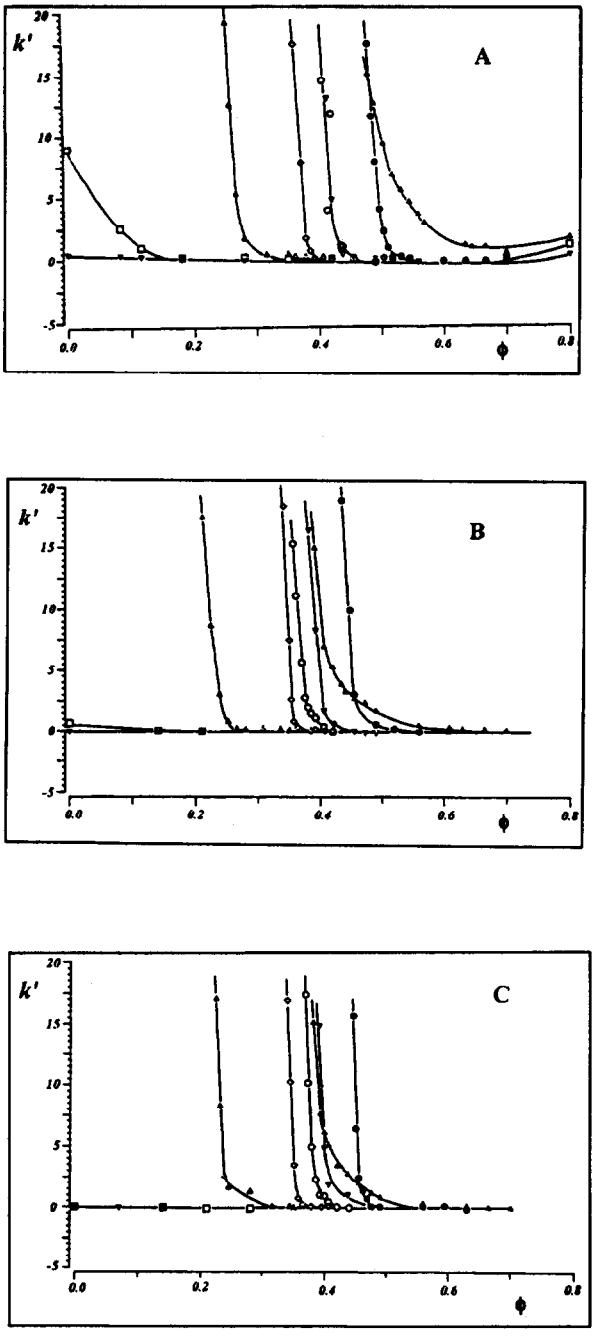

Fig. 8. Plot of capacity factors of proteins and peptides against the fraction of acetonitrile in the eluent for the polystyrene-coated Zorbax (DuPont). (A) PSM-60; (B) PSM-500; (C) PSM-1000. Eluent, acetonitrile–water containing 0.1% TFA. ▼ = Dipeptide His–Gly; ▽ = catalase; ○ = myoglobin; ● = ovalbumin; □ = pentapeptide Try–Gly–Gly–Phe–Met; △ = ribonuclease; ▲ = gramicidin; ◇ = transferrin [37].

a higher content of acetonitrile ($0.5 < \varphi < 0.8$) the proteins elute in the exclusion volume of the column, with the exception of the highly hydrophobic gramicidine. This behaviour is essentially the same as that observed for conventional monomeric RP packings. However, there is a difference in that relatively little or no enhancement of the retention of proteins at higher concentrations of acetonitrile is observed. This can be attributed to good shielding of the silica surface by the polystyrene and a decreasing contribution to the retention from silanophilic interactions [36], as is characteristic of conventional RP packings with enhanced shielding of surface silanols. In the latter instance, higher concentrations of acetonitrile ($\varphi > 0.9$) are needed to increase the retention of proteins.

For small peptides the retention is proportional to the polymer content of the packing and the content of acetonitrile in eluent. The increase in the retention of peptides with decrease in the acetonitrile content in the eluent is much less steep than for proteins. This situation again is similar to those observed with conventional monomeric RP packings.

The linear dependence of the logarithm of the capacity factor, log k', of peptides and proteins on the content of organic modifier in the eluent was found to hold for both monomeric RP packings [38] and polystyrene-coated materials. This is exemplified by the values the solvent strength parameters S_i collected in Table 4, which are comparable to those of common "brush-type" RP packings [39].

There are three main contributions to the retention of proteins on RP packings: polar-surface interactions, size-exclusion and hydrophobic interactions. With increasing pore diameter of the silica, the influence of the size-exclusion mechanism gradually diminishes. The contribution of the polar interactions with the silica surface should also decrease owing to the enhanced coating density on wide-pore materials (Table 2). Therefore, the retention of proteins on polystyrene-coated sorbents should mainly be governed by a hydrophobic interaction mechanism. Hence, the highest solvent strength values S_i were found for combinations of large and hydrophobic proteins and polystyrene-coated macroporous silica (Table 4).

The synthesized packings easily resolve mixtures of peptides and proteins under gradient elution conditions (Fig. 9) and the elution order of the solutes is essentially the same as on conventional RP packings. The good mass-transfer kinetics of polymer-coated stationary phases and the good shielding of the native surface by the polymer layer make these packings attractive for high-speed separations of proteins. This was demonstrated by protein separations in less than 20 s on polybutadiene-coated Monospher (Fig. 10A). The elution order of proteins and the high efficiency of the separations strongly support the hydrophobic adsorption–desorption mechanism. In addition, Fig. 10B shows a rapid separation on the less hydrophobic poly(ethyl methacrylate) (PEMA) phase, which maintains lysozyme native under these conditions. This provides an advantageous selectivity shift and permits a complete resolution of the three proteins examined.

TABLE 4

SOLVENT STRENGTH PARAMETER S_i FOR PEPTIDES AND PROTEINS ON POLYSTYRENE-COATED ZORBAX PSM SILICAS IN ACETONITRILE–WATER MOBILE PHASE [37]

dp = Dipeptide His–Gly; pp = pentapeptide Tyr–Gly–Gly–Phe–Met; RNA = ribonuclease; Grm = gramicidin; Ova = ovalbumin; Tyn = human thyroglobulin; Myo = myoglobin; Cat = catalase.

Silica	dp	pp	RNA	Grm	Ova	Tyn	Myo	Cat
Zorbax PSM-60	2	10	27	5	40	59	36	46
Zorbax PSM-500	–	–	35	7	35	77	39	40
Zorbax PSM-1000	–	–	51	8	54	73	35	53

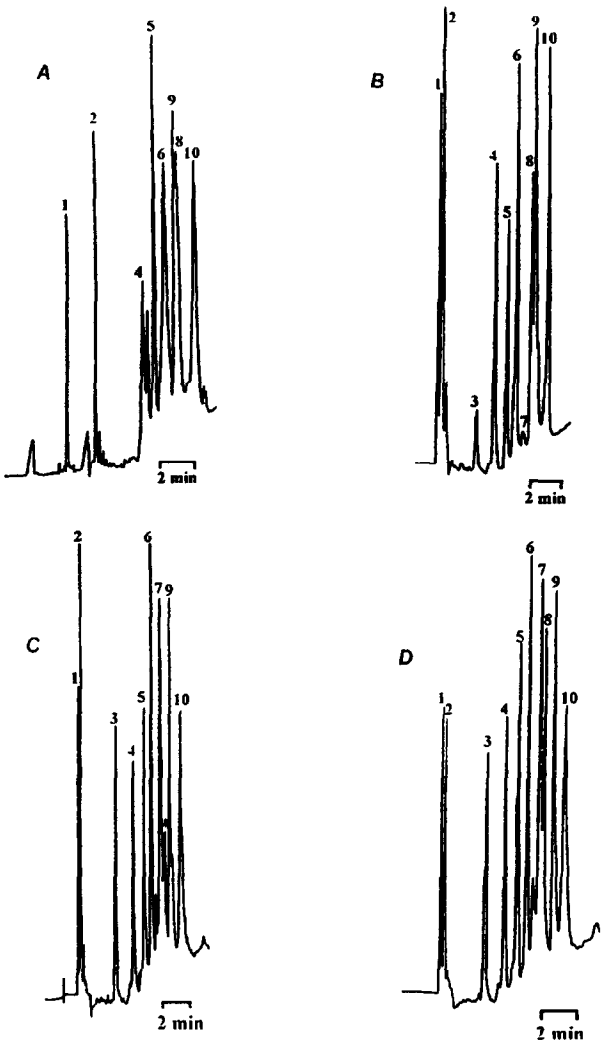

Fig. 9. Separation of a peptide–protein mixture on polystyrene-coated Zorbax (DuPont). (A) PSM-60; (B) PSM-500; (C) PSM-1000; (D) silica with a pore size of 200 nm. Column, 125 × 4 mm I.D.; flow-rate, 1 ml/min; gradient from 5% to 100% B in 10 min with A = 0.1% TFA in water, B = 0.1% TFA in acetonitrile. Solutes: 1 = dipeptide (dp); 2 = pentapeptide (pp); 3 = octapeptide Ser–Arg–Val–Tyr–Ile–His–Pro–Leu; 4 = ribonuclease (RNA); 5 = cytochrome *c* (Cyt); 6 = lysozyme (Lys); 7 = conalbumin (Con); 8 = myoglobin (Myo); 9 = catalase (Cat); 10 = ovalbumin (Ova) [37].

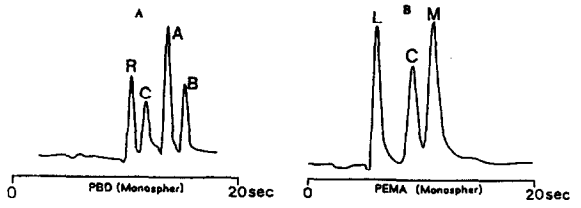

Fig. 10. Separation of proteins on polymer-coated Monospher (Merck) on 20 × 4.6 mm I.D. columns. (A) Coated with PBD, gradient from 0% to 100% B in 30 seconds, with A = water–acetonitrile (90:10) containing 0.05% TFA and B = acetonitrile containing 0.05% TFA; flow-rate, 5 ml/min. (B) coated with PEMA, gradient from 0% to 100% B in 15 s, with A = water–acetonitrile (85:15) containing 0.05% TFA and B = acetonitrile containing 0.05% TFA; flow-rate, 5 ml/min. Solutes: R = ribonuclease; C = cytochrome *c*; A = α-lactoglobulin; B = β-lactoglobulin; L = lysozyme; M = myoglobin [21].

4. CONCLUSIONS

Polymer-coated stationary phases for RP-HPLC, which historically were the first high-performance RP packings, show great potential in their separation ability, chemical stability and chromatographic performance. Compared with traditional monomeric alkylsilanized packings, the procedures for the synthesis of polymer-coated packings allow additional flexibility in tailoring the hydrophobic surface properties. Even supports such as alumina, zirconia and titania can be used as parent packings [44,45]. By increasing the load of the immobilized polymer one can change the initial pore structure of oxides. Thicker polymer films bear the disadvantage that the column efficiency may decrease owing to the slower mass-transfer kinetics of analytes in the swollen polymer layer.

Polymer-coated sorbents with "pure" hydrophobic coatings demonstrate the same separation mechanism as observed for their monomeric silanized analogues. The shielding effect by the polymer coating diminishes the contribution from the surface of the native support to the retention of the solutes, especially for solutes of high molecular mass. Additional selectivity in the application of polymer-coated RP phases can arise from the microporosity of the polymer layer, which still remains to be explored.

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