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Polyvinylpyrrolidone-coated silica packings for chromatography of proteins and peptides

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Abstract

Polyvinylpyrrolidone (PVP)-coated silica sorbents were synthesized by interaction of a copolymer of vinylmethyldiethoxysilane and vinylpyrrolidone with LiChrospher Si 300 and LiChrospher Si 500 silicas. The coating procedure retains the wide-pore structure of the starting silicas, as was shown by nitrogen adsorption, mercury porosimetry and inverse size-exclusion chromatographic measurements. Good selectivity and separation ability of the synthesized packings toward proteins and peptides were demonstrated in the hydrophobic interaction chromatographic mode of separation. Aromatic compounds undergo a specific interaction with bonded PVP chains which can be used for the preconcentration or selective recovery of polycyclic aromatic hydrocarbons.

1. Introduction

Chromatographic methods are one of the most powerful tools for the separation of complex multi-component mixtures of natural and biologically active substances. Hydrophilic packings are usually used for such separations, as they operate with water-rich eluents compatible with bioactive compounds and prevent denaturation of sensitive bioanalytes. These packings are often polysaccharides (*e.g.*, Sephadex, Sepharose), polyacrylamides (*e.g.*, Separon) or polyalcohols (*e.g.*, TSK-polymer gel, Fractogel). The pressure resistance of these sorbents is generally not sufficient for HPLC separations and many attempts have been made to combine the excellent chemical properties of hydrophilic polymers with the outstanding mechanical stability of silica gel. Many different methods of synthesis of composite materials have been developed and described elsewhere [1,2]. Polyvinylpyrrolidone (PVP)-coated materials are of particular interest for pharmaceutical and medical applications.

The first attempt to synthesize PVP-coated silicas was made by Caude and Rosset [3], who polymerized monomeric vinylpyrrolidone (VP) on the surface of silica modified with vinyltrichlorosilane. A number of protein separations by means of hydrophobic interaction chromatography (HIC) were demonstrated with this packing and a relatively high hydrophobicity of the coating were reported. The concentration of vinyl groups on the surface was obviously too

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high, which contributed to the retention of some proteins. In addition, the porosity of the starting silica was substantially changed during the polymer bonding procedure.

Ivanov *et al.* [4] suggested modifying γ-aminopropylated silica by a presynthesized copolymer of VP and acryloyl chloride. Only a certain part of the amino groups on the silica surface and chloroanhydride groups of the copolymer took part in the reaction. The unreacted functional groups had to be deactivated, *e.g.*, by reaction with acetyl chloride and ethanolamine, respectively. As a result, a polyfunctional packing with badly reproducible properties was synthesized.

An original method of immobilization was suggested by Köchler [5]. According to his observations, PVP adsorbed on the silica surface could not be removed by extraction, provided that the composite was heated to 200°C. The chemical nature of the irreversible immobilization is not clear, but Köchler proposed an opening of some lactam rings and the formation of chemical bonds with the surface. The synthesized sorbent was successfully used in the analysis of low-molecular-mass compounds by normal- and reversed-phase (RP) chromatography.

An American patent [6] described the synthesis of a PVP-coated packing by the modification of silica with the copolymer of VP and vinyltrichlorosilane. No attempts were made to purify the copolymer and a rough mixture of homo- and copolymers were used. The synthesized packing exhibited a relatively high hydrophobicity owing to the binding of vinyltrichlorosilane and its oligomers to the surface. Nevertheless, the sorbent could be applied to the separation of proteins by means of size-exclusion chromatography (SEC) with appropriate eluents.

In this paper an improved synthetic procedure for coating silica with PVP using isolated and purified VP–vinylsilane copolymer is described. The properties of these packings were studied in the separations of low- and high-molecular-mass analytes. The changes of the porosity of the silica matrix during the binding of the copolymer were studied and are discussed.

2. Experimental

2.1. Materials

A mixture of polycyclic aromatic hydrocarbons (PAHs) was prepared from individual compounds supplied by Merck and Aldrich.

The proteins used were ribonuclease A, lactate dehydrogenase, catalase, ferritin, ovalbumin, cytochrome *c*, insulin, myoglobin, lysozyme, thyroglobulin, human serum albumin, conalbumin, β-lactoglobulin, chymotrypsinogen A, trypsin and calf thymus and were supplied by Merck and Serva. The peptides were angiotensin I (human, Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu), oxytocin (Cys–Tyr–Ile–Glu–Asn–Cys–Pro–Leu–NH_2), neurotensin (pGlu–Leu–Tyr–Glu–Asn–Lys–Pro–Arg–Arg–Pro–Tyr–Ile–Leu) and somastatin (Gly–Cys–Lys–Asn–Phe–Phe–Trp–Lys–Thr–Phe–Thr–Ser–Cys) and were purchased from Sigma.

All solvents were of LiChrosolv grade from Merck and used as received.

2.2. Synthesis of copolymer

The copolymerization of vinylpyrrolidone and methylvinyldiethoxysilane was carried out in sealed ampoules under a nitrogen atmosphere. A solution of monomers and initiator in toluene was placed in an ampoule, frozen, evacuated, filled with nitrogen and then frozen out. This operation was repeated twice before sealing the ampoule. Polymerization was then performed for 7 h at 100°C. The concentration of monomers was 40% (w/w) in a ratio of 1:1. Dicumyl peroxide was used as the initiator at a concentration of 1% (w/w) with respect to the monomers. After the polymerization, the ampoule was cooled and the solution of homo- and copolymers was dropped into diethyl ether. The precipitated copolymer was filtered off and reprecipitated. The purified copolymer was dried under vacuum at 50°C. The molecular mass, determinated by means of ultracentrifugation (Model M-20 ultracentrifuge, Radelkis, Szeged, Hungary), was found to be 6500. The ratio of vinylpyrrolidone to vinylsilane monomer units in

the copolymer was 8:1, as determined by elemental analysis. The high sensitivity of the copolymer to the presence of water in solvents is worth noting. Traces of water cause cross-linking of the copolymer, which may lose its solubility in any solvent after reprecipitation.

2.3. Coating procedure

The initial silica was dried by azeotropic distillation of water with toluene. The solution of the copolymer in toluene was dropped into a suspension of silica in toluene boiling under reflux. A 0.25-g amount of copolymer was taken for modification of 1 g of LiChrospher Si 300 silica and 0.15 g for modification of 1 g of LiChrospher Si 500 silica. The suspension was boiled under reflux for an additional 5 h and the hot suspension was then filtered through a porous glass frit. The coated silica was washed thoroughly with two portions of hot toluene and dried under vacuum at 50°C. End-capping was carried out with hexamethyldisilazane according to standard procedure described elsewhere [7]. The elemental analysis data for the synthesized packings are presented in Table 1.

2.4. Physical measurements

Nitrogen adsorption isotherms were measured with an ASAP-2400 system (Micromeritics). The pore-size distribution and the specific pore volume were calculated from the adsorption isotherms by using standard mathematical software provided with the ASAP-2400 and are given in Table 1.

Mercury porosimetric measurements were performed with a Model 2000 porosimeter (Carlo Erba). The mean pore diameters and pore volumes of the packings determined from these measurements are given in Table 1.

The mean thickness of the polymeric layer was calculated according to the equation

$$d\ (\mathrm{nm}) = 10^3 m/\rho S(1-m)$$

where m is the polymer content in the composite (g/g), ρ is the density of the polymer (1.0 g/cm^3), S is the specific surface area of the native silica (m^2/g) and $1-m$ is the silica content in the composite (g/g).

2.5. Chromatography

The columns were packed by the slurry technique with a 4% (w/w) suspension of sorbent in tetrahydrofuran–tetrachloromethane (1:1) and methanol as the displacement liquid. The pressure during the packing procedure was increased to 350 bar with a Shandon packing device.

The chromatographic system used was an L-6200 intelligent low-pressure gradient pump, an L-4200 variable-wavelength UV detector and a D-2500 integrator (all from Merck). Sample injection was performed with a Rheodyne Model 7125 injection valve equipped with a 20-μl injection loop.

The polystyrene standards used in the inverse SEC experiments were of M_r in the range 3 340 000–580 with a polydispersity of 1.04–1.10 from Merck, Polymer Laboratories and Polymer Standard Service. In low-molecular mass range, benzene ($M_r = 78$), toluene ($M_r = 92$) and 2,3-diphenylbutane ($M_r = 162$) were used as markers (all from Merck). The pore-size distribution was calculated from SEC data according to the method described by Knox and Ritche [8]. The values of the pore volume and specific surface area were determined per millilitre of packing in the column. In order to recalculate these values to a per gram of packing basis, it was assumed that the density of a packing is a linear function of its composition:

$$\rho_{\mathrm{comp.}} = \rho_{\mathrm{silica}} m'_{\mathrm{silica}} + \rho_{\mathrm{polymer}} m'_{\mathrm{polymer}}$$

where ρ is the density of the composite, silica or polymer and m' is the mass of polymer or silica in the composite.

3. Results and discussion

3.1. Physical and chemical properties of PVP-coated packings

Properties of the starting silicas and synthesized coated sorbents are given in Table 1. The

Table 1
Physico-chemical properties of synthesized packings

Silica	Elemental analysis (%)			Polymer content (mg/g)	Thickness of polymer layer (Å)	Coating density μmol/m^2	Low-temperature nitrogen adsorption[a]			Mercury porosimetry[a]			Inverse SEC[a]		
	C	H	N				V (ml/g)	D^b (Å)	S (m^2/g)	V (ml/g)	D (Å)	S^b (m^2/g)	V (ml/g)	D (Å)	S^b (m^2/g)
LiChrospher Si 300, native	–	–	–	–	–	–	2.00	320	250	1.80	380	170	2.10	480	175
LiChrospher Si 300, coated with PVP	13.1	2.7	2.4	200	10	14.0	1.33	407	130	1.21	320	151	2.30	476	195
LiChrospher Si 300, coated and end-capped	12.8	2.7	2.1	200	10	14.0	–	–	–	–	–	–	–	–	–
LiChrospher Si 500, native	–	–	–	–	–	–	0.80	550	45	1.00	500	80	1.05	470	90
LiChrospher Si 500, coated with PVP	5.8	0.9	1.1	89.5	22	13.5	0.64	463	55	0.82	500	65	1.42	442	79
LiChrospher Si 500, coated and end-capped	5.9	0.9	1.0	89.5	22	13.5	–	–	–	–	–	–	–	–	–

[a] V = specific pore volume; S = specific surface area; D = pore diameter.
[b] Calculated according to $V/S = D/4$.

polymer content of the composites was calculated from carbon elemental analysis. It was found to be of about 200 mg of PVP per gram of composite based on LiChrospher Si 300 and of about 90 mg per gram of composite based on LiChrospher Si 500. These values correlated with the specific surface area of the modified silicas. The coating density calculated as micromoles of monomer units per square metre of modified surface is approximately the same for both packings (Table 1), amounting to about 14 μmol/m^2, which is much higher than is typical for silanazed silicas [7]. The end-capping of the coated silicas did not cause any significant increase in the composite carbon content (Table 1) and provided good shielding of the silica surface by the polymer.

The low-temperature nitrogen adsorption measurements revealed that the specific surface area of LiChrospher Si 300 decreased by about 35% after modification. In contrast, a small increase in the specific surface area was observed for LiChrospher Si 500 coated with copolymer (Table 1). Both initial silicas demonstrated a wide pore-size distribution, extending towards small pores for LiChrospher Si 300 but towards large pores for LiChrospher Si 500 (Fig. 1). This pore-size distribution was not significantly changed after the modification (Fig. 1) and it seems to be an indication that a relatively thin polymeric layer was deposited on the pore surface (Table 1). Probably the smallest pores of diameter ≤30 Å, which could not be evaluated by means of the common nitrogen adsorption technique, were partly blocked during the modification by PVP and this caused the large de-

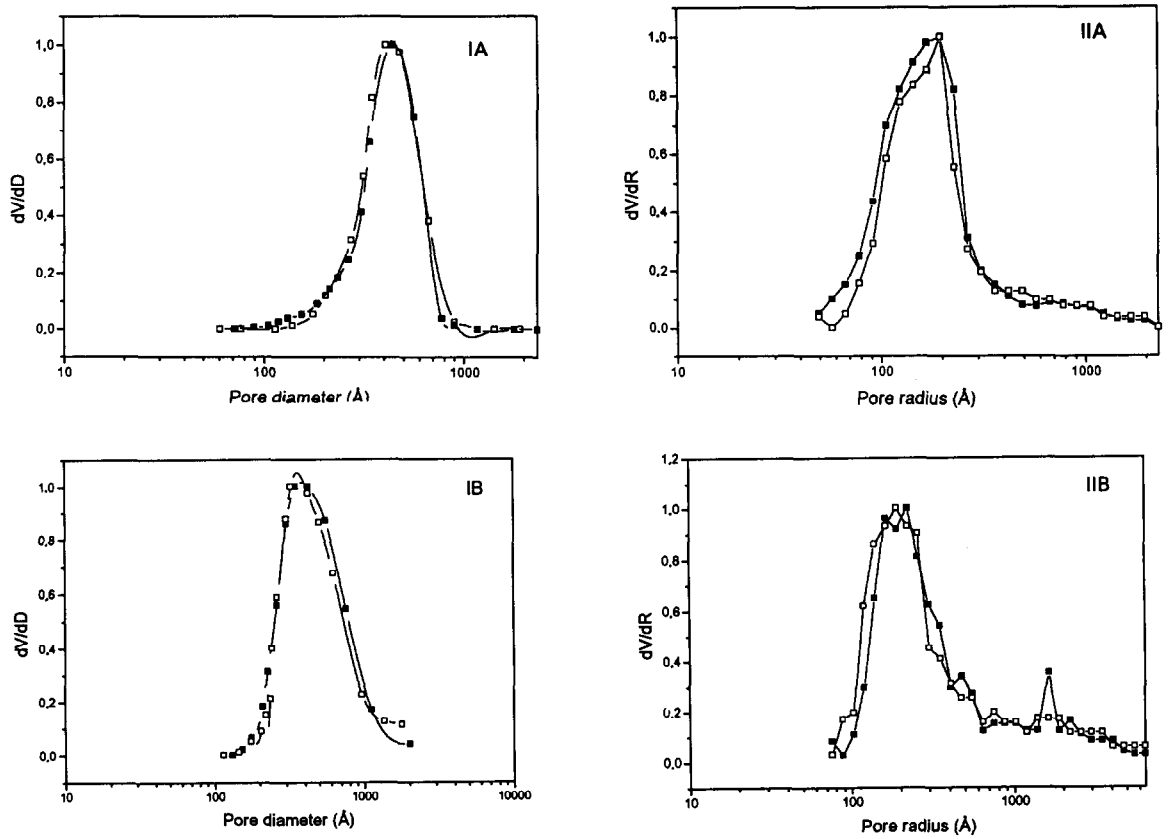

Fig. 1. Pore-size distribution of the (□) native and (■) PVP-coated silicas (A) LiChrospher Si 300 and (B) LiChrospher Si 500 from (I) nitrogen adsorption and (II) mercury porosimetry measurements.

crease in the specific surface area of LiChrospher Si 300 after modification. The same reason can explain the increase in the mean pore diameter of the modified LiChrospher Si 300. For the packing based on LiChrospher Si 500 the contribution of small pores seems to be insignificant, as indicated by the much lower value of its specific surface area. The pore volume of the packings is mainly connected with large pores and it was changed on coating LiChrospher Si 300 to a smaller extent (about 17%) than the surface area. For LiChrospher Si 500 the pore volume decrease was about 10%.

The specific pore volumes of packings based on 300 Å silica determined by means of mercury porosimetry were slightly lower than those measured by nitrogen adsorption, but slightly higher values were found for native and PVP-coated LiChrospher Si 500 packings. These differences could be expected, owing to the different limitations of these two methods. The changes in the porosity of native silicas after modification with the copolymer determined by mercury porosimetry were less pronounced than those found by nitrogen adsorption measurements.

In SEC with polystyrenes as test molecules, both modified and native silicas demonstrated a wide range of molecular mass discrimination (Fig. 2), as could be expected from the wide pore-size distribution determined by nitrogen adsorption or mercury porosimetry. The calibration graphs for PVP-coated packings are slightly lower than those for native silicas (Fig. 2), indicating some decrease in the mean pore diameters of the packings after modification (Table 1). The shape of the calibration graphs was not significantly changed after modification and therefore the pore-size distribution also was not much changed. This agrees with the results obtained by mercury porosimetry and nitrogen adsorption measurements (Fig. 1). The pore diameters and the specific surface areas measured by means of inverse SEC agreed fairly well with the values determined by other methods, but the pore volumes of the modified packings differed by a factor of almost two (Table 1). This major difference can arise because of the difficulties in the discrimination between the inter- and intraparticle volumes in the SEC calibration graphs, as was described by Stegeman *et al.* [9].

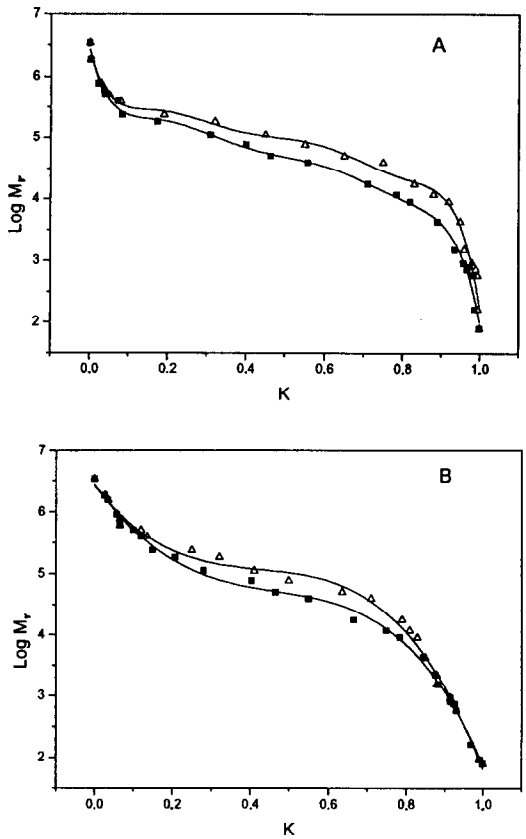

Fig. 2. SEC calibration graph for polystyrene standards on the (△) native and (▲) PVP-coated silicas (A) LiChrospher Si 300 and (B) LiChrospher Si 500. Eluent, tetrahydrofuran; flow-rate, 0.5 ml/min; column, 250 × 4 mm I.D.

3.2. Chromatographic separations on synthesized packings

Normal- and reversed-phase separations of aromatic hydrocarbons

Silica-based packings with covalently bonded pyrrolidone groups can be explored in both normal- and reversed-phase modes of chromatography depending on the eluent used [10]. To prove that PVP-coated packings can be applied to the same modes of separations, some mixtures of aromatic compounds were investigated. One of these mixtures was composed of benzene and

nitrobenzene. For alkyl/aryl stationary phases one assumes that in normal-phase chromatography nitrobenzene elutes before benzene on packings with a low contribution to the retention from silanol surface groups. For polar stationary phases such as PVP-coated silica, an additional contribution to the retention can arise from specific interactions between aromatic analytes and polar groups of modifying layer. The selectivity and retention data for benzene–nitrobenzene separations on the synthesized packings are given in Table 2. The same parameters for the unmodified silica LiChrospher Si 300 and the octadecyl RP packing Superspher-100 RP-18 are also presented in Table 2 for comparison. Benzene was found to be eluted before nitrobenzene on all the sorbents studied. The smallest retention of nitrobenzene was observed on PVP-coated LiChrospher Si 300, and the highest retention, with a broad and tailing peak (the number of theoretical plates was less than 1000), was observed on PVP-coated LiChrospher Si 500. The retention was even higher than that on native silica LiChrospher Si 300 and probably arises owing to specific interactions between the nitro and amide groups because benzene gave a sharp and symmetrical peak (the height equivalent to a theoretical plate was between 3.0 and 3.5 d_p) with all the sorbents studied.

A specific mechanism of interaction between aromatic compounds and pyrrolidone moieties of the modifying layer can be easily demonstrated from a normal-phase separation of PAHs on the synthesized packings (Fig. 3). When pure *n*-pentane was used as the eluent, the copolymer-coated silicas demonstrated higher retentions of PAHs than the unmodified silica or Superspher-100 RP-18. On the column packed with coated LiChrospher Si 500 under given conditions only the first five components could be eluted in a reasonable time of about 1 h and therefore the separation is not shown in Fig. 3. The mechanism of the stronger retention of PAHs on this sorbent is not clear.

Table 2
Capacity factors and selectivity of separation of benzene–nitrobenzene mixture on synthesized sorbents

	k'		α
	Benzene	Nitrobenzene	
LiChrospher Si 300, native	0.09	1.83	20.33
LiChrospher Si 300, coated with PVP	0.15	0.82	6.13
LiChrospher Si 500, coated with PVP	0.25	3.21	12.84
Superspher-100 RP-18	0.20	1.45	7.25

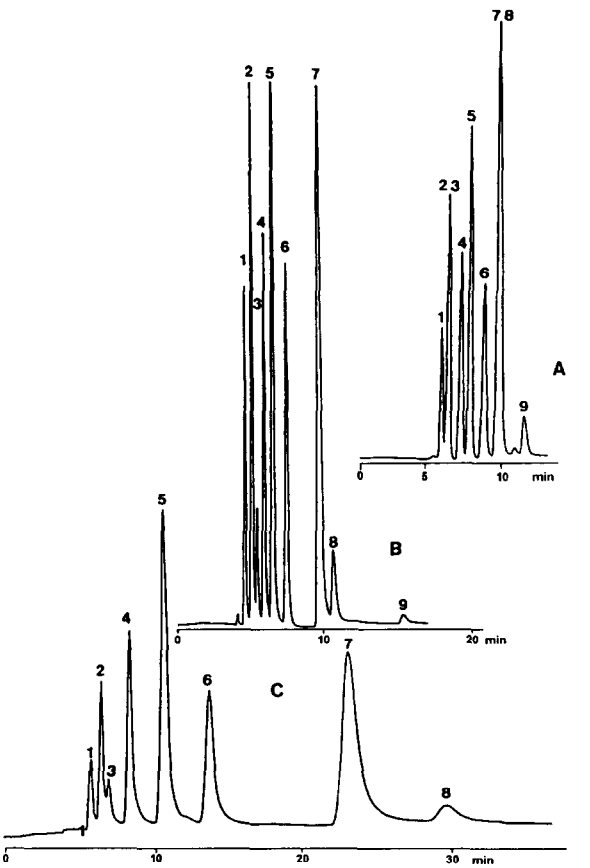

Fig. 3. Normal-phase chromatographic separation of PAH mixture on (A) PVP-coated silica LiChrospher Si 300, (B) unmodified silica LiChrospher Si 300 and (C) RP-silica Superspher-100 RP-18. Eluent, *n*-pentane; flow-rate, 0.5 ml/min; column, 250 × 4 mm I.D. Solutes: 1 = benzene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = pyrene; 6 = benz[*a*]anthracene; 7 = perylene; 8 = benzo[*a*]pyrene; 9 = coronene.

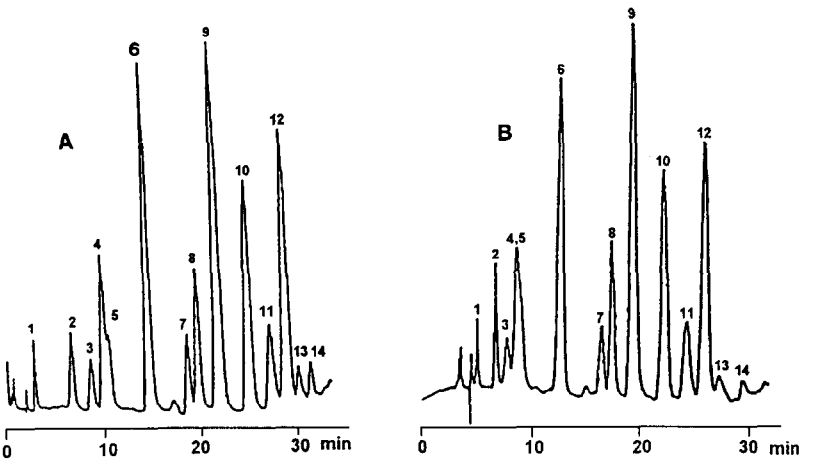

Fig. 4. RP separation of PAH mixture on (A) PVP-coated silica LiChrospher Si 300 and (B) LiChrospher Si 500. Gradient elution: 0–5 min 20% acetonitrile in water; 5–25 min from 20 to 45% acetonitrile in water; 25–30 min from 45 to 100% acetonitrile in water. Flow-rate, 1 ml/min; column, 250 × 4 mm I.D. Solutes: 1 = benzene; 2 = naphthalene; 3 = acenaphthylene; 4 = fluorene; 5 = acenaphthene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene; 9 = chrysene; 10 = benzo[*b*]fluoranthene; 11 = perylene; 12 = indenopyrene; 13 = benzoperylene; 14 = coronene.

In the RP mode of separation, there was no difference between the two PVP-coated sorbents (Fig. 4). The elution order of PAHs and their retentions are roughly the same for both packings, with the retentions being much shorter than those observed with Superspher-100 RP-18. There was a linear correlation between the k' values on the PVP-coated packings and on Superspher-100 RP-18. Hence one can propose similar retention mechanisms with these packings in RP chromatography.

From the above experimental data, one can conclude that the specific interaction between PVP and aromatic compounds in aprotic eluents is probably due to a specific association between the amide groups of PVP and the π-electron system of the benzene rings. Solvation of amide fragments by protic solvents destroys this association. The PVP-coated sorbents with polar eluents demonstrate behaviour typical of weakly hydrophobic packings.

Separation of proteins and peptides

The separation of proteins on the synthesized packings with pure water as eluent was not fully consistent with the SEC mechanism because of the noticeable adsorption of some analytes. Addition of sodium chloride to the eluent at a concentration of 0.2 *M* decreased this adsorption to a minimum. Fig. 5 shows the calibration graphs for proteins with PVP-coated packings under given conditions. For the packing with pore diameter 500 Å, the retention volumes correlate well with the logarithms of molecular mass for almost all the proteins tested (Fig. 5A). For the 300 Å packing, the largest proteins, thyroglobulin and ferritin, deviated from a straight-line correlation (Fig. 5B), eluting with smaller volumes than could be expected from the calibration graph. The basic proteins lysozyme and chymotrypsinogen A do not fit the correlation line. Nevertheless, they could be completely eluted from the columns with this simple eluent.

The SEC of proteins implies mainly a hydrophilic character of the synthesized packings. The weak ion-exchange interactions, still occurring through the thickness of the modified layer, can be effectively suppressed by adding a low concentration of salt to the eluent. A hydrodynamic mechanism of separation [11] probably contributed to the separation of large proteins also. This effect was more pronounced for the packing with 300 Å pores, as its particle size was smaller (Table 1), and for large proteins, such as calf thymus (Fig. 6), the molecular mass of which of *ca.* $1.5 \cdot 10^6$ is too high for separation according

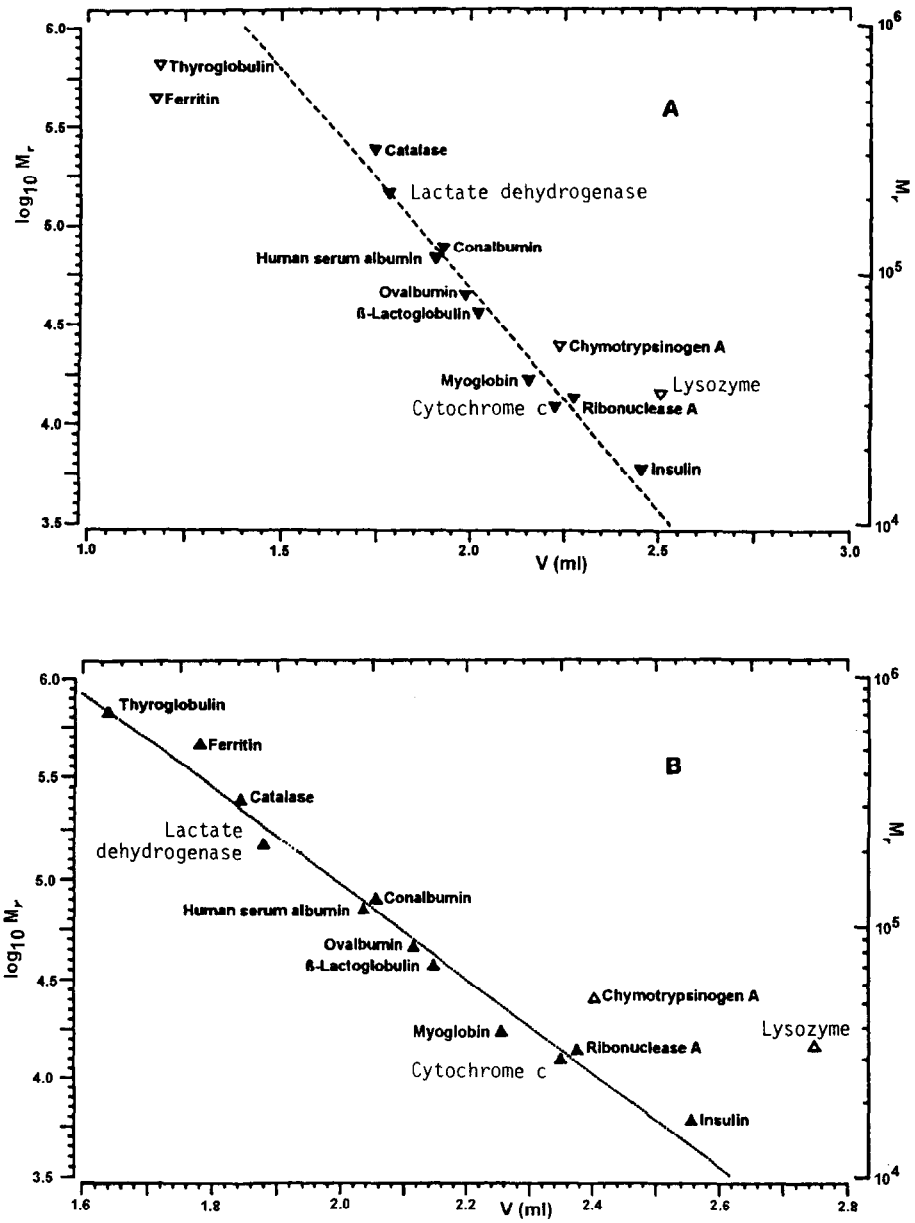

Fig. 5. Calibration graph for the retention of proteins on (A) PVP-coated silica LiChrospher Si 300 and (B) LiChrospher Si 500. Eluent, 0.2 *M* NaCl in 0.05 *M* phosphate buffer (pH 6.97); flow-rate, 0.1 ml/min; column, 250 × 4 mm I.D.

to the SEC mechanism on the synthesized packings.

With further increase in the salt concentration in the eluent, proteins will be retained again by the PVP-coated packings according to the hydrophobic interaction mechanism and they can be eluted with a decreasing salt gradient [12]. Separations of test protein mixtures by means of HIC are shown in Figs. 7 and 8. A salt concentration as high as 2.8 *M* ammonium sulphate has to be used to retain most proteins at the beginning. Nevertheless, cytochrome *c* was still weakly

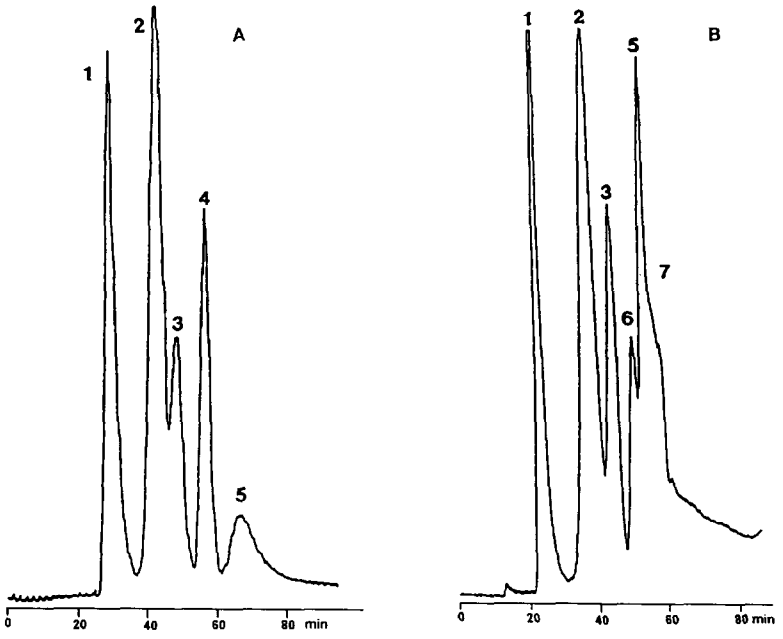

Fig. 6. SEC separation of protein mixture on (A) PVP-coated silica LiChrospher Si 300 and (B) LiChrospher Si 500. Conditions as in Fig. 5. Solutes: 1 = calf thymus; 2 = ferritin; 3 = ovalbumin; 4 = lysozyme; 5 = insulin; 6 = cytochrome *c*; 7 = sodium azide.

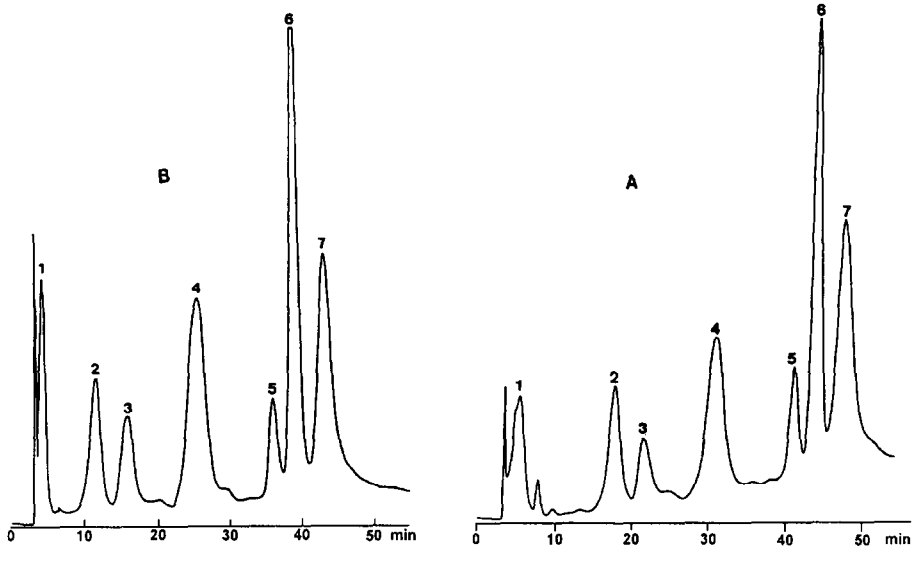

Fig. 7. HIC separation of protein mixture on (A) PVP-coated silica LiChrospher Si 500 and (B) LiChrospher Si 300. Gradient elution: 0–5 min 100% A; 5–65 min 0–100% B; A = 2.8 *M* ammonium sulphate in B and B = 0.05 *M* phosphate buffer (pH 6.97). Flow-rate, 0.5 ml/min; column, 250 × 4 mm I.D. Solutes: 1 = cytochrome *c*; 2 = myoglobin; 3 = β-lactoglobulin; 4 = ovalbumin; 5 = impurity in chymotrypsinogen; 6 = chymotrypsinogen; 7 = ferritin.

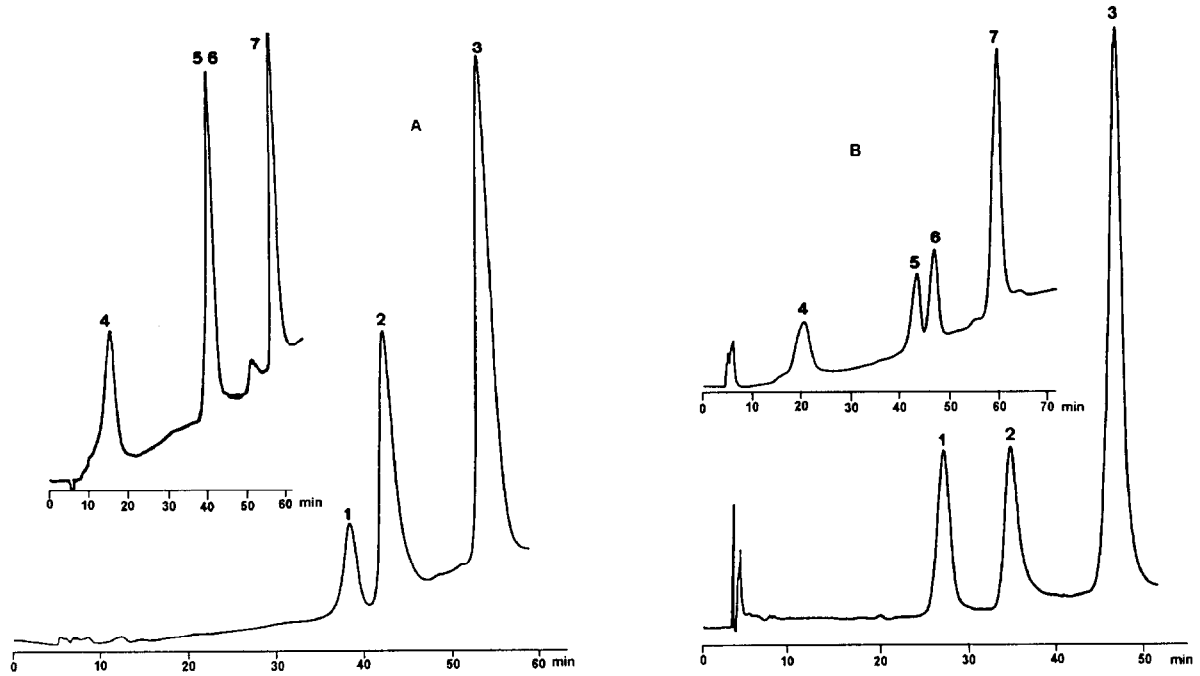

Fig. 8. HIC separation of protein or peptide mixture on (A) PVP-coated silica LiChrospher Si 300 and (B) LiChrospher Si 500. Conditions as in Fig. 7. Solutes: 1 = ribonuclease A; 2 = lactate dehydrogenase; 3 = catalase; 4 = angiotensin; 5 = neurotensin; 6 = oxytocin; 7 = somastatin.

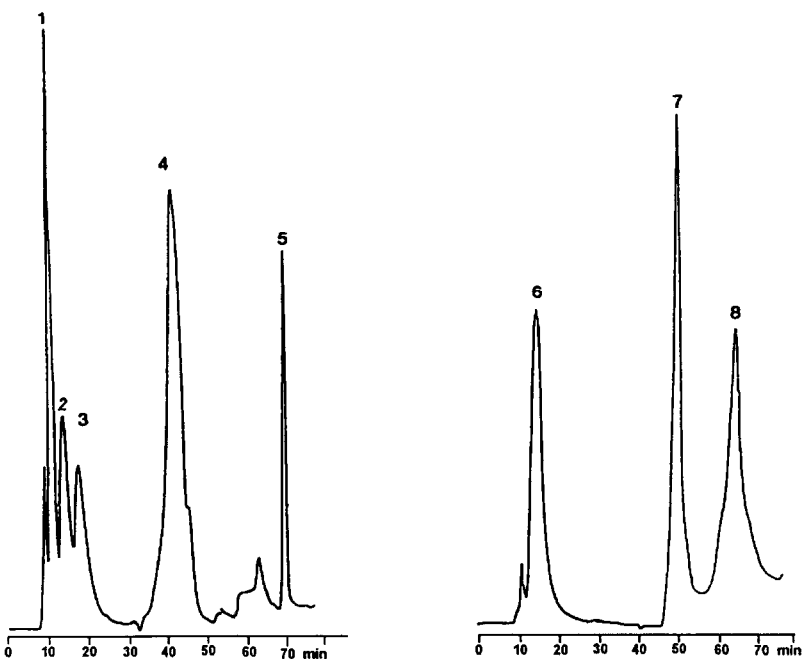

Fig. 9. RP separation of protein mixture on the PVP-coated silica LiChrospher Si 500. Gradient elution: 0–17 min 100% A; 17–47 min 0–50% B; 47–52 min 50% B; A = water–0.1% TFA, B = acetonitrile–0.1% TFA. Flow-rate 0.3 ml/min; column 250 × 4 mm I.D. Sample: 1 = ribonuclease A; 2 = β-lactoglobulin; 3 = trypsin; 4 = cytochrome *c*; 5 = myoglobin; 6 = ovalbumin; 7 = bovine serum albumin; 8 = ferritin.

retained and eluted from the column before the gradient. The elution order of proteins did not correlate with either their hydrophobicities or their molecular masses and there is a combination of different additional contributions, *e.g.*, from ion exchange and size exclusion. Probably the contribution of a size-exclusion mechanism was mainly responsible for the better separation of the pair neurotensin–oxytocin on the PVP-coated packing based on LiChrospher Si 300 (Fig. 8), as compared with the separation on the packing based on LiChrospher Si 500. However, overall there was only a minor difference between the PVP-coated packings in the HIC of proteins and comparable separations of a seven-component mixture of proteins were achieved on both packings in a similar time (Fig. 7).

An attempt to separate of proteins according to the RP mode of chromatography on the synthesized packings clearly demonstrated their hydrophilic character (Fig. 9). Ribonuclease A, β-lactoglobulin, trypsin inhibitor and even the hydrophobic ovalbumin were eluted from the column without a gradient with water containing 0.1% of trifluoroacetic acid as eluent. The elution order observed did not correlate with the hydrophobicity of the solutes, their retention being mainly controlled by other interactions. As is typical for the application of the hydrophilic packings in the RP mode of separation [13], a relatively low efficiency was observed for most of the investigated proteins. In addition, the samples were unstable under the given conditions and additional peaks emerged on the chromatograms of samples that had been standing for 1–2 h. Probably denaturation of some proteins took place during this period [13]. In any case, the synthesized hydrophilic PVP-coated packings do not meet the requirements for RP separations. The latter can be carried out with higher efficiency, *e.g.*, on hydrophobic polystyrene-coated silicas [14].

4. Conclusions

A significant result of the present investigation is that PVP-coated silica packings can be successfully used in various modes of liquid chromatography by exposing to the solute polar, H-bonding and/or apolar fragments of the bonded polymeric chains. Each chain of the copolymer is bonded by multiple covalent bonds to the silica surface. The segments between the two neighbouring links could be expected to retain sufficient flexibility, allowing the polymer to rearrange its conformation in accordance with the nature and properties of the mobile phase. This adaptation of the polymer film to the mobile phase would imply a certain change in the chemical nature of the polymer-coated column packing. In any case, the PVP chains bonded on the surface of porous silica open up new possibilities for chromatographic separations of proteins and peptides by involving a combination of possible interactions to the retention of the solutes.

5. References

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