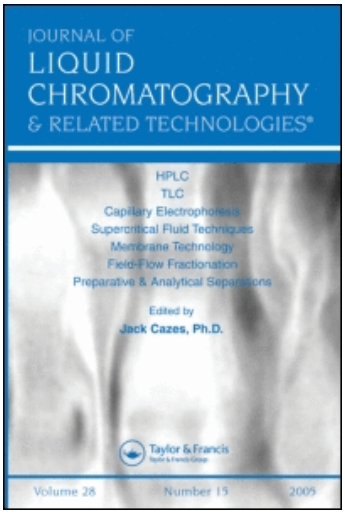

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Novel Uniformly Sized Polymeric Stationary Phase with Hydrophilized Large Pores for Direct Injection HPLC Determination of Drugs in Biological Fluids

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NOVEL UNIFORMLY SIZED POLYMERIC STATIONARY PHASE WITH HYDROPHILIZED LARGE PORES FOR DIRECT INJECTION HPLC DETERMINATION OF DRUGS IN BIOLOGICAL FLUIDS

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ABSTRACT

Novel separation media for the direct injection of complex samples have been prepared from porous 10 μm uniformly sized poly(glycidyl methacrylate-co-ethylene dimethacrylate) beads. The simple hydrolysis of all epoxide groups produces beads with separation characteristics similar to those of some restricted access media. The use of a novel size-specific modification of porous materials provides separation media with even better chromatographic properties. In this approach, hydrolysis of the epoxide groups to diols can be carried out exclusively within the large pores of the medium through the use of a polymeric catalyst. The epoxide groups remaining in the small pores after hydrophilization of the large pores were then transformed either to hydrophobic C_{18} or phenyl groups, or to more polar diethylamino groups. Due to the size specific characteristics of the modification process used in the preparation of the beads which renders the large pores hydrophilic, no protein interaction with the surface of the pores was observed, the protein recovery was high, and the retention times of proteins and of a non-retained probe was similar. Examples of separations of drugs from plasma document the good efficiency and excellent selectivity of columns packed with the novel separation medium.

INTRODUCTION

Pre-treatment of a sample by deproteinization or solvent extraction typically precedes the determination of drugs, endogenous metabolites or other small molecules in physiological liquids by high-performance liquid chromatography . This pre-treatment prevents accumulation of proteins in the column which would likely lead to clogging and fast deterioration of the separation efficiency. However, such pre-treatment is time consuming and does not always give reproducible and quantitative results. Therefore, stationary phases that allow the direct injection of complex matrices like plasma, serum, saliva, and urine into a column for the direct determination of drugs and metabolites without any pre-treatment have been developed (1,2).

All direct injection stationary phases yet described in the literature (3-11) are based on porous silica that has been modified in order to prevent any contact of the protein molecules with the hydrophobic or charged functionalities that are attached to the surface of the stationary phase. In each case, modification of the pores is uniform and affects all pores regardless of their size. Moreover, some of these silica-based packings have limited working pH range, ionic strength range, or do not allow the use of organic solvent in high concentrations in the mobile phase during the reversed phase chromatography (12). The modifications may also impair diffusion of the low molecular weight compounds that have to be separated, and the slow mass transfer results in lower column efficiencies (about 20 - 30,000 plates/m) (2) when compared to those of typical reversed phase columns.

Polymeric stationary phases have gained considerable popularity in HPLC due to their chemical stability in the entire pH range, broad variety of surface groups chemistries and polarities (13). Styrene-divinylbenzene copolymers are the most often used polymeric stationary phases (14-16). Their highly hydrophobic surface accounts for their extensive use in reversed-phase chromatography and size-exclusion chromatography in non-aqueous media. A search for more hydrophilic stationary phases is still in progress (13-16) in order to develop polymeric media for the separation of water soluble hydrophilic compounds and proteins without adversely affecting their biological activity. Though more rugged in terms of chemical resistance, the polymeric stationary phases are intrinsically less efficient than silica based phases with efficiencies seldom exceeding 30,000 plates/m for 10 μm beads. Despite this limitation, the ability to modify the chemistry within specialized polymer phases remains a very significant advantage that frequently justifies their use.

This paper concerns a new polymeric stationary phase prepared using the concept of pore-size specific functionalization. This novel phase is specifically designed for the direct injection assay of drugs in complex matrices.

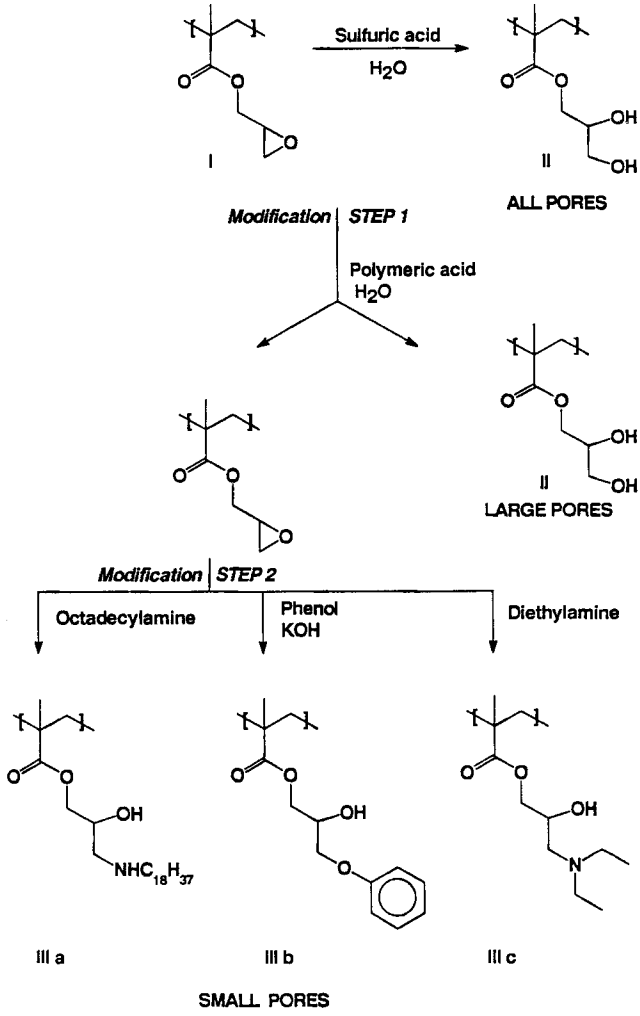
EXPERIMENTAL

Polymer Beads. Uniformly sized 10 μm porous copolymers each with a different but controlled pore size distribution were prepared by a modified shape template swelling and polymerization method described in detail elsewhere (17). Two samples were prepared from glycidyl methacrylate and ethylene dimethacrylate (monomers; 60:40 vol.%) (Resin I). Beads A were prepared in the presence of cyclohexanol as a porogenic solvent, while for beads B, butanethiol was added in order to shift the pore size distribution towards small pores. Table 1 lists the characteristics of both samples of the porous beads. Epoxide groups contents were determined by volumetric titration as follows: the beads were dispersed in 0.1 mol/l tetraethyl ammonium bromide solution in acetic acid and titrated with 0.1 mol/l perchloric acid solution in acetic acid until the crystal violet indicator indicated the blue-green end point. Pore size distributions, pore volumes and median pore diameters were calculated from retention volumes of polystyrene standards in THF (18).

Polymeric Catalyst. Poly(styrenesulfonic acid) PSSA 141 with a broad molecular weight distribution (M_W= 141,000, M_W/M_n = 1.7) was purchased from Polyscience and its molecular weight distribution was determined after an esterification to a poly(styrenesulfonic acid butyl ester).

Hydrolysis. The total hydrolysis of the epoxide groups of I into vicinal diol groups proceeds under catalysis with sulfuric acid. Resin I (3 g) was suspended in 50 ml 0.1 mol/l aqueous sulfuric acid, stirred occasionally and kept at 60°C for 10 hours to afford diol resin II (Scheme 1).

Pore Size Specific Hydrolytic Reactions. The pore size specific hydrolysis of resins I was catalyzed with 0.1 mol/l 4-toluenesulfonic acid or with a 1 wt.% aqueous solution of poly(styrenesulfonic acid) containing 0.054 mol/l sulfonic groups. The epoxide resin I (3 g) was placed in a 100-ml beaker, 25 ml of aqueous catalyst solution was added and the beaker sealed with Parafilm. The dispersion was then stirred magnetically at ambient temperature for 48 hours. The modified beads were filtered off on a fritted glass filter and washed with water until neutral. After washing with acetone the beads were dried in vacuo at room temperature.



Scheme 1

TABLE 1

Properties of Uniformly Sized Porous Poly(Glycidyl Methacrylate-co-Ethylene Dimethacrylate) Beads

Beads	A	B
Particle size (μm)	10	10
Epoxide groups (mmol/g)	2.7	2.5
Specific pore volume (ml/g)	1.1	1.0
Median pore diameter (nm)	13.8	7.0
Polystyrene exclusion limit MW	3.4×10^5	7.7×10^4

Aminolysis of Epoxide Groups. The resin containing epoxide groups (3 g) was placed in a 100-ml round bottom flask and 20 ml diethylamine was added. The mixture was heated to reflux (55 °C) for 6 hours, the resin was filtered and washed with water. The remaining unreacted epoxide groups were hydrolyzed in 50 ml 0.1 mol/l sulfuric acid at 60 °C for 4 hours. The beads **IIIc** (Scheme 1) were filtered, washed with water, then with methanol and dried.

A slightly modified procedure was used for the aminolysis of beads containing epoxide groups using octadecylamine **IIIa**. The beads (3 g) were admixed to 15 g molten octadecylamine at 70 °C and the mixture was stirred for 16 hours. The reaction mixture was then diluted with 60 ml 1,4-dioxane, stirred for 30 minutes and filtered. The resulting beads IIIa were washed with dioxane and water, and the remaining unreacted epoxide groups were hydrolyzed. The product was washed with water and methanol, and dried.

Reaction with Phenol. The resin containing epoxide groups (3 g) was dispersed in 100 ml 0.01 mol/l KOH containing phenol (3 g). The mixture was heated to reflux for 2 hours then cooled to room temperature. The resulting beads **IIIb** were washed with water and acetone and dried.

Chromatography. The stainless steel columns (150 x 4.6 mm I.D.) were slurry packed at constant pressure (10 MPa) using water as the dispersion liquid.

Testing and separations were carried out using an IBM-Nicolet Ternary Gradient Liquid Chromatograph LC 9560 equipped with a Rheodyne 7125 loop injector. The reproducibility of injection was found to be better than 2.5 %. Elution was monitored by a Hewlett Packard 1050 UV detector at 254 nm or by a Milton Roy refractive index detector. Column efficiency was determined with carbamazepine as a standard using an isocratic elution with an 80:20 mixture of 0.1 mol/l phosphate buffer solution (pH=7.0) and acetonitrile at a flow rate of 1 ml/min and ambient temperature. D_2O was used as an unretained standard for the calculation of capacity factors and column selectivities and its elution was monitored using the refractive index detector.

The plasma protein recovery was calculated from the ratio of the protein peak area measured under standard chromatographic conditions and the peak area of the same amount of protein injected into chromatographic system from which the column was removed and the inlet and outlet capillaries were connected.

The concentration of the bovine plasma (Sigma Chemical Co.) in all samples was kept constant at 70 mg/ml while the concentration of the admixed drugs was 8-40 μg/ml. Actual concentrations of individual drugs are shown in captions to Figures. The ratio between proteins and drugs was similar to that in the actual assays. The injected volume was 20 μl.

RESULTS AND DISCUSSION

Characterization of the Starting Polymer Beads. Two different batches of uniformly sized 10 μm polymer beads were prepared from glycidyl methacrylate and ethylene dimethacrylate. Batch A was prepared with cyclohexanol as a porogenic solvent, while for batch B, butanethiol was added (17) in order to shift the pore size distribution towards small pores (Table 1). The particle size, specific pore volume and polymer composition were kept constant in both polymers. The pore size distributions calculated for beads A and B from chromatographic data (Figure 1) differ considerably in the percentages of macropores (over 50 nm), mesopores (2-50 nm) and micropores (below 2 nm) and allow permeation of proteins to a different extent. For example, the radius of gyration of human serum albumin is 3.1 nm which corresponds to a "solid sphere" radius of approximately 4 nm. Due to the specific pore shape of typical porous separation media, the protein would not be able to penetrate pores with an apparent diameter less then 8 nm (4). The pore size distributions of the beads under study reveals that albumin would penetrate about 60 % of the total

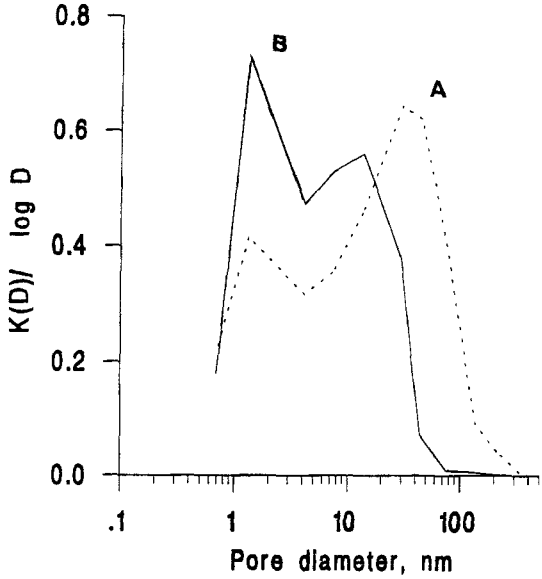


FIGURE 1. Pore size distribution of poly(glycidyl methacrylate-co-ethylene dimethacrylate) beads A and B calculated from inverse size-exclusion chromatography data.

pore volume of beads A but only about 30 % of beads B. It means that about 40 and 70 vol.% respectively of the pores will not come in contact with the protein.

Separation Media for Direct Injection Separations. In the case of complex mixtures of solutes containing molecules with very different properties such as proteins and drugs, it is very difficult to carry out a satisfactory separation. Several approaches have been suggested to avoid the absorption of proteins on silica based reversed phase columns during the separation of complex mixtures but the preparation of these separation media is tedious and their chemical stability is seriously limited (3-11). Typically, these separation media either have hydrophilic outer surfaces and very small pores that totally prevent penetration of large protein molecules into the particles, or all of their pores are covered with hydrophilic moieties. These modification methods frequently decrease the separation efficiency (2).

Evaluation of Totally Hydrolyzed Beads. First, it was determined whether or not the hydrolysis of epoxide groups is sufficient to prevent adsorption of

proteins on the surface of copolymer beads made from glycidyl methacrylate. Such a packing containing only hydrophilic surface chemistry in both large and small pores would not be expected to be suitable for direct injection separation of both proteins and hydrophobic drugs but its properties would be useful for comparison purposes.

To verify this, beads A and B were first fully hydrolyzed using sulfuric acid as a catalyst (Reaction scheme 1) and used for the separation of a mixture of bovine plasma and three drugs in a reversed-phase chromatographic mode with aqueous acetonitrile as the mobile phase. Though no interactions of both plasma proteins and drugs with the surface were expected in fully hydrolyzed beads, the separation of drugs was in fact achieved. However, the separation selectivity in the column packed with hydrolyzed beads A is relatively low, lidocaine and carbazepamine are eluted within the tail of the plasma proteins (peak 1 and 2, Figure 2a).

Since the pore size distribution in hydrolyzed beads B is shifted towards smaller pores, the major portion of these pores cannot be penetrated by the protein molecules. Therefore, the plasma proteins are eluted from beads B within a smaller elution volume than from beads A. This faster plasma elution results in a narrow protein peak that does not tail appreciably. This leads to an almost baseline separation of lidocaine (peak 1) from plasma; the separation selectivity for carbazepamine and phenytoin (peaks 2 and 3, Figure 2b) is also better as compared to that obtained for beads A. This unexpected observation led us to a more extensive examination of the chromatographic properties of the fully hydrolyzed beads B.

Figure 3 shows the dependency of log k' on the volume fraction of acetonitrile in the mobile phase for various compounds including both drugs with relatively low hydrophobicity having a capacity factor extrapolated to pure water as a mobile phase (k_w') lower than 10, and more hydrophobic drugs with k_w' in the range from about 100 to 1000. The linear relationship confirms that the separation in the column packed with hydrolyzed beads B is controlled by interactions characteristic for reverse-phase chromatography.

This unexpected behavior can be explained by the functional heterogeneity of the modified surface. In addition to their hydroxyl groups, the beads contain the hydrophobic hydrocarbon chains of the copolymer and the hydrophobic diester units of the crosslinking agent. The hydroxyl groups generated by hydrolysis of epoxide units cannot cover all of the surface of the pores with a homogeneous hydrophilic layer as their number is insufficient. The

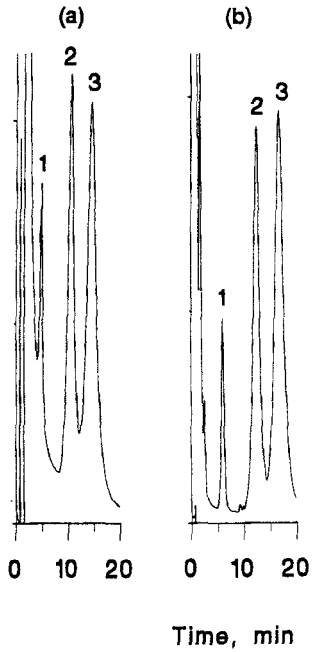


FIGURE 2. Direct injection separation of model mixtures containing drugs and bovine serum plasma.
Column 150 x 4.6 mm I.D. packed with H_2SO_4 hydrolyzed beads A (chromatogram a) and beads B (chromatogram b), mobile phase, 0.1 mol/l phosphate buffer (pH=7.0) - acetonitrile (80:20), flow rate 1.0 ml/min, UV detection 254 nm, injected volume 20 µl; peaks, 1- lidocaine (20 µg/ml) , 2- carbamazepine (8 µg/ml), 3- phenytoin (40 µg/ml).

surface is not uniformly hydrophilic but also contains hydrophobic domains interspersed within hydrophilic areas. However, the size of hydrophobic domains is smaller than that of the large protein molecules; therefore, the proteins are not adsorbed and their recoveries are high (Table 2).

Figure 1 shows that beads A contain mainly large pores and therefore the actual surface area available for interaction between surface and drug molecules is smaller than in the case of beads B which contain more of the meso- and micropores that are best suited for the separation of small molecules. This conclusion is supported by the higher capacity factors k' exhibited for all drugs by the column packed with beads B (Table 3). The capacity factors also suggest that resolution is better for the column packed with hydrolyzed beads B than

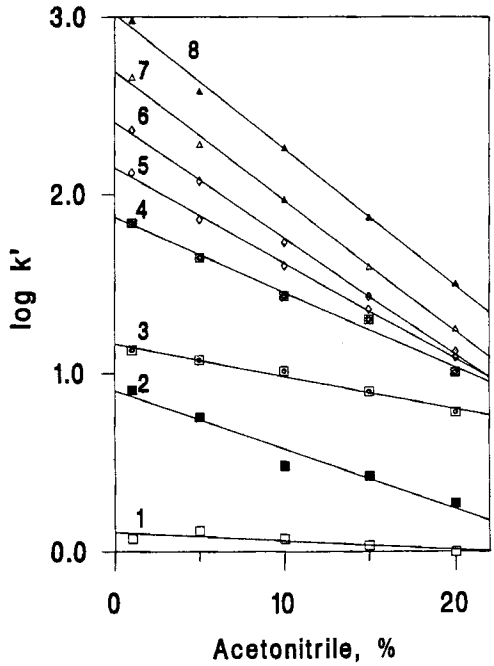


FIGURE 3. Capacity factor k' as a function of acetonitrile concentration in the mobile phase.
Column 150 x 4.6 mm I.D., packing, beads B hydrolyzed with aqueous H_2SO_4 mobile phase 0.1 mol/l phosphate buffer (pH 7) - acetonitrile (80:20), flow rate 1.0 ml/min, UV detection 254 nm; 1- acetylsalicylic acid, 2- sulfamethoxazole, 3- lidocaine, 4- chlorpheniramine, 5- quinidine, 6- carbamazepine, 7- phenytoin, 8- desipramine.

hydrolyzed beads A. This is further confirmed by a measurement of the effect of the linear flow velocity on the plate height (Figure 4). The efficiency of the column packed with beads B is twice as high as that of a column packed with beads A and the slope of the dependency is also smaller for beads B. Overall, hydrolyzed beads A are ill-suited for reversed-phase direct injection chromatography application while hydrolyzed beads B show a good performance. This is further supported by their excellent stability which does not change even after more than 500 repetitive manual injections over a period of three months.

The surprisingly good performance obtained with hydrolyzed beads B in the direct injection separation of plasma samples containing drugs are somewhat

TABLE 2

Protein Recovery from Functionalized Packings Modified in Pore-Size Specific Fashion

Hydrolysis catalyst	Surface derivatization	Protein recovery, %
	Beads A	
none	none [a]	78
Sulfuric acid	none	84
none	Octadecylamine	16
PSSA-141	Octadecylamine	81
	Beads B	
Sulfuric acid	none	100
4-Toluenesulfonic acid	Phenol	96
PSSA-141	Phenol	92
PSSA-141	Octadecylamine	96

[a] Original poly(glycidyl methacrylate-co-ethylene dimethacrylate) beads

TABLE 3
Capacity Factors k' of Drugs for a Column Packed with Beads Hydrolyzed with Aqueous Sulfuric Acid

#	Drug	k'	
		Beads A	Beads B
1	Lidocaine	1.14	2.13
2	Carbamazepine	3.40	5.78
3	Phenytoin	6.13	8.16
4	Theophyline	1.05	1.33
5	Theobromine	1.50	2.36
6	Caffeine	1.81	2.95

Column 150 x 4.6 mm I.D., mobile phase 0.1 mol/l phosphate buffer pH 7 - acetonitrile 80:20 (for #1-3); 99:1 (for #4-6), injection volume 20 µl, detection 254 nm.

similar to those obtained for shielded hydrophobic phases (5-8,11). Since the preparation of our separation medium is much easier, it may compete advantageously with many more sophisticated restricted access media.

Evaluation of Hydrophobic Beads. In contrast to the hydrolyzed beads, modification of the epoxide groups with octadecylamine should result in a material with enhanced hydrophobic interactions. Though the modification increases the overall hydrophobicity, strong adsorption of proteins in the column should also occur. Indeed, the protein recovery decreases drastically and does not exceed 16 %; the major part of the protein remains adsorbed (Table 2). The packing exhibits chromatographic properties similar to those of standard reversed phases with C18 chemistry. Such packings cannot be used for the desired direct injection separation as the proteins accumulate in pores and on the surface of the hydrophobic medium and the column becomes clogged rapidly . The column life-time typically does not exceed 200 injections (19,20).

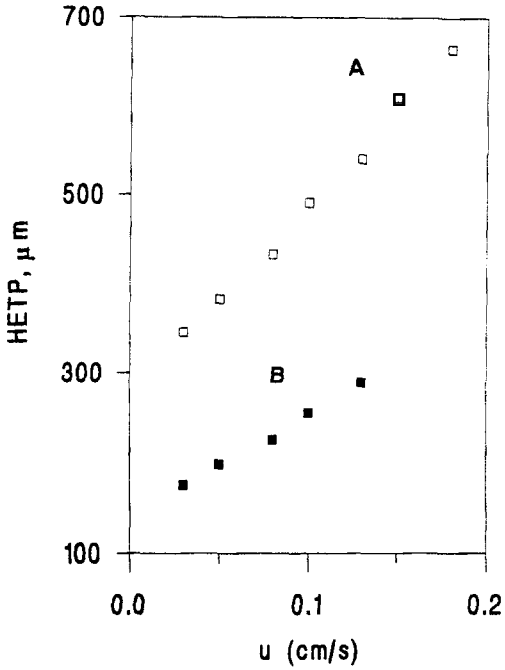


FIGURE 4. Effect of linear flow velocity on plate height.
Column 150 x 4.6 mm I.D. packed beads A or B hydrolyzed with aqueous H_2SO_4, solute carbazepamine, mobile phase 0.1 mol/l phosphate buffer (pH=7.0) - acetonitrile (80:20), UV detection 254 nm, injected volume 20 μl.

Concept of the Pore-Size Specific Modification. An ideal medium for direct injection chromatography should have both hydrophilic pores large enough to accommodate the proteins from a complex sample without risk of adsorption, and small hydrophobic or charged pores to provide for good chromatographic separation of the small molecules present in the sample while excluding proteins. In order to obtain this ideal medium, we have developed a novel concept different from the uniform pore modification processes outlined above. This concept is shown schematically in Figure 5 where the structure of a porous material is depicted as a combination of interconnected large and small pores covered with epoxy groups I, that are susceptible to acid catalyzed hydrolysis to afford diol polymer II (Scheme 1). In instances where the acid catalyst is itself a large molecule such as a polymer, its ability to catalyze reaction of the functional

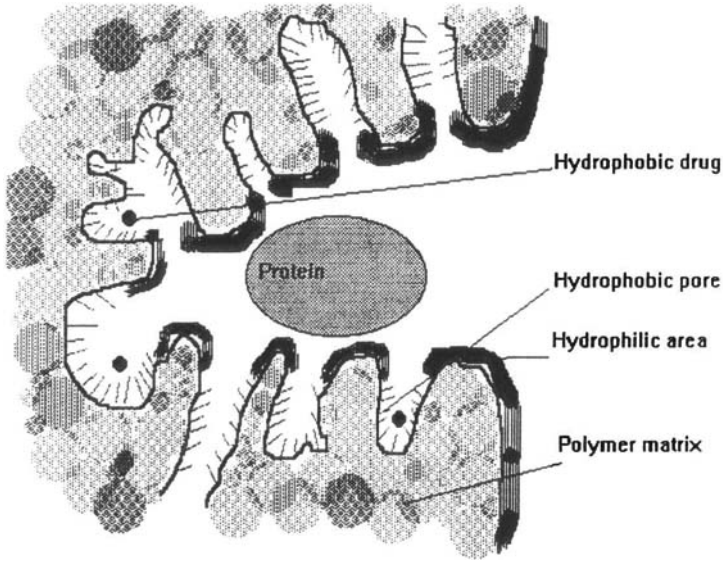


FIGURE 5. Schematic view of a separation medium modified in pore-size selective fashion using a polymeric catalyst

groups contained in the various pores will be controlled by the relative size of the catalyst molecules and the pores.

In the first step, hydrolysis of the reactive epoxide groups is carried out within the large pores only using an aqueous solution of a poly(styrenesulfonic acid) that is too large to penetrate the small pores. In the following step, reaction with a low molecular weight reagent (phenol, amine) transforms the epoxide groups that remained unchanged in the small pores to the desired functionality. In this way, beads with a bimodal distribution of surface chemistries are obtained. The process is simple and can be carried out either using a batch technique or within the column itself.

The actual separation in the bifunctional packing would proceed according to the following mechanism: all components of the injected plasma sample containing blood proteins, drugs, metabolites, etc. can penetrate all pores large enough to accommodate them. Specifically, large protein molecules enter only large pores covered with hydrophilic chemistry. Since they are not retained, they elute from the column at a volume close to V_i. Small molecules enter both large

and small pores. They also interact only weakly with the hydrophilized surface of large pores but they are retained in the small pores where the chemistry is different. Therefore, the small molecules will be eluted later and separated according to their interaction energy with the surface groups of the small pores.

Evaluation of Bimodal Beads for Direct Injection Reversed-Phase Chromatography. We have shown that the uniformly hydrolyzed diol separation media may not be sufficiently hydrophobic for all direct injection separations while the uniformly hydrophobized beads are not suitable for the separation envisioned. Therefore we used the concept outlined above to design beads containing both hydrophilic large pores and hydrophobic small pores with chromatographic properties we believe are close ideal for direct injection chromatography. The reactive epoxide groups located in the large pores were hydrolyzed in a process catalysed with a poly(styrenesulfonic acid) having a molecular weight of 141,000. The unchanged epoxide groups of the small pores were then allowed to react with octadecylamine in one case, or phenol in another, to provide hydrophobic sites.

The data presented in Table 2 confirm that the pore-size selective hydrolysis of large pores prevents the adsorption of proteins. Protein recoveries obtained from beads **IIIa** and **IIIb** are close to those of beads in which uniform hydrolysis of all epoxide groups had been carried out.

The pore-size specific hydrophilization/hydrophobization process further improves the chromatographic properties of the beads (Figures 6 and 7). The more hydrophobic surface of the modified small pores increases the retention and improves the drug separation selectivity compared to similar separations with fully hydrolyzed beads (Figure 3). Once again, the positive effect of the absence of large pores in beads B is confirmed as shown in Figure 6. Thus, the selectivity of drug separation and the peak symmetry of the octadecylamine-modified beads B are better than those for beads A. Therefore, only beads B were used in the rest of this study.

The chromatograms of Figure 7 document the effect of the catalyst size (4-toluenesulfonic acid and PSSA 141) on the chromatographic properties of the packing as a consequence of the different extents of hydrophilization. With 4-toluenesulfonic acid 94 % of all epoxide groups in beads B are hydrolyzed as opposed to only 17 % with poly(styrenesulfonic acid). Therefore, after the second step of pore-size selective modification the number of phenyl groups attached to the surface of the small pores is higher for the beads treated with aqueous poly(styrenesulfonic acid). The beads show higher hydrophobicity, the

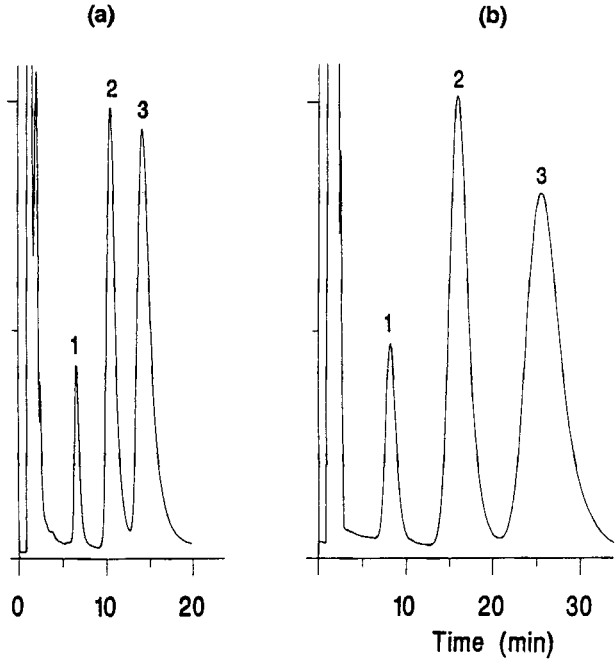


FIGURE 6. Direct injection separation of bovine serum plasma and drugs in a column packed with beads A (*a*) or B (*b*) modified by hydrolysis catalyzed by PSSA 141 then by reaction with octadecylamine.
Column 150 x 4.6 mm I.D., mobile phase, 0.1 mol/l phosphate buffer (pH=7.0) - acetonitrile (80:20), flow rate 1.0 ml/min, UV detection 254 nm, injected volume 20 µl; peaks, 1 - lidocaine (20 µg/ml), 2 - carbamazepine (8 µg/ml), 3 - phenytoin (40 µg/ml).

retention times are longer, and the separation is excellent. Column stability is also very good and equals that of the column packed with hydrolyzed diol beads.

Evaluation of Bimodal Beads for Direct Injection Ion-Exchange Chromatography. Charged compounds can be separated better in an ion-exchange chromatographic mode than in the reversed phase mode. The pore-size specific modification approach may also be used for the preparation of media for ion-exchange chromatography. After the initial hydrolysis catalyzed with polymeric acid, a reaction with diethylamine was employed in the second modification step affecting the small pores. This procedure results in a stationary phase containing diethylamino groups in the small pores (**IIIc**). Figure 8 shows

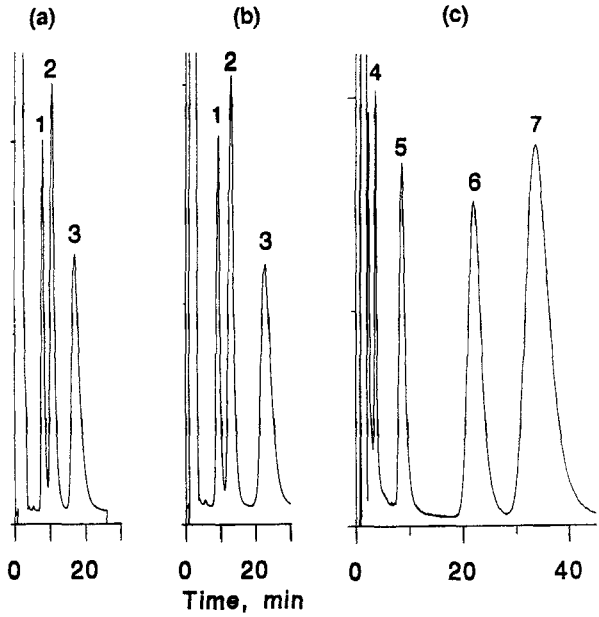


FIGURE 7. Direct injection separation of bovine serum plasma and drugs in a column packed with beads B modified by hydrolysis catalyzed by 4-toluenesulfonic acid (*a*) and PSSA 141 (*b,c*), respectively, then modified by reaction with phenol.
Column 150x4.6 mm I.D., mobile phase 0.1 mol/l phosphate buffer (pH 7) - acetonitrile 99:1 (A,B) or 80:20 (C), injected volume 20 µl, detection 254 nm; peaks, 1- theophyline (10 µg/ml), 2- theobromine (10 µg/ml), 3- caffeine (20 µg/ml), 4- barbital (10 µg/ml), 5- phenobarbital (10 µg/ml), 6- carbamazepine (8 µg/ml), 7- phenytoin (40 µg/ml).

the separation of salicylic and acetylsalicylic acids from blood plasma. The retention times strongly depend on the pH of the mobile phase (Figure 9) and confirm the ion-exchange separation mechanism. The capacity factor for salicylic acid at pH 7 is about 20 times higher than that for acetylsalicylic acid (Table 4). In contrast, a fully hydrolyzed diol column (II) or a column containing diol large pores and phenol modified small pores (IIIb) do not separate the acids and plasma at all, because there is almost no retention of the two drugs (Table 4). Due to the hydrophilization of the large pores, the protein recovery is again close to 100 % as the proteins cannot come into contact with the charged surface of the small pores.

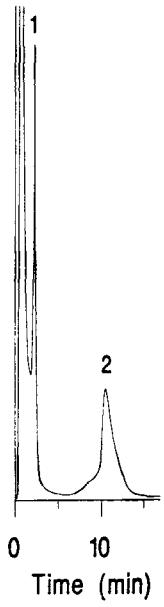


FIGURE 8. Direct injection separation of bovine serum plasma and acidic compounds in a column packed with beads B modified by hydrolysis catalyzed by PSSA 141 then modified by reaction with diethylamine.
Column 100x4.6 mm I.D., mobile phase 0.1 mol/l phosphate buffer (pH 9.3) - acetonitrile (80:20), injected volume 20 µl, detection 280 nm; peaks, 1 - acetylsalicylic acid, 2 - salicylic acid.

TABLE 4

Capacity Factors k' of Acidic Compounds for Three Columns Packed with Beads B Containing Different Functionalities in the Small Pores

	k'		
Compound	II	III b	III c
Acetylsalicylic acid	0.00	0.03	2.63
Salicylic acid	0.30	0.35	50.80

Column 150 x 4.6 mm I.D., mobile phase 0.1 mol/l phosphate buffer pH 7 - acetonitrile, 80:20, injection volume 20 µl, concentration of compounds 2 µg/ml, detection 280 nm.

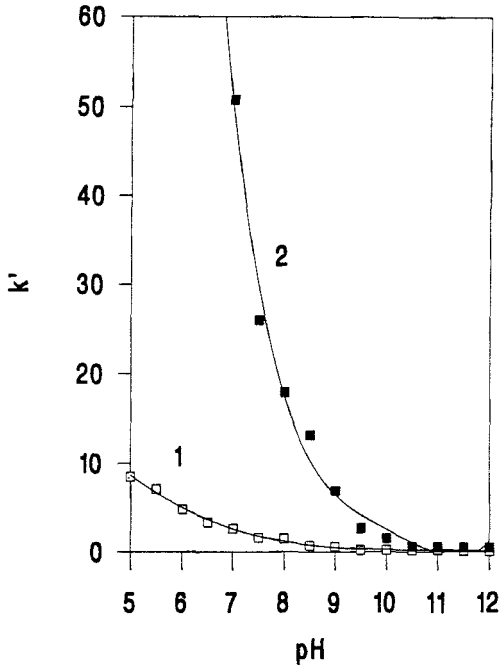


FIGURE 9. Effect of the mobile phase pH on capacity factor k' of acetylsalicylic acid (1) and salicylic acid (2).
Column 150x4.6 mm I.D., packing, beads B modified by hydrolysis catalyzed by PSSA 141 then allowed to react with diethylamine, mobile phase 0.1 mol/l phosphate buffer (pH 5-12) - acetonitrile (80:20), injected volume 20 µl detection 280 nm.

CONCLUSION

A novel polymeric stationary phases for direct injection HPLC has been prepared. The simple hydrolysis of beads having selected pore size distributions provides a medium with separation properties similar to those of commercial restricted access media. Beads with even better selectivities have been prepared by a pore-size specific modification process using catalysts that are able to recognize the sizes of the pores and promote reaction only within those pores, that are large enough to accommodate them.

Though only a few examples of preparation of separation media containing pores with different functionalities have been presented, the pore-size

specific modification method is universal and can be extended to many other combinations of functional groups.

Despite of the use of a polydisperse polymeric acid as a catalyst, the pore-size selective approach proved to be feasible. The pore surface chemistry would be controlled even better if polymeric catalysts with very narrow molecular weight distributions were used. The application of such catalysts is currently under study.

The simplicity of preparation, excellent separation of components of model mixtures similar to plasma samples, high protein recovery and very good column stability makes the novel separation media advantageous over the currently available stationary phases for direct injection.

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