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Uniform-size hydrophobic polymer-based separation media selectively modified with a hydrophilic external polymeric layer

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Abstract

A simple procedure for the preparation of macroporous hydrophobic styrene–divinylbenzene polymeric separation media with a hydrophilic outer surface has been developed. A hydrophilic monomer and water-soluble polymerization initiator are added to the reaction mixture during the final polymerization step of the preparation of size-monodisperse particles. Because the hydrophobic styrene–divinylbenzene framework of the beads is already formed, and the hydrophilic monomer does not penetrate the pores of the beads that are filled with a hydrophobic porogen, the hydrophilic layer is formed only at the surface of the beads. The hydrophilic monomers used included glycerol monomethacrylate and glycerol dimethacrylate and toluene was used as the porogen for the poly(styrene–co-divinylbenzene) beads. Comparative experiments involving beads with or without a hydrophilic medium showed that the separation selectivity of the media towards hydrophobic solutes remains unchanged. However, the modified medium with a hydrophilic layer could be used to analyse mixtures that also contained large peptide molecules as these do not adsorb at its surface as is the case with the unmodified hydrophobic beads.

1. Introduction

The control of surface chemistry is an important challenge in the design of porous materials. In the field of liquid chromatography, where polymers and C_{18} silica are used most frequently for size-exclusion and reversed-phase chromatography, respectively, difficulties are frequently encountered with analytical samples that contain proteins or other biopolymers. This is because these biopolymers are frequently absorbed irreversibly at the surface of the medium, causing the rapid clogging of its pores and a general

deterioration of the separation properties of the column. The C_{18} hydrophobic materials are not wettable with water, which precludes their use in open-column liquid chromatography. Therefore, a modification method has been developed that renders the outer surface of hydrophobic silica beads water-wettable [1]. The C_{18} chains are removed from the external surface by acid hydrolysis and glycol groups are attached to the plain silica. This material can be used for both open-column liquid chromatography using an aqueous mobile phase and for the direct-injection chromatography of complex samples [2]. A drawback of this approach is that the surface modification method is not completely site-selec-

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tive and the hydrophobicity of the internal surface is also impaired.

Polymer-based separation media such as poly(styrene-co-divinylbenzene) are stable over the entire pH range and exhibit an enhanced selectivity towards some classes of compounds, such as halogenated hydrocarbons and aromatic compounds, which is advantageous for monitoring these pollutants in the environment [3]. Unfortunately, the poly(styrene-co-divinylbenzene) media shrink in mobile phases that are rich in water and the column efficiency is therefore deteriorated. The shrinkage can be reduced through the introduction of a more hydrophilic monomer into the beads but the hydrophilic groups also decrease the required hydrophobicity. Moreover, the addition of the new monomer changes the composition of the polymerization system and affects the porous properties of the resulting beads [4].

This paper presents a new concept that allows the preparation of hydrophobic macroporous media that have a very hydrophilic external surface. The usefulness of the process is demonstrated in the preparation of novel polymeric stationary phases specifically designed for direct injection assays.

2. Experimental

2.1. Materials

Styrene, divinylbenzene (55% of divinyl monomer), methyl methacrylate and ethylene dimethacrylate were purchased from Nacalai Tesque (Kyoto, Japan). Glycerol monomethacrylate (I) and glycerol dimethacrylate (II) (Fig. 1) were gifts from Fusso Chemical (Osaka,

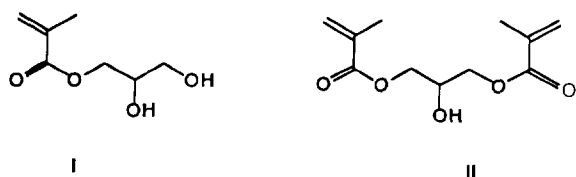


Fig. 1. Structures of glycerol monomethacrylate (I) and glycerol dimethacrylate (II).

Japan). All other reagents were purchased from Nacalai Tesque and used as received.

2.2. Preparation of monodisperse beads

Uniform-size polystyrene-shape templates were prepared and purified by the previously reported method [5]. The diameter of the particles was ca. 1 μm .

The preparation of uniformly sized hydrophobic beads with a hydrophilic surface was carried out using a multi-step swelling and polymerization method. A 1.4-ml volume of a water dispersion of the uniformly sized polystyrene seed particles ($9.5 \cdot 10^{-2}$ g/ml) was admixed with a microemulsion prepared by sonication of 0.95 ml of dibutyl phthalate used as the activating solvent for the swelling procedure, 0.085 g of benzoyl peroxide, 0.04 g of sodium dodecyl sulfate and 10 ml of distilled water. This first swelling step was carried out at room temperature while stirring at 125 rpm. Completion of the first swelling step was determined by optical microscopy. A dispersion of 5 ml of the hydrophobic monomer (e.g., styrene or methyl methacrylate), 5 ml of cross-linking agent (e.g., divinylbenzene or ethylene dimethacrylate) and 10 ml of toluene used as porogenic solvent was prepared in 90 ml of water containing 1.92 g of poly(vinyl alcohol) ($d_p = 500$, saponification value = 86.5–89 mol-%) used as the dispersion stabilizer. This dispersion was added to the dispersion of swollen seed particles and the second swelling step was carried out at room temperature for 2–24 h while stirring at 125 rpm. After this second swelling was complete, polymerization was started at 80°C under an argon atmosphere with slow stirring. After 4 h of polymerization, the hydrophilic monomers, with or without potassium peroxodisulfate, were added to the polymerizing beads. After a further 20 h of stirring at the same temperature, the dispersion of polymerized particles was poured into 250 ml of a hot water-methanol mixture and the supernatant was discarded after sedimentation of the particles. The polymer particles were redispersed into methanol and the supernatant was again discarded after sedimentation. This

procedure was repeated three times in methanol and twice in tetrahydrofuran (THF). The resulting 5–6- μm polymer particles were collected using a membrane filter, washed with THF and then with acetone and finally dried at room temperature.

The same polymerization procedure, omitting the addition of the hydrophilic monomers, was used for the preparation of porous poly(styrene-co-divinylbenzene) beads that served as a blank for comparison with the modified beads in the chromatographic experiments. The yield calculated from the amount of monomers used was ca. 85–100%.

2.3. Determination of monomer and polymer distribution between phases

Glycerol monomethacrylate, glycerol dimethacrylate or poly(glycerol monomethacrylate) was added to water-toluene (10:1, v/v) and stirred for 12 h at room temperature. The mixture was allowed to separate into aqueous and organic phases, an aliquot from each phase was taken, water or toluene was evaporated and the amount of the remaining component was determined.

2.4. Chromatography

The porous beads were slurry packed into stainless-steel columns (150 mm \times 4.6 mm I.D.) using aqueous acetonitrile or aqueous methanol as the dispersion liquid.

High-performance liquid chromatography was performed with a Jasco Model 880-PU intelligent pump or a Shimadzu, LC-4A ternary gradient pump equipped with a Rheodyne Model 7125 valve loop injector. Peak monitoring was carried out with a Jasco, Uvidec-100-III or a Shimadzu SPD-2A UV detector set at 254 or 280 nm and a Waters RI 401 differential refractometer. Peaks were recorded with a Shimadzu C-R4A Chromatopak. Recoveries of proteins were determined from their peak areas, taking the peak areas in the absence of a column as 100%.

3. Results and discussion

3.1. The concept of site-selective modification

Monodisperse macroporous particles are prepared by the swelling of “activated” shape templates with emulsified mixtures of monomers and porogenic diluents followed by polymerization. The properties of these beads are largely controlled by the properties of the monomers that are used. The traditional technique used for the preparation of monodispersed beads does not allow the modification of specific areas of the beads during their polymerization, which proceeds unabated until all the monomers are consumed. Therefore, the resulting beads are chemically homogeneous. However, we have discovered [6,7] that if another monomer is added to the polymerizing mixture once the structural framework of the beads has already been established, this monomer will not be incorporated into the bulk of the beads but will only form a layer of polymer in those areas where of the beads where it can diffuse. Typically, this would only be the external surface and the surface of the pores within the porous matrix. In addition, both size-exclusion and solubility effects can be used to localize further the site of functionalization. For example, if the aqueous phase contains a water-soluble free-radical initiator, any water-soluble monomer added to the warm aqueous suspension would start polymerizing immediately. Because the resulting polymer molecules cannot penetrate into the pores that are filled with a hydrophobic porogen, they would simply attach themselves to the outer surface of the beads. This very simple procedure allows the preparation of a macroporous medium in which each individual bead exhibits a sharp gradient of very different surface and interior chemistries.

3.2. Hydrophilization of porous poly(styrene-co-divinylbenzene) beads

A study of the preparation of porous polymer beads from styrene and divinylbenzene under conditions similar to those used in this work has

shown that nearly quantitative conversion of the monomers is achieved after 4–5 h of polymerization at 80°C [4]. Therefore, when the hydrophilic monomers are added 4 h after the start of the polymerization, the framework of the hydrophobic poly(styrene-co-divinylbenzene) beads is already well established, and phase separation between the cross-linked polymer and the porogenic solvent filling its pores has been achieved [3]. Because the hydrophilic monomer is added directly to the polymerization medium, it is expected to partition between the water phase and the porogenic solvent according to its partition coefficient.

To determine the distribution of glycerol monomethacrylate, glycerol dimethacrylate and poly(glycerol monomethacrylate) between toluene and water, separate experiments were carried out using a mixture of water and toluene in the ratio typically used for a standard polymerization mixture. The results obtained are summarized in Table 1. These experiments demonstrate that glycerol monomethacrylate, and also its polymer, partition preferentially into the aqueous phase, in contrast to glycerol dimethacrylate, which is found exclusively in the toluene phase. In practical terms, this means that both glycerol monomethacrylate and its polymer would remain in the aqueous polymerization medium but would not penetrate the pores of the poly(styrene-co-divinylbenzene) beads that are filled with toluene porogen. Therefore, if each monomer is added separately, glycerol dimethacrylate can be used to modify the internal surface of the pores, while glycerol mono-

methacrylate would only polymerize outside the beads to modify their external surface.

If the added monomer is able to partition into the toluene phase, its polymerization should lead to a decrease in the pore volume for the modified particles when compared with the pore volume of the unmodified particles. Table 2 shows the pore volumes of the particles prepared under a variety of modification conditions. As expected from its partitioning between the aqueous and toluene phases, the addition of 2 ml of glycerol dimethacrylate caused a 23% decrease in pore volume when compared with the unmodified base particles. This correlates well with the fact that the volume of added glycerol dimethacrylate represents 20% (w/w) of the toluene that was initially added to the polymerization mixture and therefore then eventually became localized in the pores.

On the other hand, the addition of glycerol monomethacrylate does not affect the pore volume despite the fact that it is incorporated completely on to the beads, as verified by elemental analysis. As seen from a comparison of scanning electron micrographs of the beads shown in Fig. 2a and b, the added glycerol monomethacrylate does not affect the size uniformity of the base particles, but it does change their external appearance (Fig. 2b), suggesting again that the attachment of poly(glycerol monomethacrylate) occurs mainly on the external surface of the poly(styrene-co-divinylbenzene) particles. These findings correlate well with the results of the partition experiments reported in Table 1.

Table 1
Partitioning of additives between toluene and water

Additive	Concentration (%. w/w) in	
	Toluene	Water
Glycerol monomethacrylate	2	98
Glycerol dimethacrylate	100	0
Poly(glycerol monomethacrylate)	8	92

The ratio of toluene to water (1:10) was selected to match that of the actual polymerization mixture.

Table 2
Pore volumes of particles modified with hydrophilic monomers

Added monomer	Volume ^a (ml)	Initiator ^b (%, w/w)	Pore volume ^c (ml/g)
None ^d	–	None	0.94
GMMA ^e	1	None	0.90
GDMA ^f	2	None	0.72
GMMA + GDMA	1 + 1	None	0.78
GMMA + GDMA	1 + 1	1	0.92

^a Volume of monomer added to the mixture during polymerization.

^b Potassium peroxodisulfate. The percentage is given relative to the added monomers.

^c Determined by size-exclusions chromatography.

^d Poly(styrene-co-divinylbenzene) beads prepared without addition of hydrophilic monomers.

^e Glycerol monomethacrylate.

^f Glycerol dimethacrylate.

Also, the addition of both glycerol monomethacrylate and glycerol dimethacrylate in the absence of a water-soluble initiator results in a 16 vol.-% decrease in the pore volume. In contrast, when both glycerol monomethacrylate and glycerol dimethacrylate are added together with a water-soluble initiator such as potassium peroxodisulfate, the resulting beads undergo no change in pore volume. They again maintain their size uniformity but their appearance (Fig. 2c) is similar to those of the glycerol monomethacrylate-modified beads (Fig. 2b). This finding suggests that the radical polymerization initiated by the water-soluble initiator proceeds mainly in the aqueous phase and the cross-linked copolymer is attached only at the surface of the poly(styrene-co-divinylbenzene) beads.

3.3. Chromatographic properties of the prepared particles

While the above findings suggest that glycerol monomethacrylate can modify only the external surface of the poly(styrene-co-divinylbenzene) particles owing to its insolubility in toluene, a column packed with these modified particles is not useful. The back-pressure increases dramatically when a mobile phase rich in water is used. This is most likely due to swelling of the water-soluble poly(glycerol monomethacrylate) at-

tached to the bead surface. The column also provides very poor efficiency.

In contrast, the modification with glycerol dimethacrylate affords particles that provide both high efficiencies and reasonable back-pressures even when water is used as the mobile phase. A previous study [8] indicated that poly-(glycerol dimethacrylate) particles are hydrophilic enough to prevent the occurrence of protein absorption. If glycerol dimethacrylate is used for the modification during the preparation of porous poly(styrene-co-divinylbenzene) beads, it dissolves in the toluene that has become localized in the pores, it then polymerizes and thus decreases the pore volume while making the pore surface hydrophilic. This is not advantageous for the preparation of separation media for direct-injection HPLC because these require that the internal surface be highly hydrophobic [9–12]. However, this approach can be used for the preparation of porous beads with completely hydrophilized internal surfaces that can find an application in other modes of HPLC such as aqueous size-exclusion chromatography.

Even if glycerol monomethacrylate is added together with glycerol dimethacrylate but in the absence of a water-soluble free radical initiator, the pore volume of the poly(styrene-co-divinylbenzene) particles is decreased, which implies that modification of the internal surface of

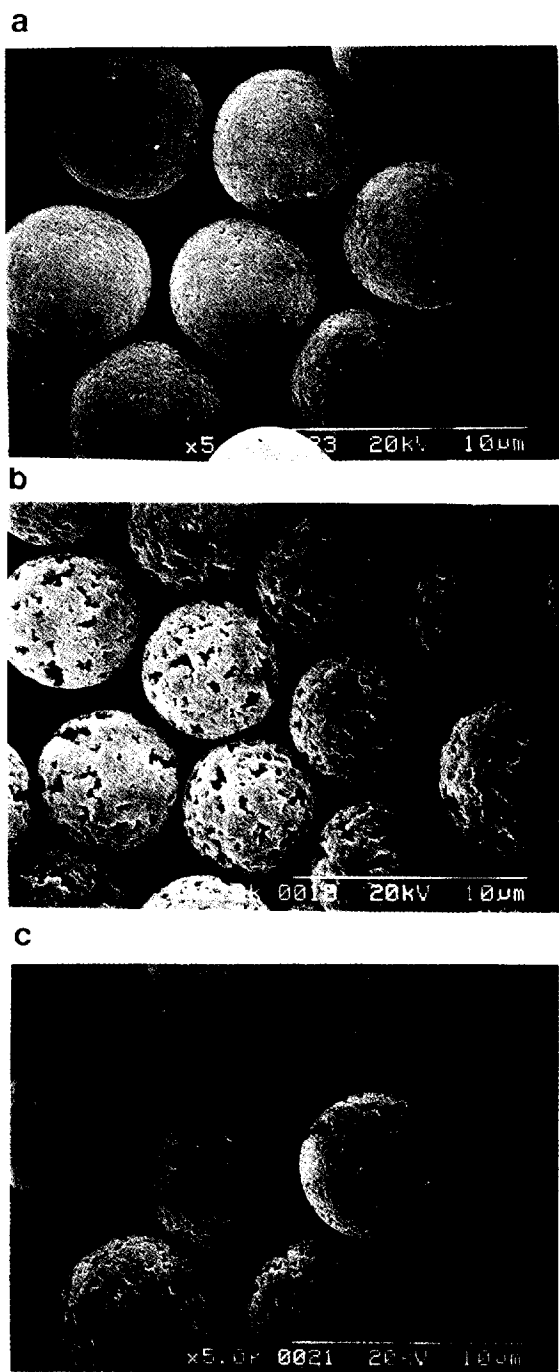


Fig. 2. Scanning electron micrographs of the particles. (a) Unmodified poly(styrene-co-divinylbenzene) beads; (b) beads modified with glycerol monomethacrylate in absence of water soluble initiator; (c) beads modified with a mixture of glycerol monomethacrylate and glycerol dimethacrylate in the presence of potassium peroxydisulfate.

the beads is also carried out and the hydrophobicity of the internal surface is decreased.

Therefore, on the basis of these results, we selected a modification method in which both glycerol monomethacrylate and glycerol dimethacrylate were copolymerized in presence of a water-soluble radical initiator. Fig. 3 shows a comparison of the separation selectivity towards hydrophobic small molecules in water-acetonitrile (80:20) for the particles modified as indicated above and the unmodified poly(styrene-co-divinylbenzene) particles. Despite the quantitative introduction of significant amounts of added monomers, the retention selectivity and hydrophobicity of the modified particles are found to be identical with those of the base particles. This observation further supports our contention that the modification occurs only at the outer surface of the beads, as only such a modification would have no effect on the re-

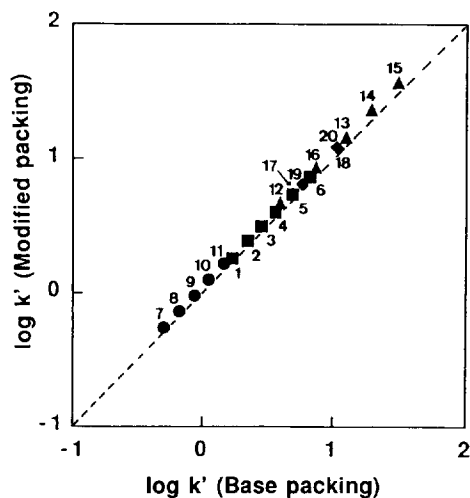


Fig. 3. Separation selectivity of unmodified poly(styrene-co-divinylbenzene) beads and the same beads modified with glycerol monomethacrylate and glycerol dimethacrylate in the presence of potassium peroxydisulfate. Chromatographic conditions: column, 150 mm \times 4.6 mm I.D.; mobile phase, acetonitrile-water (20:80); flow-rate, 1 ml/min. Solutes: 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = propylbenzene; 5 = butylbenzene; 6 = amylbenzene; 7 = hexanol; 8 = heptanol; 9 = octanol; 10 = nonanol; 11 = dodecanol; 12 = naphthalene; 13 = anthracene; 14 = pyrene; 15 = triphenylene; 16 = fluorene; 17 = diphenylmethane; 18 = triphenylmethane; 19 = triptycene; 20 = *o*-terphenyl.

tention selectivity of the small hydrophobic solutes.

The usefulness of the modification process is clearly demonstrated in separations involving mixtures in a water-rich mobile phase. For example, the separation of a mixture of two drugs and bovine serum albumin (BSA) was attempted with both the modified particles and the base particles (Fig. 4). With the unmodified particles, albumin is not eluted before the void volume of the column owing to its absorption on the hydrophobic surface of the particles. In addition, and despite their different hydrophobic properties, the two drugs are eluted nearly at the void volume of the column with no separation at all, demonstrating the inability of the stationary phase to work effectively in this mobile phase (Fig. 4a). Actually, the packed bed consisting of the unmodified base particles was found to shrink by about 10% in the column. On the other hand, the modified particles afford complete recovery of BSA before the void volume of the column and the two drugs are well separated according to their respective hydrophobicities

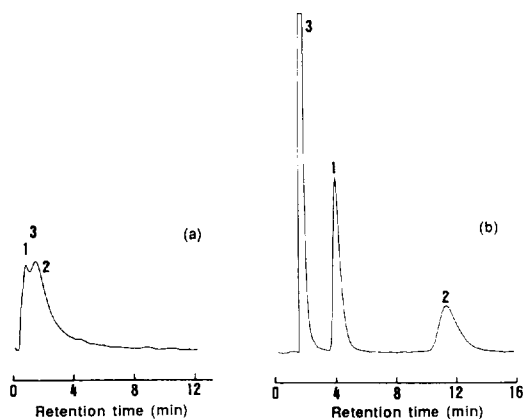


Fig. 4. Separation of drugs in the presence of BSA in a column packed with (a) unmodified poly(styrene-co-divinylbenzene) beads and (b) the same beads modified with a mixture of glycerol monomethacrylate and glycerol dimethacrylate in presence of potassium peroxydisulfate. Chromatographic conditions: column, 150 mm \times 4.6 mm I.D.; mobile phase, acetonitrile–0.02 mol/l phosphate buffer containing 0.1 mol/l sodium sulfate (pH 7) (10:90); flow-rate, 1 ml/min. Solutes: 1 = theophylline (1 mg/ml, 0.3 μ l); 2 = barbital (1 mg/ml, 5 μ l); 3 = BSA (20 mg/ml, 5 μ l).

(Fig. 4b). The complete recovery of BSA suggests that the external surface of the modified particle is hydrophilic enough to prevent the troublesome absorption of the protein.

This surface-selective modification procedure can also be applied to other hydrophobic base particles. For example, we have used this method for the modification of poly(methyl methacrylate-co-ethylene dimethacrylate) particles with glycerol monomethacrylate and glycerol dimethacrylate in the presence of potassium peroxydisulfate initiator. The modified methacrylate particles were tested in the actual analysis of drugs in human serum as shown in Fig. 5. Once again, the surface-modified particles allow the complete recovery of the proteins near the void volume of the column while the hydrophilic

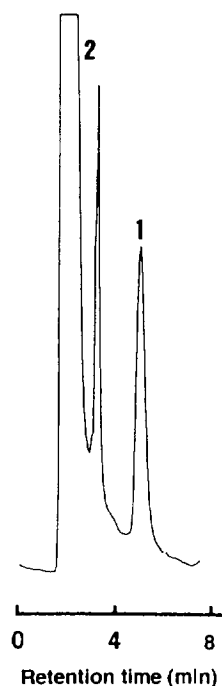


Fig. 5. Direct injection separation of a spiked human serum sample. Chromatographic conditions: column, 150 mm \times 4.6 mm I.D.; packing, poly(methyl methacrylate-co-ethylene dimethacrylate) beads modified with a mixture of glycerol monomethacrylate and glycerol dimethacrylate in the presence of potassium peroxydisulfate; mobile phase, acetonitrile–0.02 mol/l phosphate buffer containing 0.1 mol/l sodium sulfate (pH 7) (30:70); flow-rate, 1 ml/min. Solutes: 1 = theophylline (1 mg/ml, 1 μ l); 2 = human serum (20 μ l).

drug theophylline is retained by the columns and can therefore be detected. In contrast, this separation proved impossible with unmodified poly(methyl methacrylate-co-ethylene dimethacrylate) particles.

4. Conclusion

Simple concepts involving sequential polymerizations and the partitioning of monomers between immiscible aqueous and organic phases can be used to prepare surface-modified macroporous media. The surface modification with glycerol monomethacrylate of hydrophobic macroporous particles prepared with toluene as a porogen was found to occur selectively on the external surface of the base particles, in accordance with the preferred partitioning of the monomer into the water phase. However, because the separation performance of the modified particles was hampered as a result of the swelling of their surface layer in the water-rich mobile phase, an alternative modification was preferred. In this process, incorporation of both glycerol monomethacrylate and glycerol dimethacrylate was achieved in the presence of a water-soluble initiator to afford dimensionally stable modified particles. As the modification was restricted to the surface layer, the original separation selectivity and hydrophobicity of the base particles toward hydrophobic small solutes was preserved, while the presence of a hydrophilic external surface prevented the absorption of proteins. These characteristics make the surface modification process extremely useful in the design of separation media that can be used for the direct determination of drugs in blood serum.

Another advantage of this surface modification process is that it can be used to provide wettability to a polymeric sorbent without loss of its original retention properties. Because it is well known [3,13] that polymer-based separation media are better suited for the preferential retention of organohalides and also aromatic

hydrocarbons than silica-based hydrophobic stationary phases such as C_{18} , new applications of the surface-modified porous polymers are possible. For example, it is expected that our surface-modified media will be very effective for the solid-phase extraction of aqueous environmental pollutants such as halogenated hydrocarbons or toxic dioxins. This is because water wettability of the absorbent is very important in order to obtain reproducible results. Further improvements and tests of our modified macroporous media in these applications are in progress.

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