Separation of priority pollutant phenols on chemically modified poly(styrene–divinylbenzene) resins by high-performance liquid chromatography

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Abstract

A highly cross-linked porous poly(styrene–divinylbenzene) (PS-DVB) resin was prepared using the two-step microsuspension method. The retention behaviour of eleven phenols, listed as priority pollutants by the United States Environmental Protection Agency (EPA), was investigated using this resin with and without surface modification. The corresponding capacity factors (k') of these phenols were compared. Most of the tested compounds showed distinctly different elution behaviour on derivatized resins. A very fast separation of all eleven priority pollutant phenols (PPPs) could be achieved on a column packed with PS-DVB beads modified with *tert.*-butyl groups using gradient elution.

1. Introduction

The use of polymeric resins in reversed-phase high-performance liquid chromatography (RP-HPLC) has grown dramatically, since an increasing number of polymeric columns are commercially available [1]. The application of silica-based supports is limited by the low stability of silica at alkaline pH values and by the unwanted interactions between polar solutes and remaining free silanol groups not covered by the hydrophobic ligand. In particular PS-DVB-based resins show a high stability over the pH range 1–14 and provide excellent separations [2–5]. Additionally, PS-DVB particles permit the intro-duction of numerous functional groups that change their surface chemistry and hence the chromatographic selectivity [6–11].

Sun and Fritz [6] showed that the modification of a XAD-4 and a spherical PS-DVB resin by incorporating different functional groups into the polymer has a major effect on the retention behaviour of various analytes. More hydrophobic resins have been prepared by the Friedel–Crafts reaction of different alkylchlorides with the benzene ring of the polymer. In a way identical to that for silica-based supports, the hydrophobicity of the resin can be controlled by the type of functional group incorporated in the resin.

In contrast to the resin described in the present work, most of the commercially available polymeric supports for HPLC are generally prepared by suspension polymerization followed by

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a size classification procedure [12]. The PS-DVB support proposed here was polymerized using the two-step microsuspension method, which directly leads to monosized particles [13–17]. Therefore these polymer beads may be used for chromatography without any size classification procedure. Porosity and the degree of cross-linking are adjusted by the amount of an inert diluent, e.g. toluene, and the amount of a cross-linking agent, e.g. divinylbenzene, respectively, added during the polymerization step [18]. The high cross-linking degree of the PS-DVB resin studied in this paper leads to an increased ruggedness of the particles.

Numerous papers have been published dealing with the separation of substituted phenols by HPLC [5,19–29]. Some of the works investigate the retention behaviour of these solutes using different chromatographic columns, e.g. silica-based reversed-phase columns [19,28], a graphit-ized carbon column [20] or different polymer-based columns [5,6,21]. But to the best of our knowledge there are no reports addressed to the separation of all eleven priority pollutant phenols (PPPs) using a polymer-based column.

Therefore the aim of the present work was to synthesize and derivatize a highly cross-linked PS-DVB resin, using two different alkylating agents. The chromatographic behaviour of all eleven PPPs listed in the Environmental Protection Agency (EPA) Method 625 [30] was investi-gated on these polymer supports. The resin derivatized with *tert.*-butyl groups was found to be suitable for the separation of all eleven phenols.

2. Experimental

2.1. Equipment

A Waters 625 LC-system equipped with a Waters 600E system controller (Waters, Milford, MA, USA), a 9125 Rheodyne injector (Rheo-dyne, Cotati, CA, USA) equipped with a 50-μl loop, an ABI 785A programmable absorbance detector (Applied Biosystems, San Jose, CA, USA) and a HP 3359A chromatographic work-system (Hewlett-Packard, Palo Alto, CA, USA) were used for HPLC. For column packing a Knauer pneumatic HPLC pump (Knauer, Ber-lin, Germany) was used.

2.2. Reagents and chemicals

All chemicals used for the synthesis and de-rivatization of the polymeric resin were pur-chased from Merck (Darmstadt, Germany). HPLC gradient grade acetonitrile, methanol and analytical grade acetic acid were purchased from J.T. Baker (Deventer, Netherlands). Doubly distilled water was used.

Priority pollutant phenols (Merck) were dis-solved in analytical grade methanol from J.T. Baker and the solutions stored at 4°C in the dark.

2.3. Columns

PS-DVB beads were prepared by a two-step microsuspension method similar to the proce-dure described elsewhere [13]. The resulting particles had a mean diameter of 4 μm ($\pm$0.3 μm), a specific surface area between 92 and 94 m^2/g and a cross-linking degree of 50%. Part of the material was alkylated under Friedel–Crafts conditions. To a suspension of 5 g of the resin in octadecylchloride 0.5 g anhydrous aluminium chloride was added. The mixture was kept at 80°C for 20 h and then was diluted with 4 M hydrochloric acid, filtered and washed sub-sequently with water, methanol and acetone. The resulting PS-DVB particles with octadecyl-chains bound to the benzene ring of the resin were dried. In the same way *tert.*-butylchloride was reacted with the PS-DVB beads. All poly-mer resins were suspended in tetrahydrofuran, sonicated and packed into 30 × 4 mm I.D. stain-less-steel columns for 15 min at 35 MPa. Thio-urea was chosen to determine the hold-up time (t_0) of the columns in this study.

3. Results and discussion

3.1. Chromatographic behaviour

The chromatographic behaviour of eleven differently substituted PPPs has been investigated using different acetonitrile–water mixtures for isocratic elution. To both solvents 1% acetic acid was added to prevent peak tailing [29]. Fig. 1 shows the capacity factors of these phenols on a PS-DVB column as a function of the acetonitrile content of the eluent. Because of the scale of the diagram, pentachlorophenol is not shown in this plot. A flow-rate of 1 ml/min was used throughout these experiments unless specified other.
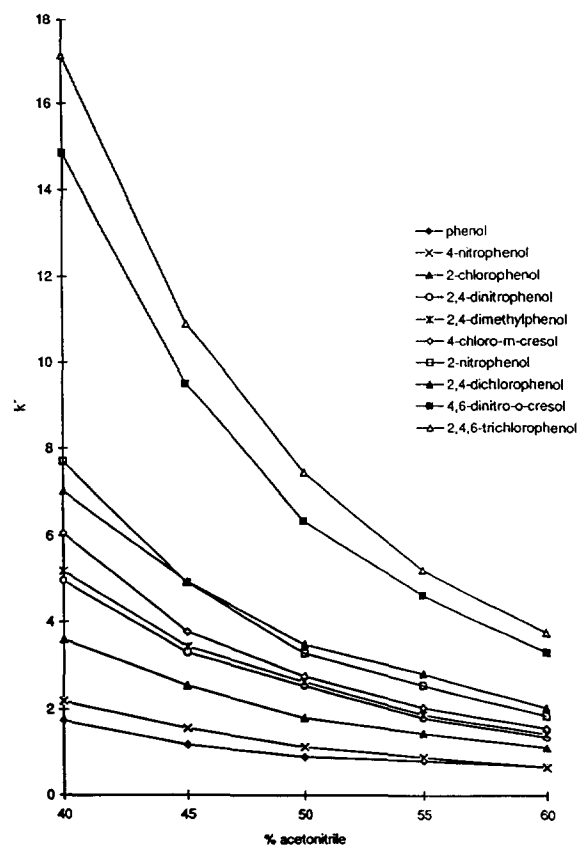

Fig. 1. The k'-values of ten PPPs as a function of the acetonitrile content of the eluent. Chromatographic conditions: column, PS-DVB, 4 μm, 30 × 4 mm I.D.; eluent, water (1% acetic acid)–acetonitrile (1% acetic acid); flow-rate, 1 ml/min.

Using the same eluent, the PPPs elute in identical order from underivatized PS-DVB as from the alkylated species. Published separations of these phenols using similar conditions but silica-based reversed-phase columns [28,29] show quite different results. The weakly retained phenols, e.g. phenol or 4-nitrophenol, as well as the strongly retained substances, e.g. 2,4-dinitro-o-cresol, 2,4,6-trichlorophenol or pentachlorophenol, show the same elution behaviour on PS-DVB-based resins as compared to silica-based materials. However, the elution order of the moderately retained compounds, starting from 2-chlorophenol and ending with 2-nitrophenol, considerably differs as compared with silica-based columns. This is clearly demonstrated in Table 1. This behaviour is believed to be caused by additional interactions between the π-electrons of the PS-DVB resin and orbitals of the solute species.

The acetonitrile content of the eluent changes the elution order of these PPPs on a PS-DVB column only in one case. As can be seen in Fig. 1, 2-nitrophenol is less retained than 2,4-dichlo-

Table 1
Comparison of k'-values of 11 PPPs on a PS-DVB and a silica C_{18} reversed-phase liquid chromatography column

Compound	Capacity factors (k')	
	PS-DVB	Silica C_{18}
Phenol	0.90	1.07
4-Nitrophenol	1.13	1.29
2-Chlorophenol	1.81	1.86
2,4-Dinitrophenol	2.55	1.77
2,4-Dimethylphenol	2.64	2.68
4-Chloro-m-cresol	2.77	3.11
2,4-Dichlorophenol	3.29	5.98
2-Nitrophenol	3.49	2.71
4,6-Dinitro-o-cresol	6.34	4.22
2,4,6-Trichlorophenol	7.45	6.85
Pentachlorophenol	17.36	18.75

PS-DVB: 30 × 4 mm I.D., 4 μm; eluent, water–acetonitrile–acetic acid (49.5:49.5:1); flow-rate, 1 ml/min.
Silica C_{18}: Radial Pak C_{18} cartridge (Waters, Milford, MA, USA), 100 × 8 mm I.D., 5 μm; eluent, water–acetonitrile–acetic acid (50:50:0.1); flow-rate 2 ml/min; data taken from Ref. [28].

rophenol on PS-DVB-based columns if the ace-
tonitrile content of the eluent is less than 45%.
Increasing the acetonitrile content of the eluent,
2,4-dichlorophenol elutes earlier than 2-nitro-
phenol. This observation does not only apply to
underivatized PS-DVB but also to the alkylated
resins used in this study.

3.2. Comparison of unmodified and modified PS-DVB resins

Alkylated and underivatized PS-DVB based
columns were investigated. Comparing the k'-
values of all eleven PPPs, it can be mentioned
that generally k'-values increase by introducing
alkyl groups to the benzene ring of the polymer
due to the increase in sorbent hydrophobicity.
As can be seen in Table 2, the resin derivatized
with C_{18} chains shows a larger increase in k'-
values than the resin substituted with tert.-butyl
groups. This may be attributed to the higher
hydrophobicity of the C_{18} chains compared with
the tert.-butyl groups. The behaviour of 2,4,6-
trichlorophenol, shown in Fig. 2, is a typical
example of this fact. However, phenols con-
taining a free nitro-group, e.g. 2,4-dinitrophenol,
4-nitrophenol or 4,6-dinitro-o-cresol, show dif-
ferent behaviour, which is illustrated in Fig. 3.
The k'-values observed with the tert.-butyl-de-

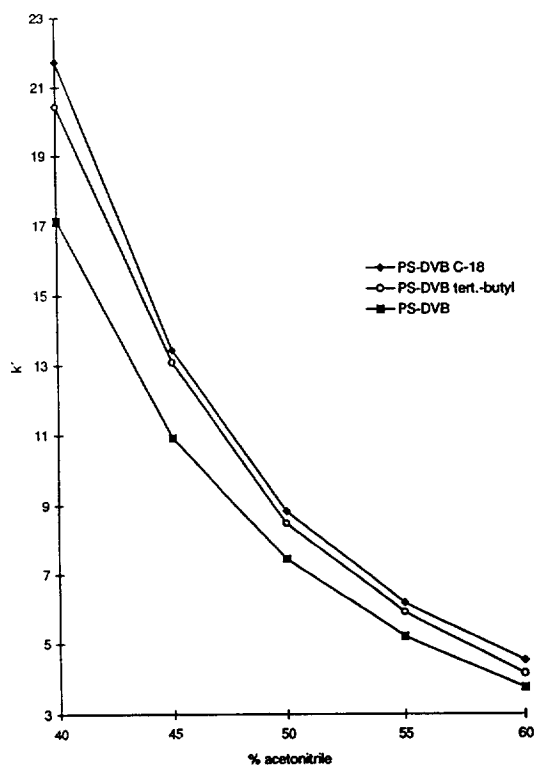

Fig. 2. The k'-values of 2,4,6-trichlorophenol as a function of
the acetonitrile content of the eluent using alkylated and
non-alkylated PS-DVB columns. Chromatographic condi-
tions: column, 30 × 4 mm I.D.; eluent, water (1% acetic
acid)–acetonitrile (1% acetic acid); flow-rate, 1 ml/min.

Table 2
Comparison of k'-values of 11 PPPs on alkylated and non-alkylated PS-DVB resins

Compound	Capacity factors (k')		
	PS-DVB	PS-DVB C_{18}	PS-DVB tert.-butyl
Phenol	0.90	1.03	1.00
4-Nitrophenol	1.13	1.23	1.10
2-Chlorophenol	1.81	2.07	1.94
2,4-Dinitrophenol	2.55	2.74	2.20
2,4-Dimethylphenol	2.64	2.80	2.66
4-Chloro-m-cresol	2.77	3.19	3.09
2,4-Dichlorophenol	3.29	4.06	3.89
2-Nitrophenol	3.49	4.12	4.15
4,6-Dinitro-o-cresol 6.34	7.24	6.07	
2,4,6-Trichlorophenol	7.45	8.83	8.47
Pentachlorophenol	17.36	24.09	21.96

Chromatographic conditions: columns 30 × 4 mm I.D.; eluent, water–acetonitrile–acetic acid (49.5:49.5:1); flow-rate, 1 ml/min.
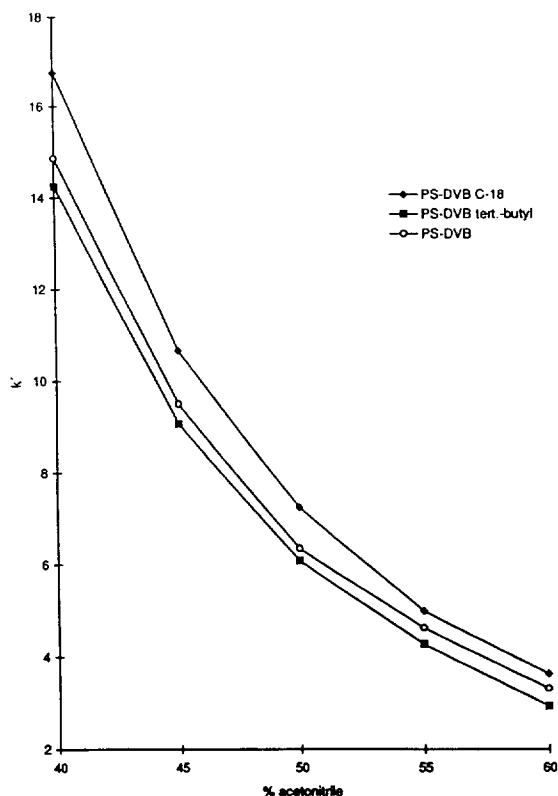
Fig. 3. The k'-values of 4,6-dinitro-o-cresol as a function of the acetonitrile content of the eluent using alkylated and non-alkylated PS-DVB columns. Chromatographic conditions: column, 30 × 4 mm I.D.; eluent, water (1% acetic acid)–acetonitrile (1% acetic acid); flow-rate, 1 ml/min.

rivatized resin are even lower than those with the underivatized resin. But it should be stressed that these more polar phenols do not reverse the retention order, as might be expected. The resin derivatized with the C_{18} chains still leads to the highest k'-values even for these PPPs containing free nitro-groups. 2-Nitrophenol behaves like the phenols without nitro-groups due to the intramolecular hydrogen bonding.

The number of theoretical plates for these columns was determined for 4-chloro-m-cresol using a mixture of 50% acetonitrile and 50% water, both containing 1% of acetic acid for isocratic elution. It was found to be 450 for the columns filled with alkylated resin and 525 for the column filled with underivatized PS-DVB.

This corresponds to a value of 15 000 respectively 17 500 plates/m.

3.3. Separation of priority pollutant phenols

To achieve complete separation of all eleven PPPs on a PS-DVB-based column isocratic conditions with a low acetonitrile content were used to separate the first eight phenols (phenol–2,4-dichlorophenol), while the strongly retained phenols, e.g. 4,6-dinitro-o-cresol, 2,4,6-trichlorophenol and pentachlorophenol, were eluted by a steep gradient up to 95% acetonitrile. When using an eluent mixture containing 40% of acetonitrile, the resolution of e.g. 2,4-dinitrophenol and 2,4-dimethylphenol is very poor for both the PS-DVB and the PS-DVB C_{18} column. The use of underivatized PS-DVB results in a resolution (R_s) of just 0.08 while the C_{18} derivatized resin gives an even worse R_s of 0.03. Whereas using the $tert.$-butyl-group containing resin increases the resolution of these two peaks to a value of 0.61. This fact is caused by the different behaviour of phenols containing free nitro-groups on the $tert.$-butyl-derivatized resin compared to that on underivatized and C_{18}-derivatized polymer. Fig. 4 shows a high-speed separation of a standard mixture of all eleven PPPs on a $tert.$-butyl-derivatized PS-DVB column. Good separation of all investigated phenols could be achieved in less than 6.5 min using the chromatographic conditions specified in Fig. 4.

4. Conclusion

The results obtained in this work indicate that PS-DVB-based resins prepared by the two-step microsuspension method are an attractive alternative to silica-based columns in reversed-phase liquid chromatography. It is apparent that the introduction of alkyl groups into these PS-DVB resins has an appreciable effect on the retention times and k'-values. In some cases derivatized columns even offer an additional selectivity parameter, which could be shown in the case of the separation of eleven PPPs on a PS-DVB-based column containing $tert.$-butyl groups. This sepa-
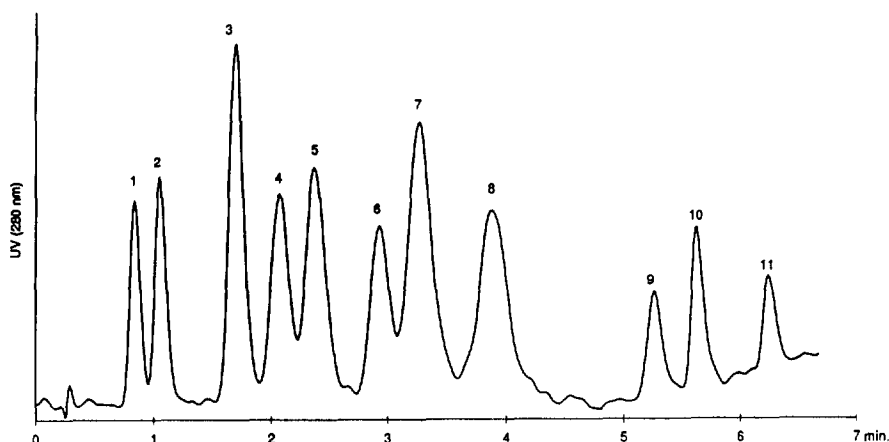
Fig. 4. Separation of a standard mixture of 11 PPPs. Chromatographic conditions: column, PS-DVB *tert.*-butyl, 4 μm, 30 × 4 mm I.D.; eluents, (A) water–acetic acid (99:1), (B) acetonitrile–acetic acid (99:1), 65% A and 35% B 4 min isocratic followed by a linear gradient to 95% B in 2 min; flow-rate, 1.5 ml/min; detection, UV 280 nm; peaks: 1 = phenol, 2 = 4-nitrophenol, 3 = 2-chlorophenol, 4 = 2,4-dinitrophenol, 5 = 2,4-dimethylphenol, 6 = 4-chloro-*m*-cresol, 7 = 2-nitrophenol, 8 = 2,4-dichlorophenol, 9 = 4,6-dinitro-*o*-cresol, 10 = 2,4,6-trichlorophenol, 11 = pentachlorophenol.

ration is less time-consuming than published separations using other chromatographic supports.

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