

A novel stationary phase for the high performance liquid chromatographic separation and determination of phenols

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A novel type of hyper-crosslinked polystyrene resin has been used as a stationary phase in the high performance liquid chromatographic separation of phenol and its derivatives; a simplified procedure for their quantitative determination on a ppb level, based on preconcentration and separation using a single chromatographic column, is proposed.

The use of polymer resins as a stationary phase in reversed-phase high-performance liquid chromatography (RP HPLC) has grown dramatically since an increasing number of polymer columns became commercially available. The reason for this growth is connected with the low stability of the most frequently used octadecylsilica (ODS) in alkaline media and undesirable interactions between polar solutes and residual silanol groups that influence the peak shape and column performance.^{1–5} The main area of application of polymer resins in RP HPLC is the separation of different biomolecules such as peptides and proteins. However, they can also be efficient for the preconcentration and separation of other organic molecules, e.g. hyper-crosslinked polystyrene has been successfully used for preconcentration prior to HPLC-determination of phenol traces in aqueous solutions.⁶

The high degree of crosslinking and new approach to the synthesis of polystyrene networks provide a reasonable mechanical stability that allows a narrow fraction of fine (down to 3–5 μm diameter) particles to be obtained. These sorbents are biporous (have a bimodal pore size distribution) and possess highly developed micropore structures. They demonstrate good kinetics and reversibility of adsorption and desorption process,^{6–8} allowing their use not only as sorbents for solid-phase extraction but also as column packing for HPLC.⁹ It is interesting that hyper-crosslinked polystyrene has a hydrophobic, but at the same time wettable by water, surface.⁷

As mentioned before, biporous hyper-crosslinked polystyrene-type sorbents show a unique affinity towards non-substituted phenol, a very high dynamic adsorption capacity (80 mg g⁻¹ on a 20×1.4 cm column with a linear flow rate over 4 cm min⁻¹) and an exceptionally high recovery of phenols from water solutions at a low concentration level (phenol 96±5%, *p*-nitrophenol 99±4%, 2,4-dinitrophenol 95±5%, *o*-nitrophenol 96±6%, 2,4-dichlorophenol 96±5%).⁶ Thus, the unique combination of physico-mechanical and chemical properties of hyper-crosslinked polystyrene opens up new facilities in the determination of phenol and its derivatives by using a single chromatographic column for preconcentration and separation.

There is a growing demand for a simple trace-level determination of ecotoxicants, such as phenol and its derivatives, in

natural waters. Determination of phenols usually includes preconcentration (extraction, solid-phase extraction) followed by chromatographic separation;^{10–12} therefore, this process is time-consuming and not too efficient. Moreover, in most cases the possibilities of a common preconcentration procedure are limited because only a small part of the preconcentrate is used for further analysis. The procedure would be more efficient if the whole volume of preconcentrate were served as a sample for chromatographic analysis. Probably, the application of HPLC columns packed with hyper-crosslinked polystyrene would allow the combination of both preconcentration and separation, which would result in a decrease in analysis time and increased accuracy, and moreover, to a decreased volume of original water sample.

Because the preconcentration of phenols by neutral non-charged hyper-crosslinked polystyrenes has already been investigated and optimal conditions chosen,¹³ we studied the chromatographic behaviour of phenol and its chloro-, nitro- and methyl-derivatives on a column packed with this sorbent as well as the possibility of carrying out both preconcentration and separation with a single column.

Retention of phenols on a chromatographic column strongly depends on the pH of the buffer solution in the mobile phase, because of partial dissociation of the molecules of phenol and its derivatives with pH change. Due to the high hydrolytic stability of hyper-crosslinked polystyrene,^{1,6–8} the retention of phenols over a broad range of mobile phase pH was investigated.

Increase of buffer pH in the eluent results in dissociation of phenol molecules, and consequently, their capacity factor k' [$k' = (t_R - t_0)/t_0$] decreases due to the lower affinity of the ionic form of phenol towards the stationary phase in comparison with the molecular form (Figure 1). The sharp change of retention corresponds to equality of the concentrations of the ionic and molecular form of phenols or their pK_a . It should be noted that at pH 5.4–6.5 a some increase in retention was observed. This effect is thought to be connected with the increasing role of salting out under a significantly increasing ionic strength of 5 mM phosphate buffer over this pH range. An approximate estimation has shown it to increase from a value of 0.05 at pH 5.4 to 0.075 at pH 6.5.

With polystyrene resin as a stationary phase the retention of organic molecules in the reversed phase mode of HPLC depends on the nature and concentration of an organic modifier much stronger as compared to ODS packings because the surface of the polymer stationary phases is more sensitive to solvation by organic solvents.^{1,7} In the case of polymer sorbents retention is generally defined by hydrophobic solute-sorbent interactions; therefore, the addition of strong solvents such as acetonitrile and THF to the mobile phase usually considerably improves the peak shape.

In this work we used acetonitrile as an organic modifier of the mobile phase. The dependence of retention (k') obtained from the acetonitrile content is described well by equation (1), proposed by Jandera¹⁴ over the range of acetonitrile concentration from 40 to 90% v/v.

$$\lg k' = a - mX + dX^2 \quad (1)$$

Table 1 Parameters [equation (1)] describing the retention of phenols on hyper-crosslinked polystyrene as a function of acetonitrile concentration in the mobile phase. Chromatographic column: hydrophobic cross-linked polystyrene (15 m), 150×3 (i.d.) mm; eluent: a mixture of acetonitrile with 5 mM phosphate buffer (pH 6.5).

Substances	<i>a</i>	<i>m</i> ×10 ²	<i>d</i> ×10 ⁴	<i>r</i>
Phenol	1.74	4.39	2.43	0.990
2,4-Dichlorophenol	3.34	7.04	3.97	0.997
4-Chloro-3-methylphenol	3.08	6.72	3.80	0.996
Pentachlorophenol	3.24	6.33	3.21	0.996
2,6-Dimethylphenol	2.79	5.92	3.14	0.999
2,4,6-Trichlorophenol	4.05	8.51	5.02	0.988
<i>p</i> -Nitrophenol	2.81	6.99	4.20	0.998
2,4-Dinitrophenol	-0.23	2.80	0.63	0.850
<i>o</i> -Nitrophenol	2.63	5.45	2.98	0.974
2-Chlorophenol	2.23	4.66	2.40	0.988

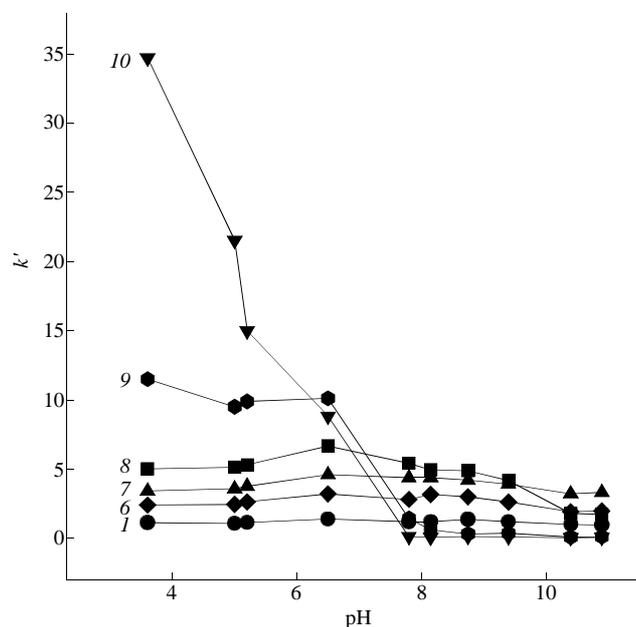
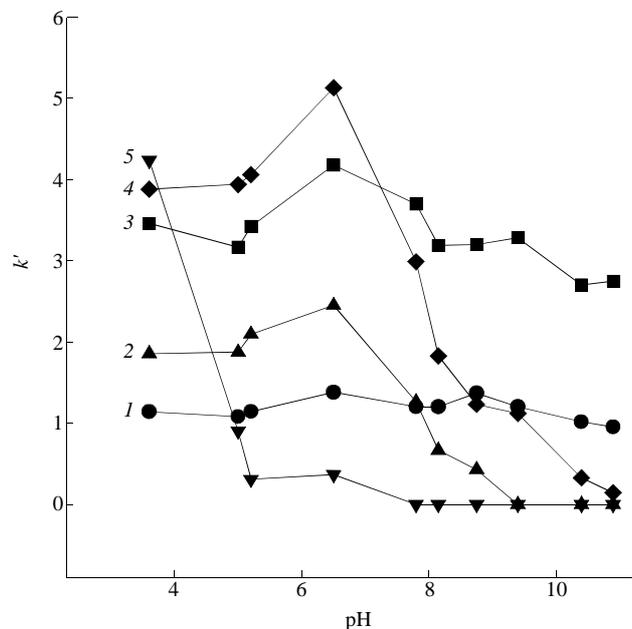


Figure 1 Plot of capacity factors of phenols versus pH of buffer solution in the mobile phase. Chromatographic column: hydrophobic cross-linked polystyrene (15 m), 62×2 mm i.d.; eluent: a 1:1 (v/v) mixture of acetonitrile with 5 mM phosphate buffer; flow rate: 0.2 ml min⁻¹; detection wavelength: 274 nm. Phenols: phenol (1), *p*-nitrophenol (2), 2,6-dimethylphenol (3), *o*-nitrophenol (4), 2,4-dinitrophenol (5), 2-chlorophenol (6), 4-chloro-3-methylphenol (7), 2,4-dichlorophenol (8), 2,4,6-trichlorophenol (9), pentachlorophenol (10).

In equation (1), X is the concentration of an organic solvent in the mobile phase in % v/v; a is a parameter corresponding to retention of phenol in water and m and d are parameters describing the change of retention with growth of X .

It should be noted that for many sorbates the value of coefficient d is negligible over the investigated range of concentration of an organic solvent and consequently, the dependence of $\lg k'$ from X is close to linear. However, the deviation of this dependence from a linear one for phenols should not be ignored. The parameters of equation (1) describing the retention of the phenols under investigation on hyper-crosslinked polystyrene are presented in Table 1. The elution order of phenol and its derivatives is in accordance with their distribution coefficients obtained earlier for adsorption on hyper-crosslinked polystyrene in static conditions.¹³

The mechanism of retention of phenols on polymer sorbents

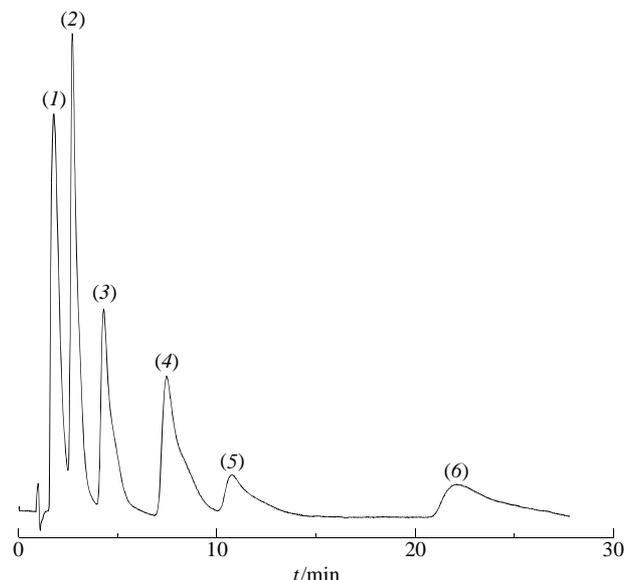


Figure 2 Chromatogram of a mixture of 2,4-dinitrophenol (1), phenol (2), *p*-nitrophenol (3), *o*-nitrophenol (4), 2,4-dichlorophenol (5), 2,4,6-trichlorophenol (6). Chromatographic column: hydrophobic cross-linked polystyrene (15 m), 150×3 (i.d.) mm; eluent: a 45:55 (v/v) mixture of acetonitrile with 5 mM phosphate buffer (pH 5.4) containing 0.01 M KCl; flow rate: 1 ml min⁻¹; detection wavelength: 274 nm.

is complex because their retention is influenced by many factors, namely: hydrophobic interactions of phenols with the surface of polymer particles, – interactions between phenols and the polystyrene matrix, the size-exclusion effect of micropores, the formation of relatively stable adducts with molecules of organic solvent in eluent, *etc.*

Some of the secondary equilibria taking place in this chromatographic system, and which are responsible for the loss of separation in the resulting chromatographic performance, can be suppressed by increasing the concentration of the common electrolyte in the eluent. So, the addition of KCl to the mobile phase at a concentration *ca.* 0.01 M markedly improves the peak shape. At the same time the retention of phenols remains practically unchanged. It should be pointed out that under the conditions chosen (Figure 2) the performance of a column (150×3 mm) packed with hyper-crosslinked polystyrene is comparatively high (11100 plates per metre, calculated based on retention of phenol). Thus, an eluent consisting of a 45:55 (v/v) mixture of acetonitrile with 5 mM phosphate buffer (pH 5.4) containing 0.01 M KCl has been found to be optimal for the separation of phenol and its two chloro- and three nitro-derivatives.

Because hyper-crosslinked polystyrene has both a high affinity to phenol and its derivatives and a reasonable selectivity in their chromatographic separation, a simple and rapid procedure for their quantitative determination at a ppb level in water using a single chromatographic column for preconcentration and separation can be proposed. As an example, the determination of phenol, 2-chlorophenol, 4-chloro-3-methylphenol and 2,4-dichlorophenol was demonstrated. The determination includes the following steps:

1. sample preparation (adjusting pH of sample to 2–3, filtration, degassing);
2. column conditioning by consecutive flushing with a mixture of acetonitrile and water;
3. preconcentration (pumping of the sample prepared as described in step 1 through the column);
4. pre-separation involving washing the column with an eluent containing 5–10 v/v acetonitrile (if necessary) to remove any components preceding the phenol peak;
5. separation of phenols (separation conditions found earlier¹⁵ to be optimal for the separation of phenol and three of its chloro-derivatives mentioned above are presented in Figure 3)

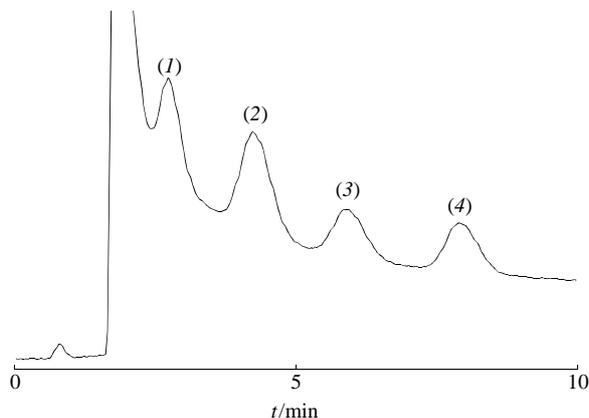


Figure 3 The chromatogram resulting after percolating 2.0 ml of a water sample spiked with phenol (25 g l^{-1}), 2-chlorophenol (25 g l^{-1}), 4-chloro-3-methylphenol (25 g l^{-1}), 2,4-dichlorophenol (100 g l^{-1}) through the chromatographic column. Eluent: a 49.5:49.5:1 (v/v) mixture of acetonitrile, distilled water and acetic acid; for other conditions, see Figure 1.

and recording of the chromatogram;

6. washing the column with an eluent of increased acetonitrile content to remove strongly retained substances from the sample.

It should be noted that chromatographs equipped with a syringe-type pump [Phoenix 20 CU (Carlo Erba) and Milichrom] are more convenient for the determination of phenols by the procedure described above. The technique proposed is quick (analysis duration including sample preparation does not exceed 30 min) and sensitive, because it avoids losing sample at the preconcentration stage and allows additional peak broadening in the chromatographic column. The chromatogram obtained after percolating 2.0 ml of water sample with a concentration of phenols at the ppb level through the chromatographic column is presented in Figure 3. It was found that loading 2 ml of water sample onto the column and separation under the conditions as stated in Figure 3 provides

quantitative determination over the following ranges: 3–100 ppb for phenol, 3–100 ppb for 2-chlorophenol, 8–200 ppb for 4-chloro-3-methylphenol and 50–1000 ppb for 2,4-dichlorophenol.

Thus, the use of hyper-crosslinked polystyrene allows a simple and quick HPLC determination of phenols in water based on their preconcentration and separation with a single chromatographic column.

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