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Transient changes of mobile phase in the high-performance liquid chromatographic separation of priority pollutant phenols

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SUMMARY

A study of the effect of cetyltrimethylammonium bromide on the separation of the eleven priority pollutant phenols is presented. Transient changes in a CTAB mobile phase produced by a sodium laurylsulphate solution plug permit the elution of hydrophobic pentachlorophenol.

INTRODUCTION

Phenols are common water pollutants because they are extensively used as pesticides, and also because they may be produced by some industrial activities[1]. The U.S. Environmental Protection Agency (EPA) has included eleven phenols in the list of priority pollutants. The priority pollutant phenols (PPP) are phenol, 2,4-dimethylphenol (24DMP), 4-chloro-3-methylphenol (43CMP), 2-chlorophenol (2CP), 2,4-dichlorophenol (24DCP), 2,4,6-trichlorophenol (246TCP), 2-nitrophenol (2NP), 4-nitrophenol (4NP), 2,4-dinitrophenol (24DNP), 4,6-dinitro-2-methylphenol (46DNOC) and pentachlorophenol (PCP)[2].

In a previous paper[3], the separation of these PPP by high-performance liquid chromatography (HPLC) with isocratic elution was proposed, but this separation may be improved if the capacity factors (k') for 24DNP and 46DNOC are larger than unity. The use of ion-pairing agents increases the k' values of acidic compounds and improves their separation. However, PCP, owing to its acidity and highly hydrophobic character, is strongly retained under these conditions.

The use of transitory mobile phase environments was applied by Gluckman *et al.*[4] to the separation of organic dyestuffs (cationic, non-ionic and anionic). In this work, cetyltrimethylammonium bromide (CTAB) was added to the mobile phase as an

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ion-pairing agent to elute all phenols except PCP. Subsequently, a short plug of mobile phase containing sodium laurysulphate (SLS) was used to elute PCP.

EXPERIMENTAL

Chemicals

The PPP were purchased from Supelco (Bellefonte, PA, U.S.A.) and were of chromatographic grade. HPLC-grade methanol was obtained from Promochem (Wesel, F.R.G.) and HPLC-grade acetonitrile from Mallinckrodt (Paris, KY, U.S.A.). Water was obtained from a Milli-Q (Millipore, Molsheim, France) purification system. CTAB was from Serva (Heidelberg, F.R.G.) and SLS from Carlo Erba (Milan, Italy). All other reagents were of analytical-reagent grade. The mobile phase was 30 m*M* ammonium acetate buffer (pH 5.0)–acetonitrile–methanol (56:34:10), unless indicated otherwise. Before use, all eluents were degassed under vacuum and filtered with an all-glass apparatus through 0.45-μm filters (Millipore, Bedford, MA, U.S.A.).

Apparatus

Analyses were performed with a Milton Roy (Riviera Beach, FL, U.S.A.) liquid chromatograph equipped with a Model CM 4000 multiple-delivery solvent system, a Model SM 4000 programmable-wavelength detector and a Model C14100 computing integrator. The injector was a Model 7125 valve (Rheodyne, Cotati, CA, U.S.A.) with a 20-μl fixed loop. For pH measurements, a Model 501 digital pH meter (Crison Instruments, Barcelona, Spain) with a glass electrode was used. Prepacked analytical columns were a Nucleosil-120, 5 μm (12 cm × 4 mm I.D.) C_{18} column (Knauer, Berlin, F.R.G.) and a Spherisorb ODS-2, 5 μm (15 cm × 4 mm I.D.) column (Tecknokroma, Barcelona, Spain).

Methods

The column was equilibrated prior to use with 0.20 m*M* CTAB mobile phase for the Spherisorb packing or 0.15 m*M* CTAB for the Nucleosil packing. The solution was passed through the column for 90 min at a flow-rate of 1.5 ml/min. Equilibrium was reached when the retention time for PPP remained constant. An SLS plug was introduced into the column by a time-programming change in the admission valve of the solvent delivery system after sample injection. The flow-rate was maintained at 1.5 ml/min. The column was reconditioned by passing CTAB mobile phase through it after the plug.

RESULTS AND DISCUSSION

Effect of CTAB concentration

CTAB affects each PPP differently because of their different capacities to form ion pairs; this behaviour is controlled by the pH of the mobile phase, as uncharged phenols do not form ion pairs. For example, phenol is uncharged at pH 5 and therefore, it was used as a reference compound. Fig. 1 shows the effect of the CTAB concentration on this separation. As can be observed, the retention time (t_r) of associated phenols decreases when the CTAB concentration increases. This effect was corroborated by Kastary and Gilpin[5] by increasing the surfactant concentration and

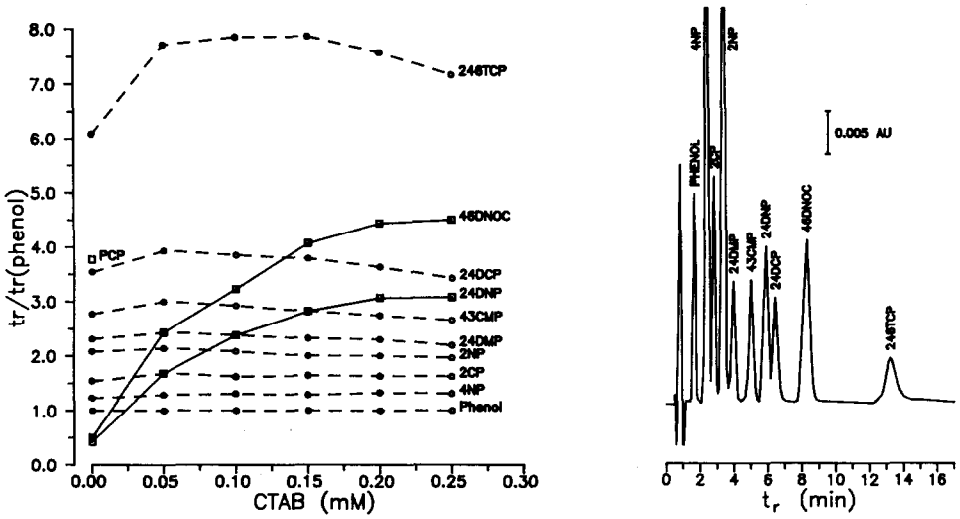

Fig. 1. Effect of CTAB on PPP separation. Column, Spherisorb ODS-2, 5 μm (150 × 4 mm I.D.); flow-rate, 1.5 ml/min; eluent, CTAB in the mobile phase (see Experimental); detection, 280 nm; injection volume, 20 μl; t_r (phenol) = 1.3–1.4 min.

Fig. 2. Separation of PPP with CTAB. Column, Nucleosil-120, 5 μm, C_{18} (120 × 4 mm I.D.); flow-rate, 1.5 ml/min; eluent, 0.15 m*M* CTAB mobile phase (see Experimental); detection, 280 nm; injection volume, 20 μl.

working with uncharged compounds. Under these conditions, PCP is partially ionized and strongly retained, as even at low CTAB concentrations an ion pair ($CTAB^+ \cdot PCP^-$) of high hydrophobic character is formed. The k' values of 46DNOC and 24DNP were also increased owing to ion-pair formation. Thus, better PPP separations were obtained with CTAB concentrations in the range 0.20–0.25 m*M*. A CTAB concentration close to 0.15 m*M* was needed to achieve a similar separation when the Nucleosil column was used (Fig. 2). Nucleosil-120 (5 μm), C_{18} and Spherisorb ODS-5 packings were chosen because of their durability when mobile phases containing CTAB were used[6].

Eluent plugs

The use of transient mobile phases allows the selective elution of strongly retained substances such as, in this instance, PCP. The eluotropic strength of the mobile phase for PCP can be enhanced in two ways, by increasing the methanol concentration or by increasing the ionic strength. When 1.0 *M* sodium chloride or 100% methanol was used, satisfactory results were not obtained. Another possibility is based on establishing a competitive equilibrium between CTAB and a high concentration of another surfactant of a different nature (anionic), which could form an ion pair with CTAB[4]. When a plug containing an excess of anionic surfactant was introduced into the column, the surfactant molecules shifted the ion-pair equilibrium between CTAB and PCP, liberating the ionized PCP. The short-chain surfactant sodium heptanesulphonate was selected in order to facilitate column re-equilibration but, after testing, the need for a more hydrophobic anionic surfactant was evident and SLS was selected. The PCP retention times *versus* the total amounts of SLS in the plug are plotted in Fig. 3 for the two above-mentioned columns. Once the determined amount

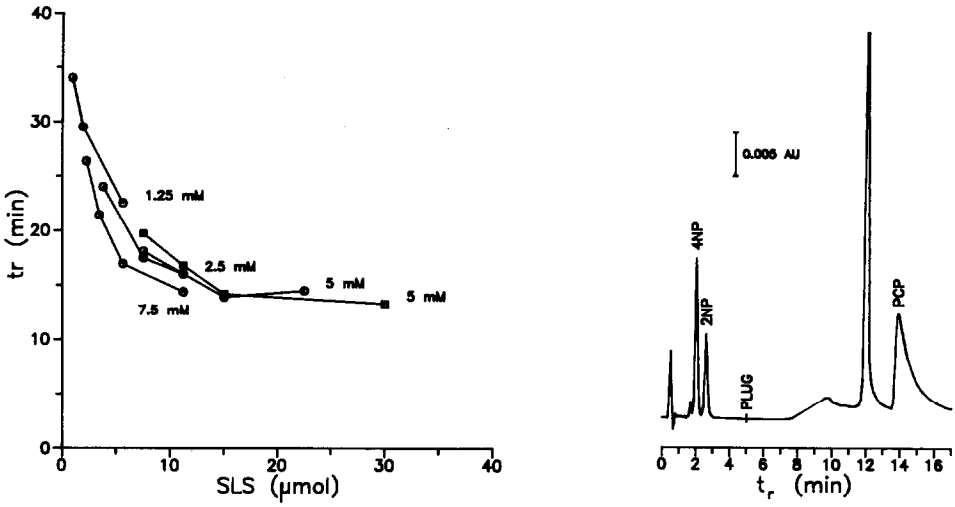

Fig. 3. Effect of the amount of SLS on elution of PCP. Eluent, 0.15 m*M* CTAB mobile phase for the Nucleosil (○) and 0.20 m*M* CTAB mobile phase for the Spherisorb (□) column (see Experimental); plug start time, 5 min; flow-rate, 1.5 ml/min; detection, 254 nm.

Fig. 4. Elution of PCP with CTAB mobile phase and SLS plug. Column, Nucleosil-120, 5 μm, C_{18} (120 × 4 mm I.D.); flow-rate, 1.5 ml/min; eluent, 0.15 m*M* CTAB mobile phase (see Experimental); detection, 254 nm; injection volume, 20 μl; plug volume, 3 ml of 5 m*M* SLS mobile phase.

of SLS had been reached, the retention time of PCP hardly changed. This may be explained as the time necessary for PCP to pass through the column according to a hydrophobic retention mechanism.

A 0.20 m*M* CTAB concentration in the mobile phase and 15 μmol of SLS in the plug were sufficient to separate all PPP and elute PCP when the Spherisorb column was used, whereas a 0.15 m*M* CTAB concentration and 15 μmol of SLS in the plug were needed for the Nucleosil column (Fig. 4). In both instances reconditioning of the column by passing CTAB mobile phase through it was needed. This was monitored by injecting 24DNP or 46DNOC and comparing the retention times with those obtained in the former chromatogram. The time necessary for column reconditioning was directly related to the amount of SLS used in the plug.

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