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CHARACTERIZATION OF PHENOLIC COMPOUNDS BY OPEN-TUBULAR LIQUID CHROMATOGRAPHY

M. P. MASKARINEC*
Analytical Chemistry Division, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, TN 37830 (U.S.A.)
and
J. D. VARGO and M. J. SEPANIAK
Department of Chemistry, University of Tennessee, Knoxville, TN 37916 (U.S.A.)
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SUMMARY

Efficient separations of isomeric alkylphenols are achieved by open-tubular liquid chromatography employing a 34 m × 50 μm I.D. capillary column coated with β,β′-oxydipropionitrile. The alkylphenol compounds present in a coal-derived liquid have been separated by this method.

INTRODUCTION

Open-tubular liquid chromatography (OTLC) has the ability to achieve chromatographic separations superior to those obtained by conventional analytical high-performance liquid chromatography (HPLC). The theoretical aspects of OTLC and its advantages over HPLC have been discussed previously[1–3]. Current research has emphasized three areas: (1) development of low-volume, high-sensitivity detectors[4–7], (2) development of stationary phases and column technology for OTLC[8–11] and (3) the use of ultra-small open-tubular columns with inner diameters less than 20 μm[4,5,12]. Noticeably lacking, however, is the application of OTLC to natural samples.

Phenols have environmental importance, as they may be introduced into the environment by paper pulp processing, waste discharges, the decomposition of pesticides and herbicides, coal liquefaction and a variety of other routes[13,14]. The separation of alkylphenols has received considerable attention[13–17]. Difficulty is often encountered when attempting to separate alkylphenols by gas chromatography[14,17]. The high polarity and low vapor pressure at moderate gas chromatographic (GC) temperatures makes analysis difficult because both factors affect retention behavior. Separation does not follow any particular substitution pattern. Likewise, thin-layer liquid chromatography has proved to be inadequate for the separation of complex mixtures of alkylphenols[15]. HPLC is probably the preferred method for separation. In normal-phase HPLC, the separation of alkylphenols is based on substitution[13,15].

Di-*ortho*-substituted alkylphenols are eluted first, followed by mono-*ortho*-, and non-*ortho*-substituted compounds. Unfortunately, conventional analytical HPLC does not possess the efficiency required to separate isomers of nearly identical polarity such as 3-ethyl- and 4-ethylphenol[13,14]. The need for a more efficient HPLC system is apparent. In this work, an OTLC column coated with β,β'-oxydipropionitrile (BOP) was used in a normal-phase manner to achieve separations of complex mixtures of alkylphenols and to characterize the phenolic content of a coal-derived liquid.

EXPERIMENTAL

The apparatus used is shown in Fig. 1. A Haskel Model 27502 pneumatic amplifier pump (Haskel, Burbank, CA, U.S.A.) was used to deliver the mobile phase. A 25 cm × 1.2 mm I.D. microbore column packed with BOP-coated silica particles (5 μm) was used to saturate the mobile phase (hexane) with BOP and supply a suitable back-pressure to the constant-pressure pump. This system allowed microliter per minute flow-rates. A three-way tee between the pump and OTLC column allowed fine adjustment of the mobile phase flow-rate by tightening or loosening the male plug. The OTLC column was connected to the tee by sealing the column inside a short piece of 1.2 mm I.D. × 1/16 in. O.D. stainless-steel tubing with epoxy resin. A brass ferrule and male nut permitted leak-free column connections at operating pressures.

Samples were injected by an on-column injection procedure, similar to that described by Tsuda *et al.*[18]. A flame was used to heat 2–4 cm of the column near the inlet. The column end was immediately immersed in the sample solution. As the column cooled, the sample was drawn into the column. This eliminated band broadening effects introduced by the injector or by injector–column connections. For a 50 μm I.D. column the volume injected was approximately 50 nl.

Detection was accomplished by using a modified absorption system at 254 nm. The 254-nm line from a UV lamp was used as the illuminating source with a photomultiplier tube as the detector. Photocurrents were processed with a picoammeter and a home-made difference amplifier. For small absorbances (as is the case for this work) the output of the difference amplifier is linearly related to the solute concentration in the flow cell. Minimum detectable injection amounts for the phenolic compounds investigated in this work were in the low nanogram range.

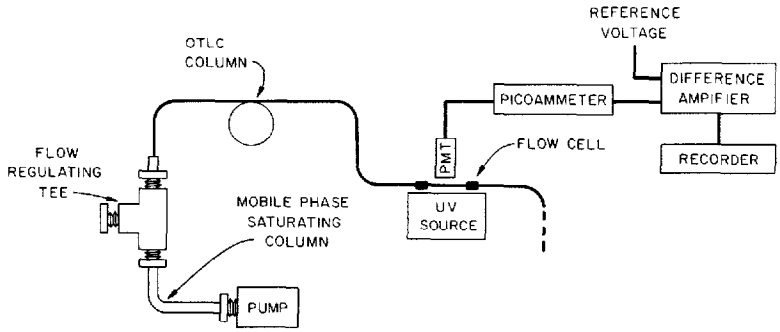

Fig. 1. Schematic diagram of OTLC system.

The detector flow cell consisted of a 0.20 mm I.D. × 0.33 mm O.D. quartz capillary tube (Vitro Dynamics, Rockaway, NJ, U.S.A.) with each end fixed with epoxy resin inside 22-gauge stainless-steel needle tubing. A short piece of heat-shrinkable tubing with a 1 mm long window cut in it was placed over the open center of the quartz capillary tube. The illuminated volume was about 30 nl. The column end was butted up against the detector cell and connected with heat-shrinkable tubing. A 1-m piece of 50 μm I.D. capillary tubing connected to the outlet side prevented degassing of the mobile phase in the detector cell. This detector cell was reused by carefully cutting the column connector with a razor blade and disconnecting it from the cell.

Glass capillary columns were drawn on a Shimadzu GDM-1 glass-drawing machine (Shimadzu, Kyoto, Japan) from standard flint-glass tubing supplied by Kimble Glass (discontinued). This standard flint glass is a low-melting glass with chemical properties similar to those of borosilicate glass. Columns were etched with gaseous hydrogen chloride at 15 p.s.i. for 2 h at 380°C. After flushing with nitrogen, the columns were coated with 100 μl of a 30% solution of BOP in dichloromethane. It was found that the capacity ratio, k', is affected by the percentage of BOP in the mixture and the total volume used for coating. After coating, the column was allowed to dry under nitrogen at 60°C for several hours. A 34 m × 50 μm I.D. OTLC column was used in this work, modified as described above.

A phenolic fraction of a coal-derived liquid was supplied by C. M. White (Pittsburgh Energy Technology Center, Pittsburgh, PA, U.S.A.).

RESULTS AND DISCUSSION

Band broadening effects and column efficiency

Column efficiency is inversely related to the height equivalent to a theoretical plate, H. The Golay equation relates H to the mobile phase linear velocity, u. The Golay equation for OTLC is given by[19]

$$H = \frac{2\,D_m}{u} + \frac{2k'd^2u}{3\,(1 + k')^2D_s} + \frac{(11k'^2 + 6k' + 1)\,d_c^2\,u}{96\,(1 + k')^2D_m} \quad (1)$$

where D_m and D_s are the diffusion coefficients of the solute in the mobile and stationary phases, respectively, d is the stationary phase thickness, d_c is the inner diameter of the capillary tube and k' is the capacity factor. Contributions to H from the first two terms, provided that d is small (as is usually the case), are normally small enough such that eqn. 1 is written as

$$H = H_m = \frac{(11\,k'^2 + 6k' + 1)\,d_c^2\,u}{96\,(1 + k')^2D_m} \quad (2)$$

where H_m is the contribution to H due to solute diffusion in the mobile phase.

In Fig. 2, H is plotted *versus* u for 2,6-dimethylphenol ($k' = 0.29$) and 2-ethylphenol ($k' = 1.05$). The broken lines represent those calculated from theory

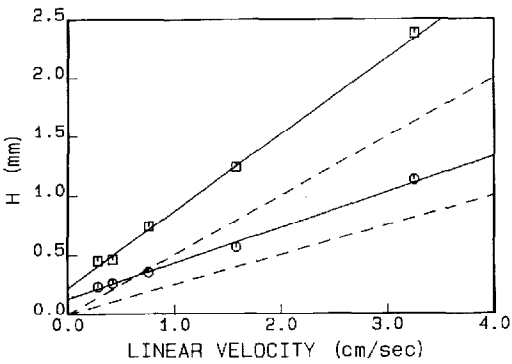

Fig. 2. Relationship between H and linear velocity. Column, 34 m × 50 μm I.D. coated with BOP; mobile phase, hexane saturated with BOP. Sample: 2-ethylphenol (□) ($k' = 1.05$) and 2,6-dimethylphenol (○) ($k' = 0.29$). The dashed lines are theoretical calculations.

using eqn. 2. The value of D_m is assumed to be $2 \cdot 10^{-5}$ cm²/sec. The experimental data are close to those calculated by theory. The lack of accurate D_m values is probably the greatest source of deviation. It can be concluded that the column has been efficiently coated.

Aside from column considerations, extra-column effects originating from injection and detection can add to the column peak variance (σ_c^2). The fractional increase in peak variance (θ) due to injection-related peak variance (σ_I^2) and detection-related peak variance (σ_D^2) can be written as

$$\theta = \frac{\sigma_I^2 + \sigma_D^2}{\sigma_c^2} = \frac{\sigma_I^2 + H_D A_D V_D}{H_c A_c V_c (k' + 1)^2} \quad (3)$$

where H, A and V are the plate heights, cross-sectional areas and volumes, respectively, of the detector flow cell (subscript D) and column (subscript C). The values of H_D (peaks are assumed to be unretained by the detector) and H_c can be calculated from previous equations. σ_I^2 is given as 0.08 V_I^2 where V_I is the volume injected[20]. Using the experimental parameters of this work, θ is 0.013 for 2-ethylphenol ($k' = 1.05$). Thus, the total increase in peak variance due to injection and detection for this system is less than 2%.

Separation of alkylphenols

The separation of dimethylphenol isomers by OTLC has previously been demonstrated[8,9,21]. In Fig. 3a, the separation of a complex mixture of twelve alkylphenols is demonstrated. Ogan and Katz[13] failed to separate the same twelve alkylphenols by either normal-phase or reversed-phase analytical HPLC. Only seven of the twelve compounds could be distinguished as separate, single-component peaks. Fig. 3a also reveals that all the components are baseline resolved or nearly so.

A more rapid separation of the same twelve alkylphenols is shown in Fig. 3b. The analysis time is reduced by more than half. All twelve of the compounds are easily distinguished, but many are no longer baseline resolved. This demonstrates the

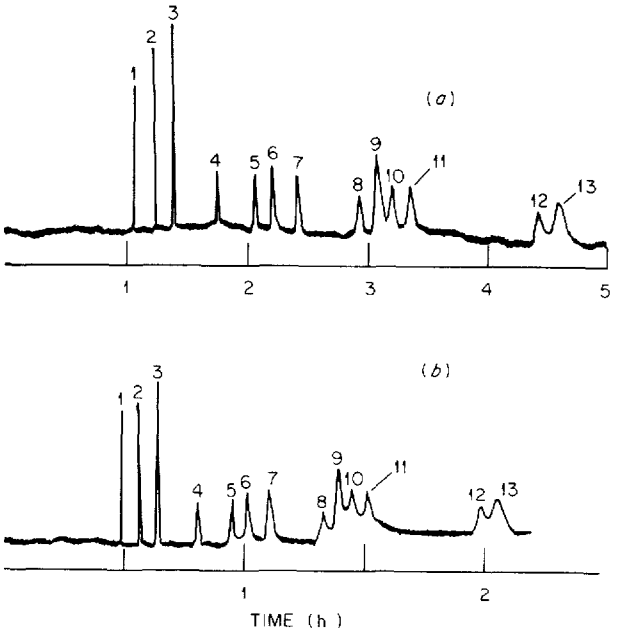

Fig. 3. Separation of alkylphenols. Column, 34 m × 50 μm I.D. coated with BOP; mobile phase, hexane saturated with BOP. Sample: 1 = solvent; 2 = 2,4,6-trimethylphenol; 3 = 2,6-dimethylphenol; 4 = 2,3,5-trimethylphenol; 5 = 2,4-dimethylphenol; 6 = 2-ethylphenol; 7 = 2,3-dimethylphenol; 8 = 3,5-dimethylphenol; 9 = 3,4-dimethylphenol; 10 = 3-ethylphenol; 11 = 2-methylphenol; 12 = 3-methylphenol; 13 = 4-methylphenol. Mobile phase linear velocity: (a) 0.83 cm/sec (1.0 μl/min); (b) 1.92 cm/sec (2.3 μl/min).

loss in efficiency at fast flow-rates as predicted by the data in Fig. 2. Lowering the flow-rates can also improve the column efficiency for packed columns. However, band broadening due to eddy diffusion can become significant or even limiting as the flow-rate is decreased in packed HPLC columns.

The three ethylphenol isomers can be separated by operating at very low flow-rates. Fig. 4 demonstrates this high-resolution separation. The resolution, R_s, for 3- and 4-ethylphenol is 1.0. Eqn. 2 indicates that the same resolution could be obtained in less than 1 h with a 20 μm I.D. column if the flow-rate is increased or the column length decreased by a factor of 6. Decreasing the column length is more desirable in terms of minimizing the column back-pressure.

Using this system, alkylphenol compounds could be tentatively identified in a phenolic fraction of a coal-derived liquid (Fig. 5). Identification is based on a comparison of k' values with those from an alkylphenol standard (see Table I). The k' values are nearly identical. No distinguishable peaks were eluted after phenol, *i.e.*, napthols, catechols, etc. No particular alkylphenol appears predominant in this sample. Very little background interference is observed (a common problem when analysing coal-derived liquid samples by HPLC).

White and Li[22] previously analysed the same fraction by capillary GC using a 30 m × 0.20 mm I.D. fused-silica capillary column coated with Superox-20 M, a poly(ethylene glycol) stationary phase. Twenty-nine phenolic compounds were identified, based on co-chromatography with standards and by GC–mass spectrometry.

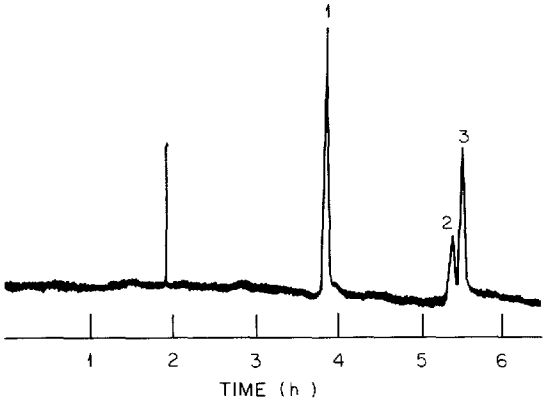

Fig. 4. High-resolution separation of ethylphenol isomers. Column, 34 m × 50 μm I.D. coated with BOP; mobile phase, hexane saturated with BOP; mobile phase linear velocity, 0.48 cm/sec (0.58 μl/min). Sample: 1 = 2-ethylphenol; 2 = 3-ethylphenol; 3 = 4-ethylphenol.

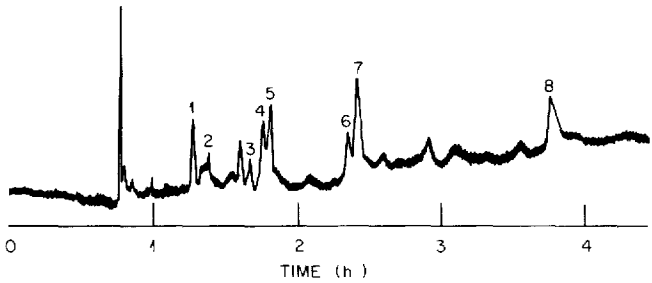

Fig. 5. Separation of alkylphenols in a coal-derived liquid by OTLC. Column, 34 m × 50 μm I.D. coated with BOP; mobile phase, 5% dichloromethane in hexane saturated with BOP; mobile phase linear velocity, 1.26 cm/sec (1.51 μl/min).

TABLE I

IDENTIFICATION OF ALKYLPHENOLS IN A COAL-DERIVED LIQUID

Chromatographic conditions as given in Fig. 5.

Peak No.	*k'*		*Identification*
	Sample	*Standard*	
1	0.65	0.63	2,4-Dimethylphenol
2	0.77	0.76	2-Ethylphenol
3	1.16	1.15	3,5-Dimethylphenol
4	1.26	1.26	3,4-Dimethylphenol
5	1.34	1.34	3-Ethylphenol
6	2.03	2.01	3-Methylphenol
7	2.10	2.10	4-Methylphenol
8	3.84	3.87	Phenol

All of the alkylphenol compounds tentatively identified here, with the exception of 2-ethylphenol, were identified by capillary GC. The use of a more sensitive detector in this OTLC apparatus (as well as a more complete collection of standard compounds) would aid in identifying the minor components of the sample. It is interesting that some of the alkylphenols are separated with better resolution by OTLC than capillary GC.

The ability of OTLC to separate a complex natural sample has been demonstrated. Separations of isomers were achieved that could not be achieved by conventional analytical HPLC. The apparent disadvantage in this experiment is the lengthy time of analysis. The use of smaller bore OTLC columns will allow the same separations to be accomplished in less time. Future work in this laboratory will focus on reducing the column dimensions and improving detector sensitivity.

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