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Note

Reversed-phase high-performance liquid chromatographic separation of substituted phenolic compounds with a β-cyclodextrin bonded phase column

C. ALLEN CHANG* and QIHUI WU
Department of Chemistry, University of Texas at El Paso, El Paso, TX 79968 (U.S.A.)
and
DANIEL W. ARMSTRONG
Department of Chemistry, Texas Tech University, Lubbock, TX, 79409 (U.S.A.)
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The high-performance liquid chromatographic (HPLC) analysis of phenols has been an active field due to its practical applications in biochemical, clinical, forensic, and wood chemistry as well as in food inspection and environmental pollution control. For example, Åkerblom and Lindgren[1] have separated *o*- and *p*-chlorophenol isomers using an octadecylsilica (ODS) column with a methanol–acetic acid mobile phase system. The United States Environmental Protection Agency has listed eleven priority phenolic pollutants with a variety of substituents such as chloro, methyl, and nitro groups. An investigation of a group of multi-substituted phenols by Buckman *et al.*[2] indicates that the more number of chlorine atoms on phenol, the longer the retention time, using an ODS column with an acetonitrile–water–acetic acid mobile phase system. Separations of several hydroxyphenols using a silica column[3] and a diamine bonded phase column[4] have also been reported. Despite all of the activities in the separation of phenols (in most cases, with ODS columns), no detailed study has been reported using the newly commercialized β-cyclodextrin bonded phase column.

It is noted that the initial effective use of cyclodextrins (CD) in chromatography was as modifiers in thin-layer chromatography (TLC)[5,6]. Polymerized CD gels were also used as stationary phases in column chromatography with varying degrees of success[7]. Attempts to prepare stable, fully derivatized high-performance CD-silica packings via different amine or ethylenediamine linkages were later reported by two Japanese groups[8–10]. However, problems such as poor hydrolytical stability, low cyclodextrin loading, low efficiency, and tedious synthetic procedures and poor reproducibility were encountered[11].

The newly commercialized β-CD-bonded phase column by Advanced Separation Technology is packed with 5-μm silica material covalently bonded with β-CD molecules. The β-CD molecule, a non-ionic, toroidal shaped and cyclic carbohydrate, is considered to be the host molecule chemically bonded to silica gel via a stable spacer (non-nitrogen-containing) 6 to 10 atoms in length[12]. In the hollow truncated cone, there are seven primary hydroxyl groups on the side with the smaller circum-

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ference and fourteen secondary hydroxyl groups, seven in clockwise directions and seven counterclockwise, on the side with the larger circumference[13]. Inside the cavity there are no hydroxyl groups which provides a hydrophobic environment. Because of the rigid cavity size of β-CD, only those guest molecules of proper size such as naphthalene can form strong β-CD inclusion complexes[14]. Molecules that do not form strong inclusion complexes with β-CD can also be separated by either a normal phase or a reversed-phase mode[15]. However, due to the hydrolytic stability of this particular β-CD-bonded phase it is often used with water–methanol eluent systems. The unique selectivities exerted by the β-CD column toward a large number of enantiomers[16] and its use for routine separations have warranted large numbers of applications. In this paper, we report its use for the efficient separation of various phenolic compounds.

EXPERIMENTAL

Apparatus

A Beckman 332 liquid chromatograph with two Altex 110 pumps and a microprocessor control unit together with a Micromeritics 786 variable-wavelength (200–600 nm) detector with a deuterium lamp was used. A Micromeritics Model 7500 LC system was also used. This latter system was equipped with a Model 750 solvent delivery system, a Model 752 ternary solvent mixer, and a Model 731 column compartment with a universal injector and a variable-temperature controller from ambient temperature to 150°C.

Pressure-Lok series C-160, 10-μl and 25-μl syringes were used for sample injection. Chromatograms were recorded with a Linear Model 555 single-channel recorder.

Materials

Partisil PXS ODS column, 5 μm, 25 cm × 4.6 mm I.D., from Whatman was used for reversed-phase separation. β-CD column, 25 cm × 4.6 mm I.D., was obtained from Advanced Separations Technology.

HPLC-grade methanol, 2-propanol, and *n*-heptane were obtained from Fisher Scientific. HPLC water (18 MΩ) was obtained by passing boiled deionized water through a Milli-Q Type I reagent-grade water system. All other chemicals were reagent grade and obtained from various sources.

Chromatographic procedures

The general procedures to study the effects of solvent composition were described in a previous publication with minor modifications[17]. Mobile phase pre-equilibration was always achieved before any separation occurred. A flow-rate of 1 ml/min was used. Published methods were used to determine the t_0 value (retention time of an unretained compound) for the β-CD column[16].

RESULTS AND DISCUSSION

Table I lists the capacity factors of several substituted phenols using a β-CD column with various methanol–water mobile phase systems. In general, the elution

TABLE I

CAPACITY FACTORS (k') OF SEVERAL SUBSTITUTED PHENOL ISOMERS IN A β-CD-COLUMN USING A METHANOL–WATER MOBILE PHASE SYSTEM

		Percentage of methanol in water (v/v)							
		10	*20*	*30*	*40*	*50*	*65*	*80*	*100*
Aminophenol	*o-*	1.08	0.76	0.57	0.41	0.28	0.17	0.13	0.11
	m-	0.72	0.51	0.37	0.24	0.14	0.10	0.08	0.08
	p-	0.59	0.43	0.29	0.19	0.10	0.08	0.08	0.07
Cresol	*o-*	2.60	1.87	1.11	0.61	0.33	0.12	0.04	0.03
	m-	3.22	2.51	1.37	0.68	0.40	0.15	0.06	0.03
	p-	4.87	3.64	2.19	1.12	0.61	0.25	0.08	0.05
Nitrophenol	*o-*	4.09	2.80	1.92	1.19	0.70	0.29	0.15	0.13
	m-	4.22	2.86	2.02	1.21	0.70	0.24	0.08	0.06
	p-	8.30	6.33	4.22	2.53	1.53	0.61	0.27	0.24
Chlorophenol	*o-*	5.33	3.08	2.20	1.06	0.54	0.22	0.06	0.04
	m-	6.92	5.10	2.99	1.43	0.72	0.29	0.07	0.05
	p-	8.05	5.85	3.64	1.87	0.96	0.33	0.09	0.06

order for the positional isomers of cresol and chlorophenol is always *ortho* < *meta* < *para* regardless of mobile phase composition. However, the sequence is *para* < *meta* < *ortho* for aminophenol isomers. On the other hand, the elution order for nitrophenols is *ortho* < *meta* < *para* when methanol in mobile phase is 10% or 15%. This changes into *meta* < *ortho* < *para* when mobile phases of 65–100% (v/v) methanol in water are used. These results are not quite the same as those of unmodified and modified CD columns prepared by the Japanese groups, because most of their studies on retention dependence on eluent composition stopped at 40% methanol in water, and their studies were also complicated by the presence of amine groups in the bonded phases[8–10].

If all compounds are considered, it is observed that *p*-aminophenol and *m*-aminophenol are eluted as the first two compounds among twelve, and *p*-chlorophenol and *p*-nitrophenol the last two, using 10–65% methanol–water mobile phase system. At higher concentration of methanol in water, *i.e.* 80–100%, most of compounds coelute with *o*-cresol and *m*-cresol showing the shortest retention times and *o*-aminophenol, *o*-nitrophenol, and *p*-nitrophenol as the last three. The best condition for the separation of all twelve compounds is when a mobile phase of methanol–water (10:90) is used. (See chromatogram in Fig. 1). Although not all the peaks are well resolved, the separation is considered most favorable as compared to those done by other systems. In fact, our preliminary test for the separation of the twelve compounds using either a silica column under a normal phase mode or an ODS column under a reversed-phase mode does not give satisfactory results. For example, Fig. 2 shows the poor separation of all twelve substituted phenols using a Partisil PXS ODS column with 10% 2-propanol–water (10:90, v/v) mobile phase system under simple optimized, isocratic binary solvent conditions. It should be noted that although further optimization of the separation can be performed on all separations, including

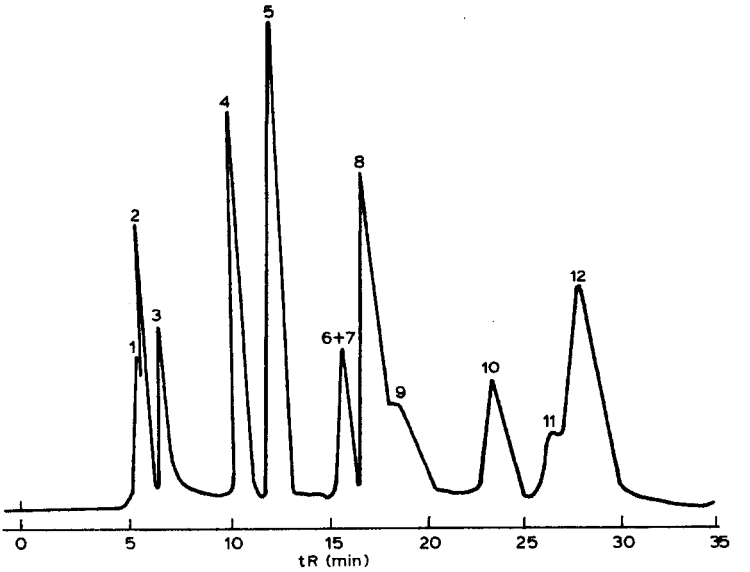

Fig. 1. Chromatogram for the separation of twelve substituted phenols, β-CD column, methanol–water (10:90), flow-rate = 1 ml/min. Peak identification: 1 = *p*-aminophenol, 2 = *m*-aminophenol, 3 = *o*-aminophenol, 4 = *o*-methylphenol, 5 = *m*-methylphenol, 6 = *o*-nitrophenol, 7 = *m*-nitrophenol, 8 = *p*-methylphenol, 9 = *o*-chlorophenol, 10 = *m*-chlorophenol, 11 = *p*-chlorophenol, 12 = *p*-nitrophenol.

the present separation of substituted phenols using a β-CD and an ODS columns, the intrinsic separation power of the β-CD column is clearly evident.

A specific feature involved in the separation using β-CD bonded phase is the possibility of inclusion process. A recent study by Chang *et al.*[18] proposed both vertical and flat inclusion configuration of several aromatic ligands and their organo-

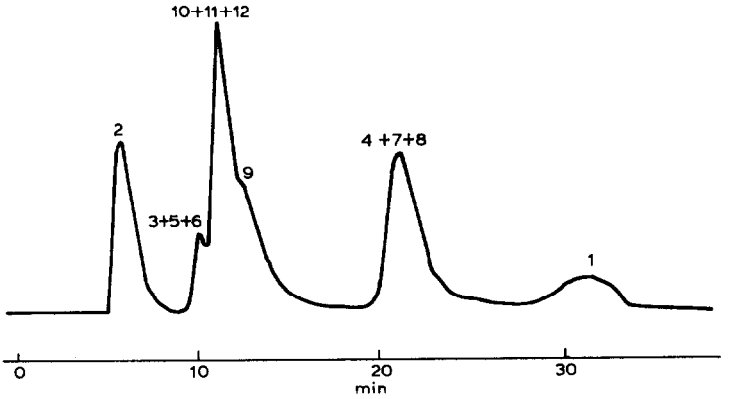

Fig. 2. Chromatogram for the separation of twelve substituted phenols, Partisil PXS ODS column, 2-propanol–water (10:90), flow-rate = 1 ml/min. Peak identification: 1 = *p*-aminophenol, 2 = *m*-aminophenol, 3 = *o*-aminophenol, 4 = *o*-methylphenol, 5 = *m*-methylphenol, 6 = *o*-methylphenol, 7 = *p*-chlorophenol, 8 = *m*-chlorophenol, 9 = *o*-chlorophenol, 10 = *p*-nitrophenol, 11 = *m*-nitrophenol, 12 = *o*-nitrophenol.

metallic compounds. In the present work, a reversed-phase behavior is observed for all phenolic compounds, *i.e.* retention time increases with increase of water content in the mobile phase. This is consistent with the inclusion phenomenon because the hydrophobic cavity of β-CD provides a reversed-phase environment. On the other hand, when 100% methanol is used as mobile phase, the inclusion process is probably not significant as evidenced by the fact that resolution of enantiomers cannot be achieved either[19]. In this case, normal phase behavior is probably dominant. Thus, mixed retention mechanisms are involved in the separation of phenols, similar to what has been found for the separation of several benzoic acids using a β-CD-bonded phase column[15]. This is advantageous when a complex separation is considered because one should be able to adjust the selectivity at a higher degree of freedom.

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