Reversed Phase Liquid Chromatographic Separation of Some Mono-Substituted Phenols with Calix[6]arene-*p*-sulfonate-Modified Eluents

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Key Words

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Mobile phase additive
Inclusion complexation

Summary

The utility of water-soluble calix[6]arene-*p*-sulfonate as a mobile phase additive has been investigated for the reversed phase liquid chromatographic separation of some monosubstituted phenol isomers. Retention factors and separation factors for regioisomers of methoxyphenol, aminophenol, and nitrophenol were measured using methanol – water and acetonitrile – water mobile phases of varying composition containing calix[6]arene-*p*-sulfonate, and the values were compared with those obtained using mobile phases containing no additive. It was observed that addition of calix[6]arene-*p*-sulfonate to both acetonitrile - water and methanol - water caused a reduction in the retention of the phenol isomers but generally increased the separation between them, thereby improving the overall separation efficiency.

Introduction

Calixarenes are cavity-shaped cyclic molecules made up of phenol units linked *via* alkylidene groups which can act as receptor molecules of widely varying size for metal cations and organic molecules [1]; because they possess a cylindrical architecture similar to that of cyclodextrins, they are expected to form inclusion complexes. Cyclodextrins (CDs) have been extensively employed in liquid chromatographic separations [2-9] because of their ready accessibility and rather unique properties in comparison with micellar inclusion [10]: unlike micelles, CDs operate by a single solubilization mechanism over the entire concentration range. As mobile phase modifiers, CDs possess the advantage over micelles in that they do not foam when purged with nitrogen.

Ready accessibility also accounts for the increasing attention received by calixarenes during the past decade [1,11,12]. Little work has, however, been performed to investigate the utility of calixarenes in chromatographic separation. To the best of our knowledge the only work on the subject is that reported by Ungaro et al. [13] who separated alcohols, chlorinated hydrocarbons, and aromatic compounds by gas-solid chromatography with *p-tert*-butylcalix[8]arene absorbed on Chromosorb. No studies of the use of calixarenes in reversed phase liquid chromatographic separations have yet been reported, mainly as a result of the insolubility of most of the calixarenes in water. Shinkai et al. have recently synthesized a number of water-soluble functionalized calixarenes and shown that they form complexes with a variety of organic molecules in aqueous systems [14-21]. It is thought that water-soluble calixarenes can thus be utilized as mobile phase additives or chemically bonded stationary phases in liquid chromatography.

In this work we report the use of calix[6]arene-*p*-sulfonate [16] (abbreviated CAPS) as mobile phase additive in the reversed phase liquid chromatographic (RPLC) separation of some substituted phenolic compounds, in order to determine whether water-soluble calixarenes can be utilized as effectively as CDs in RPLC separations. CAPS was chosen from among other water-soluble calixarenes since it can be readily synthesized and has a cavity of depth 3 Å and diameter 7.6 Å [22], similar to that of β-CD (cavity depth, 7 Å; diameter, 6-8 Å) which has been most widely utilized in liquid chromatography [3].

One drawback of using calixarenes as mobile phase additives is that they are not transparent in the UV region and the strong UV absorption essentially precludes the use of UV absorption detectors. This suggests that calixarenes should be used as chemically bonded stationary phases, which would enable the use of the more convenient and sensitive UV detector. If CAPS is found useful in the separation of phenol isomers, the covalent bonding of CAPS to a porous support can be pursued.

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Experimental

Chemicals

The chemicals used for the synthesis of calix[6]arene-*p*-sulfonate were reagent grade obtained from various sources and used without further purification. Phenolic compounds for chromatographic measurements were purified as described elsewhere [23]. Methanol and acetonitrile were of HPLC grade and purchased from Ajax (Auburn, Australia). HPLC water (18 MΩ) was obtained by passing boiled deionized water through a Milli-Q type I reagent-grade water system.

Synthesis of Calix[6]arene-*p*-sulfonate

Calix[6]arene-*p*-sulfonate was synthesized according to the method of Shinkai et al. [16]; the structure of the compound was confirmed by IR and NMR spectrometry, and elemental analysis.

Chromatography

Retention measurements on an octadecylsilane (ODS) column (μ-Bondapak C-18, 3.9 × 300 mm, 10μm, Waters, Milford, USA) were obtained with an HPLC system comprising a Waters Model 510 pump, a Rheodyne injector (Model 7125) equipped with 10 μL sample loop, and a Waters Model R-400 differential refractometer. Chromatograms were recorded with a Hewlett-Packard 3396 Series II integrator.

The column was placed in a water jacket, and the temperature was controlled at 30 ± 0.2 °C. The eluents used were methanol - water or acetonitrile – water in different proportions. Mobile phases containing CAPS were obtained by dissolving CAPS in the methanol – water or acetonitrile – water mixtures at a concentration of 10^{-3} M. The concentrations of the phenolic solutes dissolved in the eluents were half that of CAPS in order to ensure that the latter was in excess. Mobile phase preequilibration was always achieved before any separation was performed. The eluent flow rate was 1 ml/min. The column void volume was determined by injection of methanol. Capacity factors calculated from the mean retention times obtained from injections in triplicate are listed in Table I.

Results and Discussion

Inspection of the capacity factors of phenol isomers listed in Table I indicates that all the phenols studied were less retained in mobile phases containing CAPS than in unmodified mobile phases. The formation of inclusion complexes between host and guest molecules is mainly responsible for the reduction in solute retention: it is believed to be a consequence of the increased solubility of the phenols in the mobile phases as a result of the formation of inclusion complexes with CAPS [16]. The reduction of phenol retention observed in the CAPS-containing aqueous methanol decreases in the order methoxy < amino < nitro. A similar trend is observed in acetonitrile - water system. This indicates that interactions between CAPS and phenols in the inclusion complexes are affected by the type of the substituent on the phenols.

Table I Capacity factors measured for phenol isomers with methanol – water and acetonitrile – water mobile phases.

		Methanol – Water		Methanol – water + CAPS		Acetonitrile – water		Acetonitrile – water + CAPS	
		40/60	50/50	40/60	50/50	40/60	50/50	40/60	50/50
Methoxyphenol	*o-*	2.34	1.56	2.32	1.17	2.36	1.96	1.54	1.23
	m-	2.41	1.57	1.58	0.95	2.29	1.91	1.19	0.87
	p-	1.90	1.31	1.88	1.04	1.96	1.64	1.34	1.09
Aminophenol	*o-*	1.58	1.56	0.97	0.81	1.53	1.46	0.08	0.03
	m-	0.99	0.80	0.71	0.49	1.52	1.04	0.67	0.59
	p-	1.32	0.87	0.56	0.45	1.15	0.95	0.55	0.37
Nitrophenol	*o-*	4.26	2.39	0.97	0.77	2.78	2.01	0.84	0.52
	m-	3.70	2.05	0.70	0.39	2.12	1.56	1.20	0.81
	p-	3.43	1.75	0.14	0.07	1.92	1.46	0.21	0.15

Table II Separation factors for closely eluted phenol isomers.

		Methanol – Water			Methanol – water + CAPS			Acetonitrile – water			Acetonitrile – water + CAPS	
		40/60	50/50		40/60	50/50		40/60	50/50		40/60	50/50
Methoxyphenol	*m/o*	1.03	1.00	*p/m*	1.19	1.10	*o/m*	1.03	1.02	*o/p*	1.15	1.12
	o/p	1.23	1.19	*o/p*	1.23	1.12*	*m/p*	1.17	1.17	*p/m*	1.12*	1.26
Aminophenol	*o/p*	1.19	1.82	*o/m*	1.38	1.64*	*o/m*	1.00	1.40	*p/o*	6.96	13.07
	p/m	1.34	1.08	*m/p*	1.25*	1.10	*m/p*	1.32	1.09	*m/p*	1.22*	1.58
Nitrophenol	*o/m*	1.15	1.16	*o/m*	1.38	1.96	*o/m*	1.31	1.29	*m/o*	1.43	1.55
	m/p	1.08	1.17	*m/p*	4.85	5.60	*m/p*	1.10	1.07	*o/p*	3.93	3.51

*separation factor is reduced in the CAPS-containing eluent

With the exception of nitrophenols in the methanol - water system, changes in the order of elution of the phenol isomers are also observed upon addition of CAPS to the mobile phases. It is speculated that changes in elution order are also related to differences in the stability of the inclusion complexes formed between CAPS and the different phenol isomers, although mechanistic aspects of the chromatographic behaviour of CAPS remain to be investigated by measurement of formation constants for inclusion complexation of CAPS with the phenol isomers.

Notwithstanding that the phenols were less retained when the CAPS-modified mobile phases were used, separation factors between closely-eluted phenol isomers were, in general, much improved in comparison with those obtained using unmodified eluents (Table II). Only for five of twenty four closely-eluting pairs of isomers did the separation factor decrease slightly. *Ortho* and *meta* methoxy phenols are hardly separated by methanol - water eluents but all three isomers were better resolved in the presence of CAPS. The selectivity for the separation of nitrophenol isomers, in particular, was greatly improved.

These results indicate that CAPS and possibly other water-soluble calixarenes can be effectively utilized in RPLC separation of monosubstituted phenol isomers. As noted above, strong UV absorption of calixarenes in the mobile phases means that the use of the more convenient and sensitive UV detector will be enabled only by use of the calixarenes as chemically bonded stationary phases. Work is in progress in our laboratory to develop such phases.

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