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# High-performance liquid chromatographic analysis of sulfonated aromatics using a β-cyclodextrin-bonded phase

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**ABSTRACT**

**A cyclodextrin-bonded phase and methanol-aqueous ammonium acetate mobile phase were used in the liquid chromatographic analysis of a number of sulfonated aromatics. This separation system offered two advantages over the more conventional ion-pair approach to the liquid chromatographic analysis of these highly polar compounds. It achieved the resolution of positional isomers of sulfonated aromatics and allowed the use of thermospray liquid chromatography/mass spectrometry for the characterization of these compounds. Chromatographic selectivity for these solutes was found to be most significantly influenced by the ionic strength of the mobile phase mixture.**

## INTRODUCTION

Sulfonated aromatics are a group of polar, water-soluble compounds that are difficult to analyze by liquid chromatography. The conventional means for separating highly ionized compounds of this type is ion exchange or ion-pair chromatography [1]. These techniques will adequately perform separation by classes, e.g., mono- from disulfonated substituted aromatics, but may not be able to resolve positional isomers of sulfonated aromatics.

Cyclodextrin-bonded phases have demonstrated an often remarkable selectivity in the liquid chromatographic separation of isomers [2–7]. In particular, β-cyclodextrin-bonded phases have proven to be especially useful for the resolution of positional isomers of a variety of disubstituted aromatics, including aromatic carboxylic acids [8–12]. The same sterically driven separation mechanism that resolves non-ionized disubstituted aromatics might also be expected to separate positional isomers of sulfonated aromatics.

This study reports the use of a conventional reversed-phase mobile phase system (a methanol-0.1 *M* ammonium acetate mixture) and a β-cyclodextrin column to accomplish the non ion-pair separation of sulfonated aromatics. This system possesses two advantages when compared to the ion-pair separation of this class of compounds. It achieves the separation of positional isomers of substituted sulfonated benzenes -a separation not obtained with the ion-pair, reversed-phase system. The second advantage arises from the use of the aqueous ammonium acetate buffer as a constituent of the mobile phase mixture. This mobile phase is the optimal choice for the use of mass spectrometric detection using the thermospray interface and so permits the on-line structural characterization of sample components.

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## EXPERIMENTAL

### *Apparatus*

The β-cyclodextrin column used in this work was a 250 mm x 4.6 mm Cyclobond I column from Advanced Separations Technologies. A Hewlett-Packard Model 1090 liquid chromatograph equipped with autoinjector and column oven was used for solvent delivery. All data were acquired using a column temperature of 35 ± 0.1°C. Sample injection size was 10 μl.

Two modes of detection were used. Monitoring of UV absorbance at 220 nm was the primary routine means of detection. This was accomplished using a Hewlett-Packard 1040 diode array detector. The ability to acquire the UV spectra of each of the components was utilized in arriving at the optimal wavelength, 220 nm, for the analysis. The peak purity function of the detector was also used to assure that no unresolved components were present. The acquisition and storage of individual spectra proved to be particularly useful in the verification of peak identity during the separation optimization portion of this work. Elution order changes made it imperative that some means of definitively identifying each component peak be employed. In addition, the UV spectra assisted in subsequent structural characterization of several unknown sample components. A Finnigan model 4600 mass spectrometer with a Vestec thermospray LC-MS interface constituted the second means of detection employed in this work. It was used to provide mass spectral data to help definitively identify unknown sample components. The interface was used in the discharge mode and positive ions were monitored to produce the mass chromatogram.

### *Chemicals and reagents*

All sulfonated samples were produced at Eastman Chemicals. Reference samples of *ortho* and *para* sodium phenolsulfonate and 4,4′-dihydroxydiphenolsulfone were laboratory prepared and purified and characterized by NMR.

The methanol, ammonium acetate and acetic acid used to prepare mobile phase mixtures were obtained from J.T. Baker (Phillipsburg, NJ, USA) and were "HPLC" grade. Millipore (Milford, MA, USA) Milli-Q filtered, deionized water was used in the ammonium acetate and acetate buffer solutions.

## RESULTS AND DISCUSSION

Sodium phenolsulfonates (SPSs) are useful industrial intermediates. A knowledge of the levels of the three positional isomers in reaction

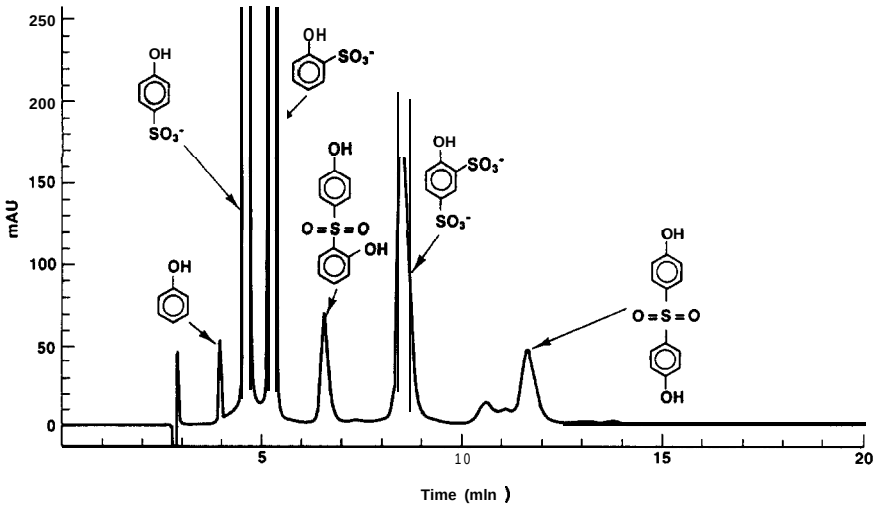


Fig. 1. Chromatogram of a reaction mixture sample of sodium phenolsulfonate. Conditions: column, 250 × 4.6 mm I.D. Cyclobond I; mobile phase, methanol-O.1 M aqueous ammonium acetate (4357); temperature, 35°C; flow-rate, 1 ml/min; detection, UV at 220 nm.

samples of sodium phenol sulfonates, as well as the amount of disulfonated material and residual phenol, is important in optimizing the reaction to produce these materials. A liquid chromatographic method was developed to provide information for process control and product purity determinations. Fig. 1 presents the chromatogram of a typical crude reaction mixture sample containing SPSs and related impurities. It can be seen that the *para-* and *ortho-*SPS isomers are well resolved from each other and from phenol, disulfonated phenol (di-SPS), and two sulfone impurities. This chromatogram was obtained using a mobile phase mixture of methanol-O. 1 *M* ammonium acetate in water (43:57) at a flow-rate of 1.0 ml/min. These conditions were optimal in terms of speed of analysis and resolution of components. Verification of known sample components as well as identification of unknown impurities in the reaction mixture sample was obtained using LC-MS with a thermospray interface. The choice of ammonium acetate as the buffer was partially dictated by the need for verification of peak identity by MS. A buffer system containing a volatile component, like ammonium acetate, is necessary for thermospray LC-MS. In the course of achieving this optimized separation, it was observed that the retention of three classes of components of interest -monosulfonated phenols, disulfonated phenol and the sulfones— were affected differently by changes in the ionic strength of the mobile phase mixture, percentage of methanol in the mobile phase and temperature.

### *Effect of buffer ionic strength*

The most striking change in retention behavior was produced by changing the ionic strength of the mobile phase. Ionic strength was adjusted by the addition of ammonium acetate to the aqueous mobile phase component to provide concentrations from 0.005 to 0.300 *M. The* effect on retention time for three of the classes of compounds in this separation, *i.e.,* monosulfonated phenols, disulfonated phenols and sulfones, vs. concentration of ammonium acetate can be seen in Fig. 2. It may be seen from the plot that a decrease in concentration of ammonium acetate, and hence a decrease in the ionic strength of the

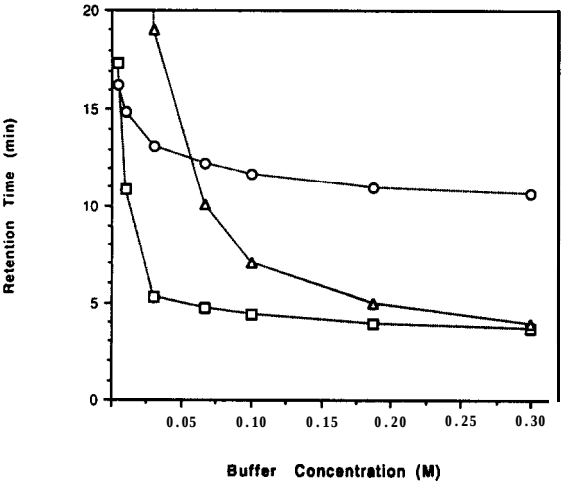


**Fig. 2. Variation of retention time with change in ammonium acetate buffer concentration. Column, Cyclobond I; mobile phase, methanol-ammonium acetate buffer (4357) at different concentrations; temperature, 35°C; flow-rate, 1 ml/min; detection, UV at 220 nm; Cl =*para-SPS;* A = 2,4-di-SPS; 0 = 4,4′-bis-diphenolsulfone.**

mobile phase, produces an increase in retention time for each of the three solute classes. The increase is most pronounced for the ionizable solutes with the disulfonated phenol showing a greater effect than the monosubstituted phenol. At ammonium acetate concentrations below 0.02 *M,* the disulfonated phenol is essentially noneluted. The monosulfonated phenols show a qualitatively similar behavior, but still may be eluted (although with a retention time of 17 min) at an ammonium acetate concentration of 0.005 *M. The* uncharged bis-sulfone displays a much smaller change in retention time with change in buffer concentration. Recent work by Beeson and Vigh [13] has shown that ionized carboxylic acids may be more strongly retained than the unionized acids on a β-cyclodextrin bonded phase. This is in agreement with our findings in this study.

### *Effect of methanol content*

*The* plot of change in retention time with change in methanol cosolvent content at a fixed buffer concentration (0.1 *M)* is shown in Fig. 3. No attempt was made to keep the buffer ionic strength constant; that is, the addition of methanol was allowed to effectively dilute the

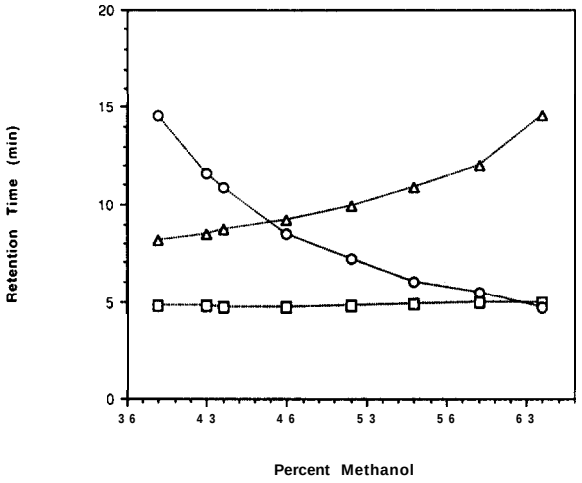


**Fig. 3. Variation of retention time with change in percent methanol. Column, Cyclobond I; mobile phase, methanol at different concentrations-O.1 *M* ammonium acetate buffer; temperature, 35°C; flow-rate, 1 ml/min; detection, UV at 220 nm; □ = p-SPS; A = 2,4-di-SPS; 0 = 4,4′-bis-diphenolsulfone.**

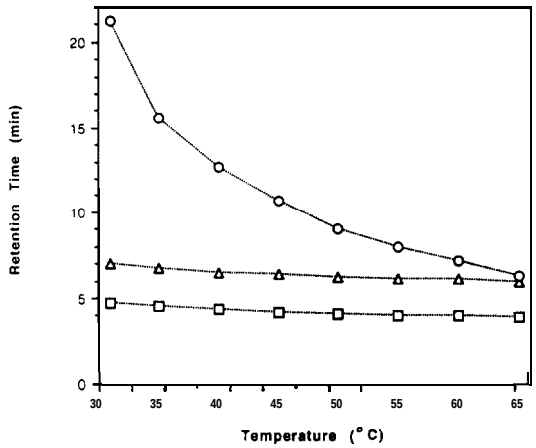


**Fig. 4. Variation of retention time with change in temperature. Column, Cyclobond I; mobile phase, methanol-O.1 M ammonium acetate buffer (43:57); temperature, 31–65°C; flow-rate, 1 ml/min; detection, UV at 220 nm; Cl = *p*-SPS; A = 2,4-di-SPS; 0 = 4,4′-bis-diphenolsulfone.**

aqueous ammonium acetate buffer concentration. It may be seen that the effect of methanol on retention time is less dramatic than that seen in Fig. 2 when buffer ionic strength was adjusted. An interesting trend is seen, however, in the different response obtained for the sulfone vs. sulfonic acid-containing solutes. The sulfone behaves as one would expect when an uncharged solute molecule is subjected to a "stronger" eluent, i.e., one richer in methanol; its retention time decreases. The retention times for the sulfonated solutes increase and the increase is roughly consistent with the dilution of the ammonium acetate buffer (and hence dilution of ionic strength) by methanol.

*Effect of temperature*

The effect of temperature on retention was also investigated. Fig. 4 displays a plot of temperature vs. retention time of the three solute classes. The neutral sulfone undergoes the largest change in retention time, with the charged solute classes relatively less affected by temperature.

These data suggest that the neutral sulfone solute behaves according to accepted and well documented theory for the inclusion complexation of solutes in the cyclodextrin cavity. The equilibrium for this inclusion complexation is shifted, as expected, when the mobile phase environment is altered by the change in the methanol content and change in temperature. The retention mechanism for the sulfonated solutes, however, is less straightforward and appears to be dominated by a quantitatively stronger interaction, perhaps ion exchange. It has been documented that ions may be separated on a $\beta$-cyclodextrin bonded phase [14,15]. It is not clear from the data whether this effect works in concert with the conventional complexation mechanism or is an extraneous artifact produced by ion-exchange groups on the surface of the silica gel. The fact that we are able to separate positional isomers of the sulfonated phenol suggests at least some contribution to retention and resolution from the cyclodextrin moiety. The preparation of the bonded phase does not involve the use of potentially ionizable, e.g., amine, functionalities that may act as fixed ion-exchange sites [16]. It is possible that a dynamic ion exchange mechanism may be involved. This might arise through inclusion of cationic constituents of the mobile phase -in the present case ammonium ion— in the cyclodextrin cavity, which then acts as a partially encapsulated ion-exchange site. We have not found literature

evidence for a strong ion-exchange effect internal to the cyclodextrin cavity -one in which a charged mobile phase ion (for example, ammonium) is trapped in the cyclodextrin cavity— but the results obtained in this work makes such an explanation attractive.

### *Effect of stronger electrolyte*

To further explore the possibility of an ion-exchange retention mechanism for the sulfonic acid-containing solutes, the use of a stronger (in the context of ion exchange) mobile phase electrolyte was examined. Ammonium sulfate replaced ammonium acetate in the aqueous portion of the mobile phase solution. Theory indicates that sulfate should have more affinity for a hypothetical anion-exchange site than would acetate and so sulfate would be less easily displaced than acetate by sulfonate-containing solutes [17]. This would result in a reduction of retention time for the sulfonated solutes. Fig. 5 shows retention of the three classes of solutes as a function of ammonium sulfate concentration. The retention times for the solutes are diminished at a given electrolyte concentration compared to the ammonium acetate mobile phase. The difference is particularly well displayed for

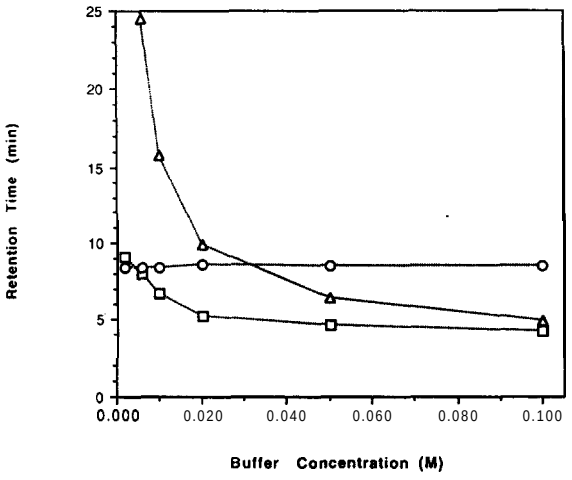


**Fig. 5. Variation of retention time with change in ammonium sulfate buffer concentration. 'Column, Cyclobond I; mobile phase, methanol-ammonium sulfate buffer (4357) at different concentrations; temperature, 35°C; flow-rate, 1 ml/min; detection, UV at 220 nm; □ = *p*-SPS; A = 2,4-di-SPS; 0 = 4,4'-bis-diphenolsulfone.**

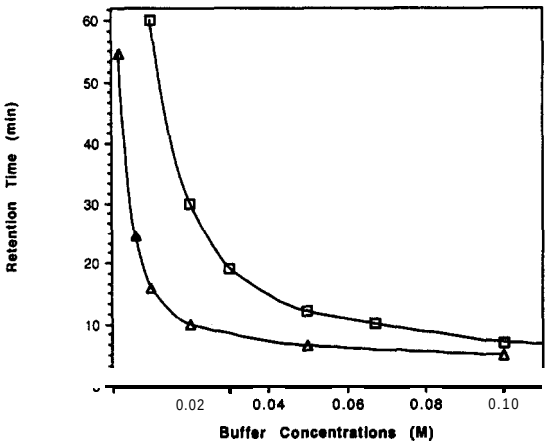


**Fig. 6. Comparison of retention time for 2,4-disulfonated phenol in ammonium acetate vs. ammonium sulfate buffer. Column, Cyclobond I; mobile phase, methanol-buffer (4357) at different concentrations; temperature, 35°C; flow-rate, 1 ml/min; detection, UV at 220 nm; □ = acetate; A = sulfate.**

the 2,4-disulfonated phenol solute in Fig. 6. As mentioned above, these data are consistent with an ion-exchange mechanism where the stronger sulfate anion should compete more favorably with the sulfonic acid-containing solutes for the anion-exchange sites.

### *Effect of different counter-ions*

*The* effect of different counter-ions on retention was also investigated. All other conditions in this investigation were unchanged and were the same as that used to achieve the optimal separation, ***i.e.,*** 57% methanol, temperature, 35°C, flow-rate, 1 ml/min. Changing the cation from ammonium to sodium produced very little change in retention behavior. The substitution of potassium for ammonium, however, had a greater effect. No change in the retention of the neutral sulfones was seen, but the retention times of sulfonated solutes were reduced, with the effect on the disulfonated phenol being the greatest. Its retention time was reduced from 8.5 to 6.8 min.

## CONCLUSIONS

This work has demonstrated the utility of a cyclodextrin-bonded phase for the separation of

highly ionized organic solutes. The aqueous ammonium acetate-methanol mobile phase system is particularly well suited to detection and characterization of analyte by thermospray LC–MS. Finally, a qualitative difference in the retention mechanism for neutral vs. ionizable solutes was observed. An analysis of the influence of ionic strength, percent methanol and temperature on solute retention suggests that an ion-exchange-like mechanism may be superimposed with inclusion complexation in the chromatographic retention of the sulfonated phenols.

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