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SEPARATION OF *m*- AND *p*-CRESOL AND OTHER METHYLATED PHENOLS IN SHALE OIL BY REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

A novel reversed-phase approach is described for the separation of phenol, the cresols, the xylenols, and other methylated phenols in real samples such as shale oil. The approach, based on the fundamental optimization of mobile phase pH to provide enhanced selectivity, enables *m*-cresol and *p*-cresol and many xylenol isomers to be baseline resolved for the first time in a reversed-phase analysis of shale oil. Selectivities obtained with our reversed-phase method for the difficult-to-separate isomers are compared with those observed using other reversed-phase methods. The moderate oxidation potentials of the phenols allows them to be detected selectively at the unusually high (but optimum) pH of 10.82 in the presence of redox-active and redox-inactive interferents. Although unnecessary with our chromatographic method, *m*-cresol and *p*-cresol can also be resolved electrochemically via judicious control of the working electrode potential.

INTRODUCTION

Phenols constitute a large, diverse class of compounds that are found in both natural and man-made environments. Some are present in plants as building blocks and metabolites; others are used in the chemical industry as raw materials for the manufacture of insecticides, herbicides, pharmaceuticals, as well as for various types of phenol–formaldehyde products.

An important sub-class of phenols are the methylated phenols, in particular the cresols and xylenols, some of which are recognized as priority pollutants by the United States Environmental Protection Agency[1]. As noted in an extensive review[2] and elsewhere[3–7], a variety of methods, including colorimetry, gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), normal-phase liquid chromatography (NPLC), and LC with cyclodextrin stationary phases[8] have been applied to the separation and determination of the cresol and xylenol isomers in a wide range of samples.

One very popular LC method that, until now, has not been very successful for the separation of the mono- and dimethylphenols, is reversed-phased liquid chro-

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matography (RPLC)[2]. This should come as no surprise since RPLC in general is not nearly as good as normal-phase high-performance liquid chromatography (HPLC) in separating geometrical isomers[9]. To our knowledge, no RPLC separation of *m*-cresol and *p*-cresol has ever been achieved in a real sample, and separation of many xylenol isomers have been marginal at best. Despite the formidable challenge, however, an RPLC separation of the cresols and xylenols is highly desirable because (i) it would not suffer the drawbacks occasionally or frequently observed with the other methods; and (ii) it would provide an independent method of analysis in instances where more than one method is required to ensure validation. Drawbacks of the other methods include (but are not limited to): the peak tailing of the (underivatized) phenols commonly observed in GC and NPLC with silica stationary phases, the incompatibility of GC and NPLC mobile phases with aqueous samples, and the low peak capacity/plate count of cyclodextrin bonded phases.

In a previous report[10], we presented preliminary results for the first RPLC separation of *m*-cresol and *p*-cresol and many difficult-to-separate xylenol isomers, using a synthetic mixture of those compounds to illustrate the feasibility. In the present work, we discuss more fully the optimization of this separation and various aspects of the electrochemical detection of these compounds, and show that our reversed-phase method can be applied to their separation in real samples such as shale oil. The selectivities obtained with our reversed-phase method for the difficult-to-separate isomers are compared with other reversed-phased methods.

EXPERIMENTAL

LC system

A ternary liquid chromatograph (Rainin Instruments, Woburn, MA, U.S.A.) was used, along with a Model 7125 injection valve (Rheodyne, Cotati, CA, U.S.A.) with 20-μl loop, a Model LC-4B thin-layer electrochemical detector (Bioanalytical Systems, Lafayette, IN, U.S.A.), and a Model CR-3A computing integrator (Schimadzu, Kyoto, Japan). In some instances a Model V[4] UV–VIS absorbance detector (Isco, Lincoln, Nebraska, U.S.A.) was also employed at a wavelength of 280 nm. A 150 × 4.1 mm I.D. PRP-1 column (Hamilton, Reno, NV, U.S.A.) was used throughout the study. It was washed periodically with 100% acetonitrile to remove any build-up of highly retained compounds that might have been present along with the phenols in our standards or in the shale oil samples. The column was thermostated at 25.0 ± 0.1°C using a glass water jacket and a Model RMS-6 circulating bath (Brinkmann Instruments, Westbury, NY, U.S.A.).

Mobile phase preparation

Our mobile phase consisted of an aqueous sodium carbonate–sodium bicarbonate buffer and 30% acetonitrile. The 25 m*M* solutions of sodium bicarbonate and sodium carbonate were segregated so that the pH of the mobile phase could be changed by varying the proportions of the two solutions. The sodium bicarbonate solution also contained 50 m*M* sodium perchlorate so that the ionic strength would remain constant as the pH was varied (*ca.* 50 m*M* after the buffer was mixed with acetonitrile). All mobile phase solutions were filtered through 0.22-μm Nylon-66 filters (Rainin Instruments, Woburn, MA, U.S.A.) before use. Subsequent degassing

of the mobile phase, for use with the electrochemical detector, was accomplished by placing the solutions in a heated ultrasonic bath and then applying a partial vacuum for just a few minutes. Continous degassing (*e.g.*, by sparging with helium) was avoided in order to minimize loss of the carbonate buffer.

Reagents and standards

Mobile phase components acetonitrile and water were HPLC-grade, as was the sodium carbonate and sodium perchlorate. The sodium bicarbonate was ACS reagent grade. All chromatographic solutes were reagent grade or better and used without further purification. They were dissolved either in the aqueous buffer or in a minimal amount of acetonitrile and then diluted with the aqueous buffer. For the optimization of the separation, mixtures of standards were made to give a signal of *ca.* 40 nA or 0.05 a.u. for each peak. For the standard solutions used to estimate the linear dynamic range and the limit of detection, concentrations ranged from about 10 to 1000 μg/g (0.2 to 20 ng injected).

Measurement of pH and limiting capacity factors

A Model 920 research grade pH meter (Orion, Cambridge, MA, U.S.A.) was calibrated at pH 7.00 and then at pH 10.00 before measuring the pH of the mobile phase to within 0.01 pH unit. Limiting capacity factors (k') were measured as described previously[11] and were subsequently used to determine the optimum pH for the separation (*vide infra*).

Sample preparation

The shale oil sample was prepared according to the method of MacCrehan and Brown-Thomas[6]. This procedure, which takes less than 15 min, consists of a liquid–liquid extraction followed by a solid phase extraction.

RESULTS AND DISCUSSION

The secondary equilibrium/high pH approach

Because of the difficulties previously encountered for the separation of *m*- and *p*-cresol and other methylated phenolic isomers by RPLC[2], a new approach was taken. In our study, we used a mobile phase pH sufficiently high (10.82) to partially ionize the phenolic compounds, which have pK_a values ranging from 9.98 to 10.89. By exploiting this secondary acid–base equilibrium in the mobile phase, a dramatic improvement in selectivity and resolution was achieved. Due to the high mobile phase pH, traditional silica-based reversed-phase stationary phases could not be employed, and a macroporous polystyrene–divinylbenzene copolymer (PS–DVB) stationary phase was used instead. The PS–DVB phases exhibit reversed phase retention characteristics and are stable over a pH range of 1 to 13.

Optimization

The mobile phase pH was optimized by use of the following equation[10]:

$$\mathrm{pH_{opt}} = \mathrm{p}K_{\mathrm{avg}} + \tfrac{1}{2}\log(k'_{\mathrm{HA}}/k'_{\mathrm{A}^-})_{\mathrm{avg}} \quad (1)$$

TABLE I

OPTIMIZATION OF MOBILE PHASE pH FOR THE SEPARATION OF PHENOLIC COMPOUNDS

No.	*Compound**	pK_a**	k'_{HA}	k'_{A^-}	*Optimum pH****
1	Phenol	9.98	3.94	0.25	
2	3-Methylphenol	10.09	7.46	0.50	10.71
3	4-Methylphenol	10.26	7.10	0.59	10.82
4	2-Methylphenol	10.26	9.07	0.92	10.87
5	2,5-Dimethylphenol	10.40	17.45	1.89	10.91
6	2,3-Dimethylphenol	10.54	15.39	2.49	11.00
7	2,4-Dimethylphenol	10.59	15.48	2.72	11.04
8	2,6-Dimethylphenol	10.62	18.81	3.03	11.08
9	2,3,5-Trimethylphenol	10.67	29.08	5.23	11.12
10	2,4,6-Trimethylphenol	10.89	34.44	9.74	11.19

* Compounds are listed in order of elution.

** Ref. 15.

*** $pH_{opt} = pK_{avg} + \frac{1}{2} \log(k'_{HA}/k'_{A^-})_{avg}$, see text for further explanation.

where pK_{avg} is the average of the *effective* pK_a values of the two compounds and $(k'_{HA}/k'_{A^-})_{avg}$ is the average of the quotients of limiting capacity factors for the two compounds. Limiting capacity factors were measured as described previously[11]. The phenolic compounds we studied are reported in Table I in order of their elution, along with their pK_a values, limiting capacity factors, and the optimum pH for the separation of adjacent pairs of these compounds.

As shown in Table I, the optimum mobile phase pH was different for each pair of adjacent compounds. Moreover, the optimum pH increases monotonically from 10.71 to 11.19, suggesting the use of a pH gradient rather than a fixed mobile phase pH. We recently reported a theory for the optimization of pH gradient elution in RPLC[12]. In the present study, however, we decided to use a fixed mobile phase pH rather than a pH gradient for two reasons. First, although the UV detector baselines were comparable, the electrochemical detector baseline was more stable with a constant mobile phase pH[13], consistent with previous findings[14]. Second, the separation of one pair of compounds, *m*-cresol and *p*-cresol, was considerably more difficult to achieve and was also arbitrarily deemed more important than the separation of the other compounds. Under these circumstances, a fixed mobile phase pH based on the optimum pH for the critical pair of compounds is more appropriate than a pH gradient[10].

Detection

All of the phenols studied absorbed light sufficiently at 280 nm to permit the use of an absorbance detector during method development with standard mixtures. In the shale oil samples, however, non-phenolic compounds that were not of interest in this study tended to co-elute with the cresols and xylenols and thus interfere with their detection, particularly since the interferents were much more concentrated than the phenols. This interference was eliminated by using an electrochemical detector that responded selectively to the phenols and other easily oxidized substances, but

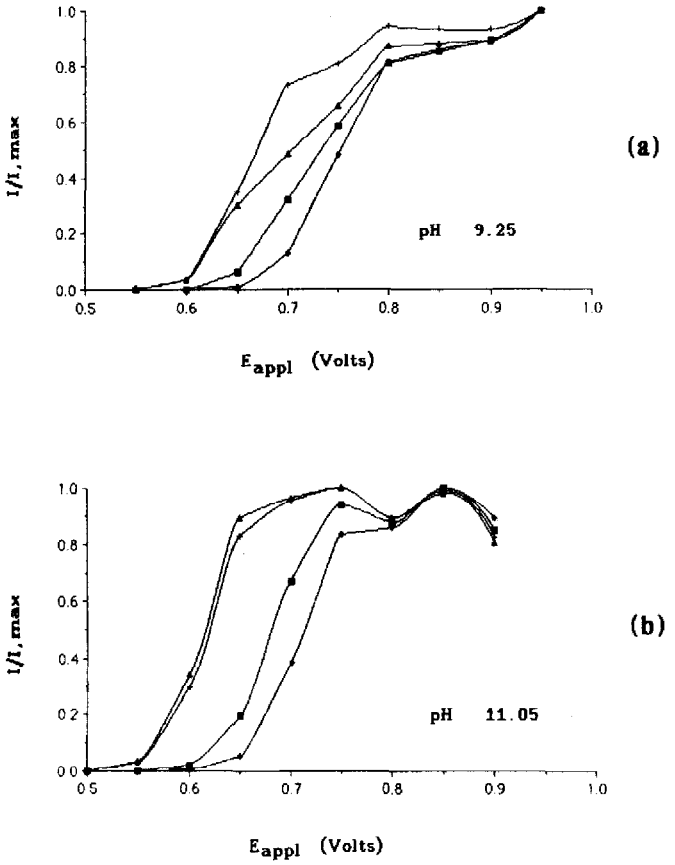

Fig. 1. Hydrodynamic voltammograms for phenol (●), *o*-cresol (+), *m*-cresol (■), and *p*-cresol (▲): (a) at pH 9.25, where they exist primarily in their protonated forms; and (b) at pH 11.05, where they exist as anions. Column: Hamilton PRP-1. Mobile phase: acetonitrile–aqueous sodium bicarbonate/carbonate buffer (30:70) with added sodium perchlorate (overall ionic strength, 50 m*M*).

not to the major interferents in the sample. In the present work, a glassy carbon working electrode and an Ag/AgCl reference electrode proved to be quite satisfactory, provided that the working electrode was pretreated consistently[14].

The hydrodynamic current/potential response curves for phenol and the cresols are shown in Fig. 1 for two different mobile phase pH values, one in which the protonated forms of phenols exist predominantly (pH 9.25), and another in which the phenolate anions are predominant (pH 11.05). Curent responses (peak heights) were normalized to the maximum response obtained at a given mobile phase pH. For a given amount injected, the response at pH 11.05 was *ca.* 1.4 times greater than the response at pH 9.25. As seen in Fig. 1, both forms of the phenols can be oxidized, and the best results were obtained at an applied potential of +0.85 V *vs.* Ag/AgCl. Although increases in E_{appl} beyond +0.95 V gave larger peak currents, they also resulted in exponential increases in background current and noise, and are not shown in Fig. 1. The exponential rises in background current at $E_{appl} > +0.95$ V are at-

tributed to the oxidation of the solvent (mostly water) and electroactive impurities in the mobile phase, which can occur at relatively modest applied potentials due to the unusually high pH of the mobile phase.

Other than the similar optimum E_{appl}, the hydrodynamic voltammograms in Fig. 1 for the protonated phenols (pH 9.25) and the phenolate anions (pH 11.05) are significantly different: First, the electrochemical waves are somewhat broader (shallower slopes) at pH 9.25, indicative of a less reversible oxidation, and consistent with the somewhat smaller currents we observed. Second and most importantly, the resolution between the electrochemical waves of *m*-cresol and *p*-cresol is greater at pH 11.05, and in fact is sufficient to permit their resolution by judicious control of the working electrode potential ($E_{appl} \approx 0.6$ V) in instances when they cannot be resolved chromatographically. Finally, the hydrodynamic voltammogram at pH 11.05 exhibited reproducible dips at $E_{appl} = +0.80$ and $+0.90$ V. These dips did not affect our results at $E_{appl} = 0.85$ V and were not investigated further. Overall, the better electrochemical detection was obtained at pH 11.05 and $E_{appl} = +0.85$ V.

Hydrodynamic voltammograms obtained at pH 10.82 were very similar to those shown in Fig. 1 at pH 11.05. Thus the electrochemical method for resolving *m*- and *p*-cresol at pH 11.05 mentioned above can also be used at pH 10.82, the mobile phase pH determined to be the optimum for the separation. However, since we were able to achieve near-baseline resolution ($R_s \approx 1.4$) via chromatographic techniques, we did not utilize this electrochemical method of resolution in the present study.

Phenol separations

Shown in Fig. 2 are the separations we achieved for phenol, the cresols, xy-

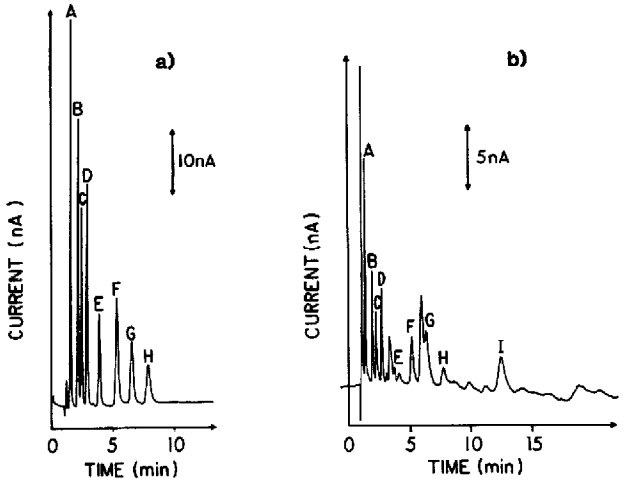

Fig. 2. Separations of (a) a mixture of selected phenol standards; and (b) phenols in a shale oil sample. $E_{appl} = 0.85$ V *vs.* Ag/AgCl, other conditions as in Fig. 1, with mobile phase pH 10.82. Compound identification: A = phenol; B = *m*-cresol; C = *p*-cresol; D = *o*-cresol; E = 2,5-dimethylphenol; F = 2,4-dimethylphenol; G = 2,6-dimethylphenol; H = 2,3,5-trimethylphenol; I = 2,4,6-trimethylphenol. Note that 2,4,6-trimethylphenol was inadvertently omitted from the mixture of Fig. 2a but was identified in Fig. 2b (compound I) and is reported in Table I. Likewise, 2,3-dimethylphenol was omitted from Fig. 2a but is included in Table I. It was not found in the shale oil sample of Fig. 2b.

lenols, and other methylated phenols in (a) a mixture of standards; and (b) in a shale oil sample. All of the compounds in Table I are shown in Fig. 2, except for 2,3-dimethylphenol which was inadvertently omitted from the standard mixture and was not found in the shale oil sample. The peak prior to peak A in each chromatogram is a void peak commonly observed with electrochemical detectors. Baseline or near-baseline resolution was achieved for all the isomers listed in Table I in less than 15 min. Although somewhat better for the mixture of standards than the sample, baseline stability for the latter was sufficient for good quantitation. Retention times and peak areas of the phenolic compounds in both the standard mixtures and samples were reproducible to within 0.5 and 2%, respectively. Linearity of the calibration curves for various phenols was better than $r^2 = 0.99$ over a concentration range of 10 to 1000 μg/g. The limit of detection, defined as the amount of compound injected to produce a signal three times the standard deviation of the baseline, was estimated to range from *ca.* 120 pg for phenol to *ca.* 650 pg for 2,4,6-dimethylphenol.

As noted in the Experimental section, we found it necessary to wash the analytical column periodically (*e.g.*, twice a day) with 100% acetonitrile, the stronger mobile phase component, to eliminate the excessive build-up on the column of a few neutral, highly retained compounds that remained in the sample after the solid phase extraction. Otherwise, the build-up and gradual elution of these compounds resulted in a gradually deteriorating baseline. Although this might be viewed as a minor drawback of our method, it is actually a minor disadvantage shared by all isocratic separation methods when they are applied to complex samples.

On the other hand, a unique advantage of the secondary chemical equilibrium approach is that the elution order of a set of related compounds can usually be predicted on the basis of their equilibrium constants[10], in some cases allowing for the tentative identification of unknown peaks in a chromatogram, subject to confirmation by another method. As shown in Table I and Fig. 2, phenol, the cresols, xylenols, and other methylated phenols do elute approximately in the order of their pK_a values. Note that *o*-cresol elutes after *p*-cresol due to an *ortho*-substituent effect discussed previously[11].

TABLE II

COMPARISON OF SELECTIVITIES ACHIEVED IN RPLC FOR DIFFICULT TO SEPARATE CRESOL AND XYLENOL ISOMERS

MP = Methylphenol (cresol); DMP = dimethylphenol (xylenol) Numbers indicate the ring position of the methyl group(s) on the phenol ring. The separation of *o*-cresol from its *meta*- and *para*-isomers is relatively easy and is not considered here. Likewise, other straightforward xylenol isomer separations have been excluded. NA = No attempt was made to separate these isomers.

Compound pair	*Reference*					
	This work	*3*	*4*	*5*	*6*	*7*
3-MP/4-MP	1.245	1.000	1.000	1.000	1.000	NA
2,3-DMP/2,5-DMP	1.170	1.148	1.071	1.037	NA	1.108
2,3-DMP/2,6-DMP	1.317	1.000	NA	1.000	NA	1.036
2,4-DMP/2,5-DMP	1.295	1.047	1.012	1.000	NA	1.023
2,4-DMP/2,6-DMP	1.215	1.096	NA	1.037	NA	1.045

The selectivity obtained using our pH-optimized reversed-phase method for the difficult-to-separate cresol and xylenol isomers is compared with other recent reversed-phase investigations in Table II. For each isomer pair, the selectivity we observed was much greater than that observed with the other reversed-phase methods, thus clearly illustrating the superiority of our pH-optimized approach, which takes advantage of the acid–base secondary chemical equilibrium in the mobile phase to greatly increase the selectivity.

CONCLUSION

The novel reversed-phase approach we have described for the separation of phenol, the cresols, the xylenols, and other methylated phenols is well suited for petroleum samples such as shale oil, but it should be even better for aqueous environmental samples since much of the sample pretreatment could probably be eliminated. In terms of separation selectivity, our approach is significantly better than any other reversed-phase method published to date.

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