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Separation of structural homologues of alkylphenols and isomers of 4-nonylphenol by cyclodextrin-modified micellar electrokinetic chromatography

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Abstract

The potential of cyclodextrin-modified micellar electrokinetic chromatography (CD/MEKC) for separating structural homologues of alkylphenols and isomers of 4-nonylphenol (4-NP) was evaluated and compared with ordinary MEKC. The addition of cyclodextrins to the separation buffer containing sodium dodecyl sulphate (SDS) significantly improved the resolution of the alkylphenol (AP) homologues with long hydrocarbon chains, and especially those of 4-NP isomers, while reducing the analysis time. The operating conditions in the CD/MEKC were optimized, with an emphasis on investigating the effects of the type and concentration of CD, SDS concentration, acetonitrile concentration and sample stacking on the separation of 4-NP isomers.

Keywords: Buffer composition; Alkylphenols; Nonylphenol

1. Introduction

Nonylphenols (NP) have useful applications in industry and they also appear as pollutants in the environment. The highly-branched nonylphenols are the main raw materials used to manufacture nonylphenol polyethoxylates (NPEO) which are widely used as non-ionic surfactants in industrial cleaning products and are to a great extent also present in household detergents [1]. In the process of manufacturing polyvinylchloride by the suspension polymerization technique, 4-NP, which acts as the inhibitor for the reaction promoted by the dissolved oxygen, is added in the recovery system [2,3]. In addition, nonylphenols are also applied as emulsifiers in pesticide formulations [4].

Investigations of full-scale sewage treatment plants and rivers in Switzerland have revealed that 4-NP, together with 4-NP mono and diethoxylate, are major refractory constituents in mechanically or biologically treated sewage effluents and in the Blatt River [5,6]. Investigations of organic pollutants in waste water and natural waters in the USA showed the presence of NP at extraordinarily high levels in sewage sludge, particularly after anerobic stabilization by mesophilic and methanogenic digestion [7–9].

The considerable use and consequently widespread occurrence of 4-NP have promoted studies on NP. NP are generally produced by the alkylation of phenols with propylene. The reaction produces many

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isomers due to the various possible positions of the double bond along the hydrocarbon chain [10]. Laboratory studies have shown that 4-NP with highly branched hydrocarbon chains work better as inhibitors for the reactions promoted by dissolved oxygen because the branched hydrocarbon chain induces more activity of the OH group of the phenolic unit. Therefore, it is preferable to have branched isomers of 4-NP in industrial applications. However, from the environmental point of view, highly branched isomers of 4-NP are not desirable because of their very poor biodegradability [11]. Increasing toxicity has been found to be related to the higher lipophilicity of the phenols. Hence, detailed studies of these isomers are of great interest in terms of both industrial production and environmental protection. Hitherto, the available methods of separation and characterization were based on techniques such as GC, LC, MS, GC–MS and GC–FTIR [11–14]. LC is preferable to GC with regard to the determination of the total amount of 4-NP isomers [1,15–17]. However, it is not possible to separate these isomers, especially chain isomers with this technique. GC has been reported to resolve about 30 peaks of 4-NP isomers [11]. This method, however, requires the conversion of the 4-NP into volatile derivatives before analysis, and the analysis time is relatively long.

Cyclodextrin-modified micellar electrokinetic chromatography (CD/MEKC) was first introduced by S. Terabe and co-workers for the separation of highly hydrophobic and closely related compounds [18,19]. Most previous reports focused on the separations of optical isomers [20–23] and aromatic ring isomers [18,24–26]. Separation of chain isomers of alkylsulphate with hydrocarbon chain lengths of C_4 using MEKC has also been reported [27]. A recent paper discussed the separation of homologues of long-chain linear saturated free fatty acids [28]. To our knowledge, no work has been reported on the successful separation of long hydrocarbon chain isomers.

The main purpose of this preliminary work was to investigate the application of CD/MEKC to the separation of 4-NP isomers (consisting of mainly long hydrocarbon chain isomers) in which the substituted alkyl group contains nine carbon atoms. The feasibility of using MEKC for this purpose is indicated by a criterion we set ourselves, that is, to resolve as many peaks as possible in this complex isomeric mixture under the limited conditions (MEKC with two types of cyclodextrins) available to us. We aimed to obtain a separation at least comparable to an existing GC procedure [11]. The difficulty of separating 4-NP isomers mainly results from its long substituted hydrocarbon chain which makes it so soluble in SDS that separation selectivity is difficult to achieve in ordinary MEKC. This problem can be conceivably resolved by the addition of cyclodextrin which can selectively include the 4-NP isomers. Taking into account the fact that 4-NP often coexists with other alkylphenols, we first investigated the separation of alkylphenol homologues, and then focused on studying the separation of 4-NP isomers.

2. Experimental

2.1. Apparatus

Both MEKC and CD/MEKC were performed on a Prince CE system equipped with the Butler buffer exchanger (Prince Technologies, The Netherlands), with detection by UV at 220 nm on a Lambda 1000 spectrophotometer (Bischoff, Leonberg, Germany). A Chromatopac C-R6A integrator (Shimadzu, Kyoto, Japan) was used for data processing. The fused-silica capillary used was 80 cm long (effective length 64 cm) with an I.D. of 50 μm.

2.2. Reagents

4-Nonylphenol (technical grade with branched isomers) was used as received from Tokyo Kasei (Tokyo, Japan). 4-Methylphenol, 4-ethylphenol, 4-propyphenol, 4-butylphenol, 4-heptylphenol and 4-*tert.*-octylphenol were purchased from Fluka (Buchs, Switzerland).

Sodium tetraborate was purchased from Merck (Holtenau, Germany). Phosphoric acid was purchased from Carlo Erba (Milan, Italy). Sodium dodecyl sulfate (SDS) and β-cyclodextrin were purchased from Fluka. Hydroxypropyl-β-cyclodextrin (ave. MS=0.8) was bought from Aldrich (Milwaukee, WI, USA).

HPLC-grade acetonitrile was bought from J.T. Baker (Phillipsburg, NJ, USA). The water used for the preparation of the sample and buffers was purified by a Milli-Q system (Millipore, Bedford, MA, USA).

3. Results and discussion

3.1. Separation of structural homologues of alkylphenols

We first studied the influence of the acetonitrile concentration on the resolution of seven alkylphenols (AP) in a synthetic mixture. The SDS concentration was set at 12.5 m*M*, and the pH of the liquid medium was set at 9 using a borate–phosphoric acid buffer. Separation was carried out at a voltage of 25 kV.

Generally, the migration times of the AP increased on the addition of acetonitrile. The extent of this increase depends on the alkylphenols, as shown in Fig. 1. It is indicated in the figure that the addition of acetonitrile improves the resolution of the more hydrophobic AP with longer hydrocarbon chains. However, this improvement in the resolution of 4-HP, 4-OP and 4-NP (see Fig. 1 for an explanation of the abbreviations) was achieved at the expense of a decrease in the quality of separation of the less hydrophobic AP with shorter hydrocarbon chains. In fact, the resolution was poorer than that with no acetonitrile present for 4-MP, 4-EP and 4-PP. With the addition of 10% of acetonitrile in the liquid medium, 4-MP and 4-EP co-eluted as one peak while the most hydrophobic pair, 4-OP and 4-NP could not be baseline-separated (Fig. 2).

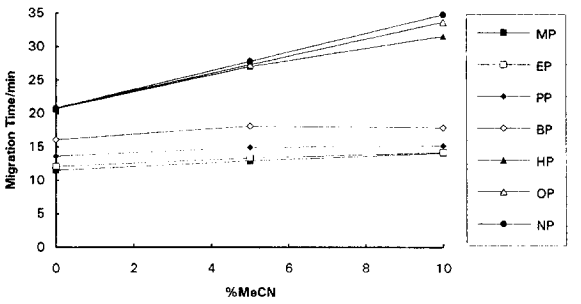

Fig. 1. Influence of acetonitrile (MeCN) content in the buffer on the migration time of seven alkylphenols. Borate–boric acid buffer, 12.5 m*M* (pH: 9); concentration of SDS, 25 m*M*; fused-silica capillary (80 cm×50 μm I.D.); voltage: 25 kV; UV detection, 220 nm; hydrodynamic injection, 300 mbar s. Analytes: 4-MP=4-methylphenol; 4-EP=4-ethylphenol; 4-PP=4-propylphenol; 4-BP=4-butylphenol; 4-HP=4-heptylphenol; 4-OP=4-*tert.*-octylphenol; 4-NP=4-nonylphenol.

In order to improve the quality of separation, we investigated the effect of adding HP-β-CD. We found that addition of HP-β-CD also improved the resolution of the more hydrophobic AP with longer alkyl chains while degrading the resolution of the less hydrophobic AP with shorter chains. However, the migration times for all the AP were reduced with the addition of HP-β-CD (Fig. 3). This indicates that the AP were included in the CD and the interactions between the CD and AP were different among the AP tested. The results showed that the optimal concentration of HP-β-CD was 1.0 m*M*, allowing virtually baseline separation for all the compounds except for the pairs 4-EP and 4-PP, as shown in Fig. 4.

A comparison between the electropherograms in Fig. 2 and Fig. 4 shows that use of HP-β-CD in

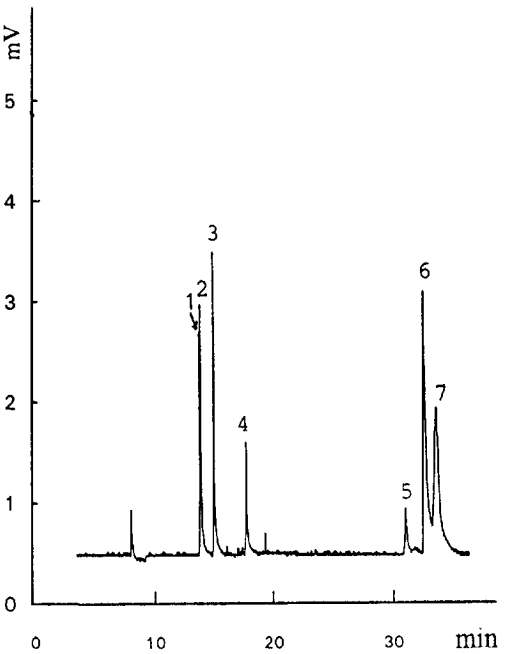

Fig. 2. Electropherogram of seven alkylphenols using MEKC with an acetonitrile content of 10% in the buffer. Other conditions as in Fig. 1. Peaks: (1) 4-MP, (2) 4-EP, (3) 4-PP, (4) 4-BP, (5) 4-HP, (6) 4-OP, (7) 4-NP.

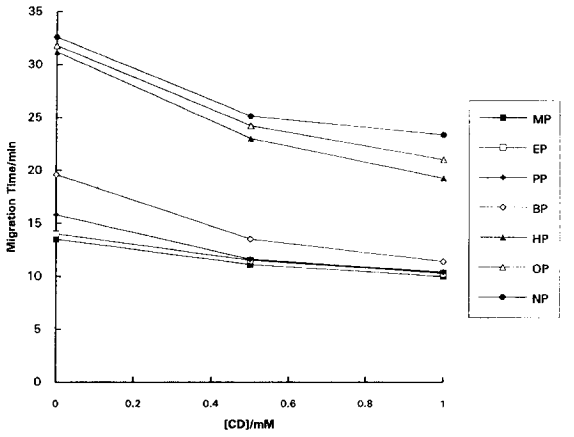

Fig. 3. Effect of HP-β-CD concentration on the migration times of seven alkylphenols. Acetonitrile concentration: 8%. Other conditions as given in Fig. 1.

MEKC can improve the resolution of AP with long hydrocarbon chains while reducing the overall analysis time.

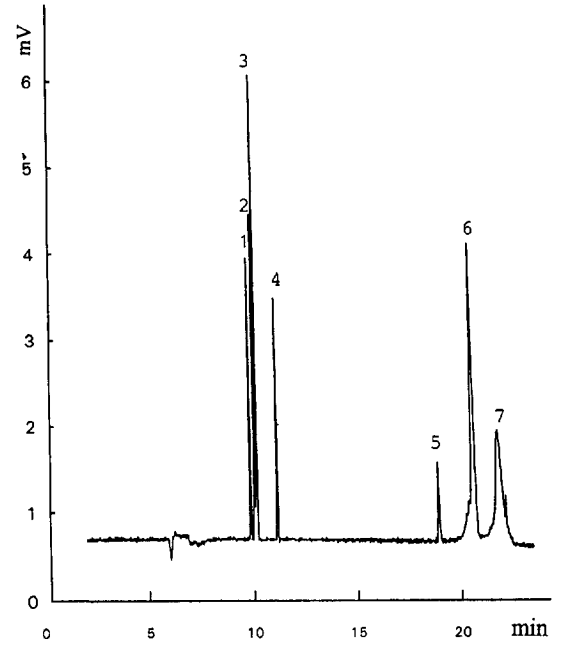

Fig. 4. Electropherograms of seven alkylphenols using CD/MEKC with HP-β-CD concentration of 1.0 m*M*. Acetonitrile concentration: 8%. Other conditions as in Fig. 1.

3.2. Separation of isomers of 4-nonylphenol

3.2.1. Micellar electrokinetic chromatographic (MEKC) separation of isomers of 4-nonylphenol

The separation of 4-NP isomers using MEKC without CD was first investigated. As previously shown in Fig. 2, since resolution of the most hydrophobic AP was improved with the increase in concentration of acetonitrile, we expected that the separation of 4-NP isomers should be performed at a higher concentration of acetonitrile. The results showed that 4-NP gave a broad peak (electropherogram not shown) accompanied by a few small peaks when 20% of acetonitrile was used in a 25 m*M* buffer. The migration time was unacceptably long (50–60 min). Therefore, good separation of the isomeric mixtures of 4-NP could not be achieved by using MEKC only, a result different from that of the separation of butylbenzene sulphate isomers which were almost completely resolved by MEKC [27]. Efforts were subsequently made to improve the separation of 4-NP isomers by investigating the effect of the addition of cyclodextrins to the SDS solution.

3.2.2. Cyclodextrin-modified micellar electrokinetic chromatographic (CD/MEKC) separation of isomers of 4-nonylphenol

The separation of 4-NP isomers using CD/MEKC can be affected by many factors. In this part of the work, we investigated the effect of several important factors, including the type and concentration of CD, organic modifier (acetonitrile) concentration, surfactant (SDS) concentration and sample stacking.

Throughout the subsequent experiments, the borate buffer concentration was set at 25 m*M*, pH at 9.0, applied voltage at 25 kV. For this preliminary work, we chose to vary one parameter at a time ignoring the interactions of the parameters, and used the number of distinguishable peaks (NDP), total analysis time and peak shapes to estimate the quality of the separation. A distinguishable peak means that the top of the peak is not obscured by other peaks. In the present case where the number of completely overlapping peaks is relatively high due to the large number of the components to be separated, NDP may be used as a convenient and effective response

function to reflect the quality of the chromatogram [29].

3.3. Effect of cyclodextrin type

The technical grade of 4-nonylphenol considered in this work consists of many isomers (about 90% are *para*-isomers (4-NP itself) with the rest being *ortho*- and *meta*-isomers). These isomers can be classified into 2 categories: ring isomers (*ortho*-, *meta*- and *para*-) and chain isomers. The overall molecular shape, determined by the different substitutions on the aromatic ring (for ring isomers) and/or along the hydrocarbon chain (for chain isomers), is a major factor affecting the stability of the CD/4-NP inclusion complex, and, hence, the migration order of these isomers.

The cyclodextrins employed in the work reported here were native β-CD and derivatized hydroxypropyl-β-CD (HP-β-CD). Native β-CD is the most widely used homologue for separation of enantiomers, diastereomers and structural isomers. HP-β-CD is one kind of β-CD derivative, which can be made inexpensively and is much more soluble in water than β-CD. In addition, HP-β-CD has been found to show better enantiomeric selectivity for some chiral compounds than native β-CD [30].

In the present work, we investigated the effect of adding β-CD and HP-β-CD to the SDS solution on the separation of 4-NP isomers. Fig. 5(a) and Fig. 5(b) show the separation of 4-NP isomers in the presence of β-CD and HP-β-CD. It is clear that the addition of either β-CD or HP-β-CD resulted in a significant improvement in resolution and a reduction in the migration times of all isomers, compared with those in the SDS solution without CD. This result indicates that there was a certain selectivity for the isomers afforded by the CD. Since CD has a hydrophilic external surface due to the primary and secondary hydroxyl groups, it is not solubilized by the SDS micelle and is transported with almost the same velocity as that of the electro-osmotic flow. Therefore, the formation of stable inclusion-complexes between the CD and isomers brings about faster migration. Furthermore, due to the different relative stabilities of the inclusion complexes, attributable to the steric configuration of individual isomers, the migration times were reduced to varying

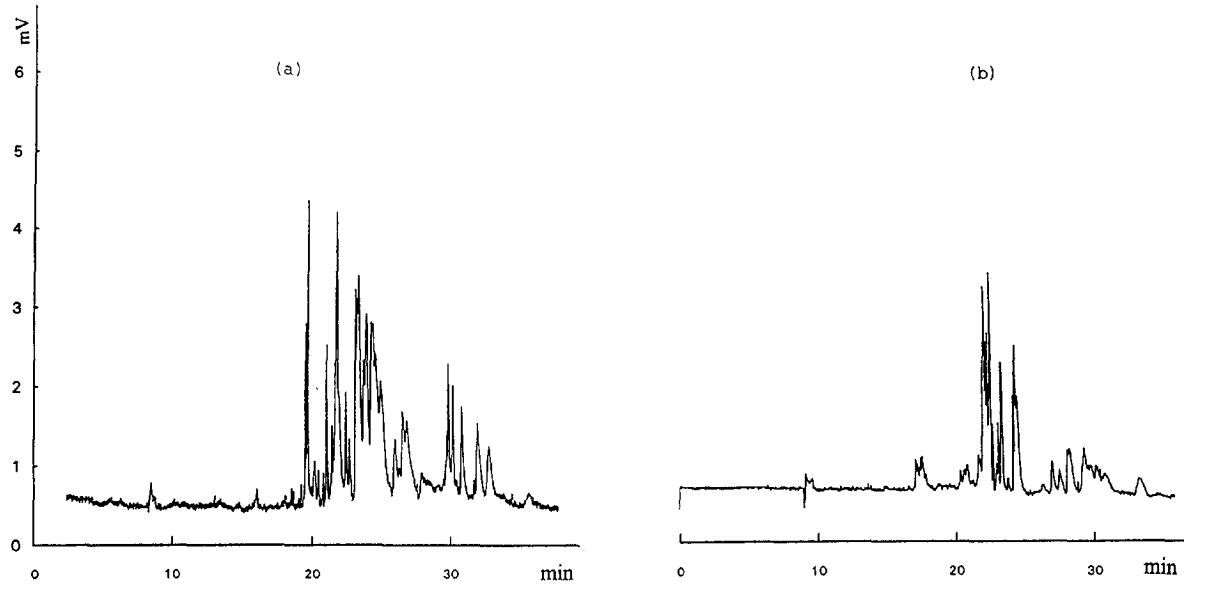

Fig. 5. Separation of 4-NP isomers by the addition of CD. Fused-silica capillary (80 cm×50 μm I.D.); detection, 220 nm; hydrodynamic injection, 300 mbar s; voltage, 25 kV; borate–boric acid buffer, 25 m*M* (pH 9); SDS concentration, 20 m*M*; acetonitrile concentration, 20%. (a) 10 m*M* HP-β-CD (b) 10 m*M* β-CD.

extents, permitting improved separation of the components.

In addition, the selectivity shown by β-CD and HP-β-CD was different in the separation of isomers. It was, therefore, conceivable that the appropriate combination of β-CD and HP-β-CD may provide better resolution. However, our preliminary results showed no improvement in the separation as a whole.

3.4. Effect of cyclodextrin concentration

Isomeric resolution depends on the concentration of the CD in the buffer system. HP-β-CD was used for further study as it showed better resolution of 4-NP isomers and was more soluble in aqueous solution, which made our study possible over a wide range of HP-β-CD concentrations.

Fig. 6 presents a series of electropherograms obtained at different concentrations of HP-β-CD in the buffer. For convenience of discussion, observed peaks were arbitrarily divided into 3 groups, A, B and C. At low concentrations (4 m*M*) of HP-β-CD, the migration times were long (in the range 30–55 min) (6a); the resolution was good for group A peaks, but unsatisfactory for those in the other groups. As the HP-β-CD concentration was increased, the migration time was concomitantly reduced, and the resolution for the peaks in group B and C was improved. The optimal resolution of most isomers was obtained at about 10 m*M* HP-β-CD (Fig. 6b), where the migration times for all peaks were in the range 15–35 min. The NDP was approximately >30, and the resolution for the peaks in group A was maintained while the resolution for the peaks in the other 2 groups improved with most peaks baseline-separated. Peak shapes were also markedly improved. Further increases in the concentration of HP-β-CD resulted in a shorter overall analysis time. However, the resolution was compromised. At 16 m*M* HP-β-CD, the elution time range of all isomers was reduced to 12–25 min at the expense of a decrease in the quality of separation of most isomers, with NPD reduced to about 15. The guest–host complex formation between 4-NP and HP-β-CD is driven by the tendency of the CD cavity to selectively accommodate the isomers of 4-NP. In the case of partial complex formation, the differences in stability constants among isomers result in an increase in the difference between the effective mobilities of these isomers. However, if the CD concentration is increased up to the point where complex formation becomes complete, the effective mobilities of 4-NP isomers become almost identical owing to the high molecular mass of the CD, and the resolution is degraded [18].

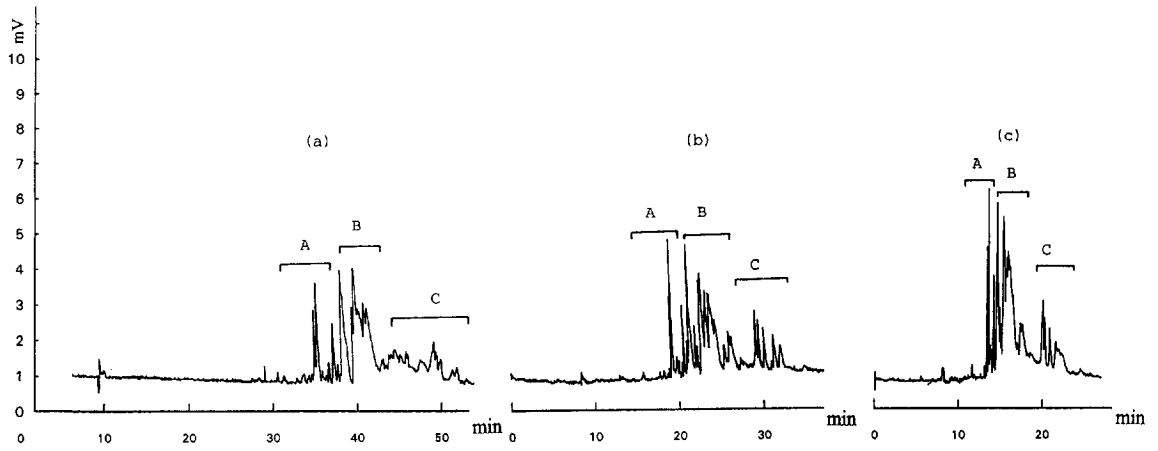

Fig. 6. Effect of HP-β-CD concentration on the separation of 4-NP isomers. HP-β-CD concentration: (a) 4 m*M*, (b) 10 m*M*, (c) 16 m*M*. Other conditions as in Fig. 5.

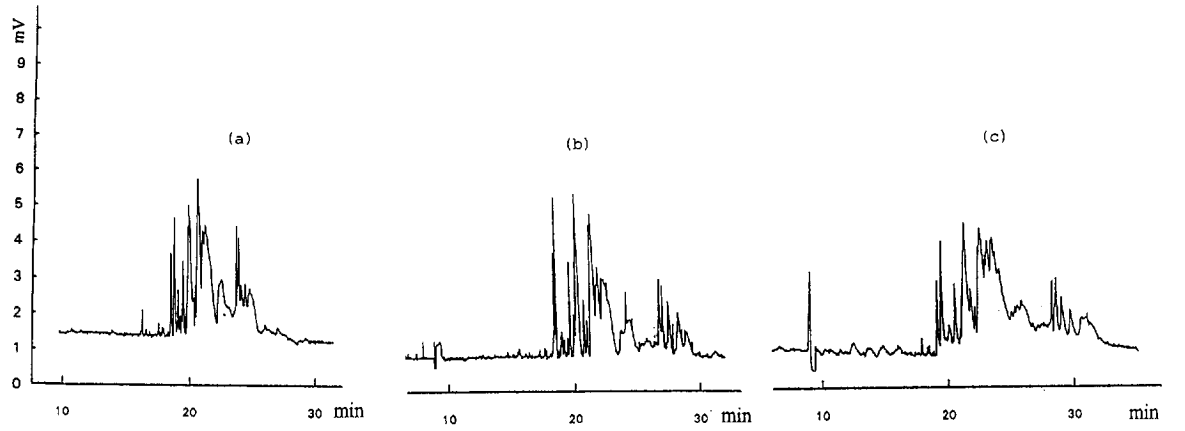

Fig. 7. Effect of acetonitrile concentration on the separation of 4-NP isomers in the presence of CD. Acetonitrile concentration: (a) 15%, (b) 20%, (c) 25%. HP-β-CD concentration: 10 m*M*. Other conditions as in Fig. 5.

3.5. *Effect of organic modifier-acetonitrile*

In buffer solutions of SDS with CD, adding acetonitrile can change the distribution of analytes among three phases, that is, the aqueous phase, the micellar phase (SDS), and the CD cavity, making lipophilic solutes, e.g. 4-NP isomers, more soluble in the aqueous phase, less soluble in the micelle phase and more weakly included in CD.

Fig. 7 shows the influence of acetonitrile on separating 4-NP isomers in the presence of both SDS and CD. Firstly, the migration times were increased on the addition of the acetonitrile, a result similar to the results when CD was not used. However, the increase in the migration times was less with increasing acetonitrile concentrations. One reason for this may be that the 4-NP isomers formed inclusion complexes with CD, which thus migrated at the velocity of the aqueous phase and hence to some extent offset the acetonitrile's effect on increases in the migration times.

Secondly, the resolution of the 4-NP isomers was progressively improved on increasing the acetonitrile concentration. As the concentration of the acetonitrile was increased, we noted that more and more peaks were observed with significant improvement on resolution of almost all these peaks. When the acetonitrile concentration was 20%, an electropherogram with over 30 distinguishable peaks was obtained within 35 min (Fig. 7b). A further increase of the acetonitrile content to 25%, however, brought about longer migration times for all isomers and the baseline became unstable along with peak broadening.

3.6. *Effect of SDS concentration*

In CD/MEKC as well as MEKC, the retention and the selectivity of the solutes can be manipulated by the total concentration of the surfactant in the buffer system. Fig. 8 shows the effect of the SDS concentration on the isomeric separation in the presence of HP-β-CD. We started with 8 m*M* SDS (the critical micelle concentration of SDS in water), then increased the concentration in 4 m*M* steps, to 28 m*M*.

At low (8 m*M*) concentrations, all isomers coeluted as a single peak at approximately 10 min, that is, similar to the migration time of the electroosmotic flow marker. This indicated that no SDS micelles formed under this condition, probably because of the destabilizing effect of the organic solvent, acetonitrile. As the SDS concentration was increased, more and more peaks were revealed and resolved, with a progressive increase in migration times. The optimal resolution for most peaks (more than 30 peaks were distinguished) was obtained at about 20 m*M* SDS (Fig. 8b). Further increase in the

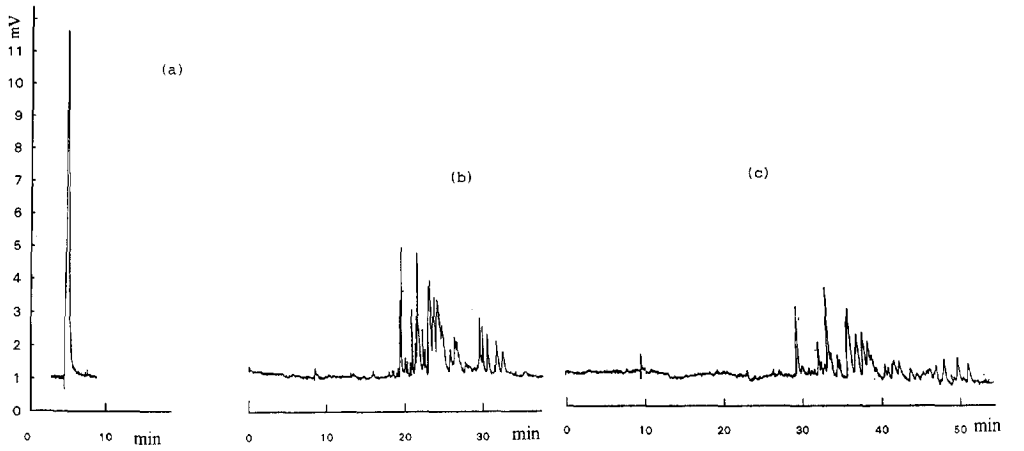

Fig. 8. Influence of SDS concentration on the separation of 4-NP isomers. SDS concentration: (a) 8 m*M*, (b) 20 m*M*, (c) 28 m*M*. HP-β-CD concentration: 10 m*M*. Other conditions as in Fig. 5.

concentration resulted in longer migration times and broadened peaks.

3.7. Effect of sample stacking

Sample injection is a crucial procedure in capillary electrophoresis. Even an optimized electrophoretic system produces unsatisfactory separation if sample injection is performed carelessly. Several specific injection techniques, e.g. sample stacking, have been reported by Burgi and Chien [31]. In this study, we kept the injected amount constant in all runs, and focused on investigating the effect of the sample solution conductivity on the separation of the 4-NP isomers. When 4-NP was dissolved in pure methanol, the electropherogram shown in Fig. 9a was obtained. All the isomers appeared clustered together even though the optimized buffer was used. Then 4-NP was dissolved in water–methanol (50:50) containing 12.5, and 25 m*M* SDS solution whose conductivities were respectively about 1/12, and 1/6 of that of the separation buffer containing 25 m*M* borate and 20 m*M* SDS. The resulting electropherograms are presented in Fig. 9b,c. The results indicated that the

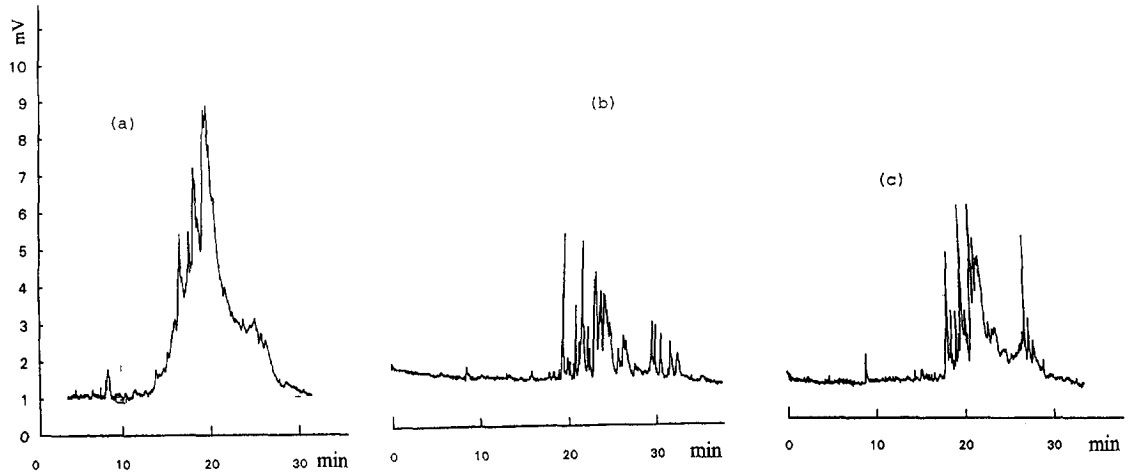

Fig. 9. Effect of sample stacking on the separation of 4-NP isomers. Sample solvents: (a) methanol (MeOH), (b) MeOH–H_2O (50:50) containing 12.5 m*M* SDS (c) MeOH–H_2O (50:50) containing 25 m*M* SDS, HP-β-CD concentration: 10 m*M*; Other conditions as in Fig. 5.

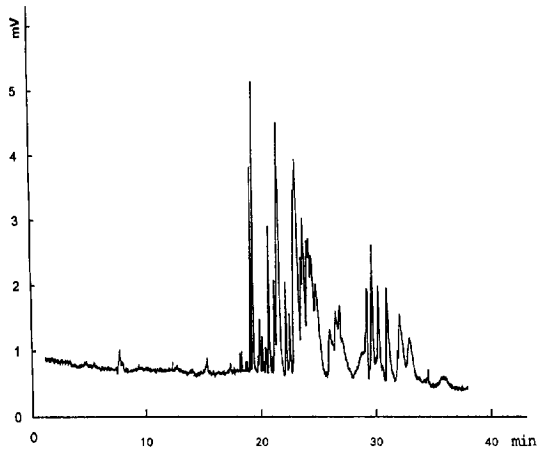

Fig. 10. Optimized separation of 4-NP isomers. Fused-silica capillary (80 cm×50 μm I.D.); detection, 220 nm; hydrodynamic injection: 300 mbar s; sample solvent, MeOH–H_2O (50:50) containing 12.5 m*M* SDS; voltage, 25 kV; borate–boric acid buffer, 25 m*M* (pH 9); SDS concentration, 20 m*M*; acetonitrile concentration, 20%; HP-β-CD concentration, 10 m*M*. Concentration of 4-NP, 50 ppm.

optimum SDS concentration was 12.5 m*M*, at which most peaks were fully separated (Fig. 9b). For higher SDS concentrations, the resolution of all isomers was considerably degraded.

Compared with the separation of most other compounds in CE, the separation of 4-NP isomers was more strongly affected by the sample solution conductivity. This may be due to the very small differences in effective mobilities among these isomers. Hence, particular attention should be paid to the selection of the sample solution conductivity so that the optimal separation could be obtained, at least for this series of analytes.

Summarizing the above results, we obtained the optimal separation conditions as shown in Fig. 10. More than 30 distinguishable peaks were observed with most peaks baseline-separated. This result is comparable with that obtained by GC which resolved about 30 peaks [11].

4. Conclusions

A capillary electrophoresis method has been developed to separate the isomers of 4-nonylphenol. CD/MEKC was found to be much more useful than MEKC in the isomeric separation of alkylphenols with relatively long hydrocarbon chains. The novel method does not need time-consuming derivatization, and even allowing that this is only a limited and preliminary study at this stage with no further attempt at complete optimization of the separation conditions, it is comparable to GC in terms of being able to resolve a large number of 4-NP components.

The capability of CD/MEKC to resolve a complex mixture of 4-NP alkyl chain isomers further extends the applicability of the technique and implies that CD/MEKC is effective not only for separating the aromatic ring isomers but also for separating long alkyl chain isomers. It is thus reasonable to suggest that the technique may be applied to separate isomers with even longer alkyl chains, such as, for example, WITCO TRS 10-80 which consists of homologues and isomers of alkylbenzene sulphonates with alkyl chain lengths between C_6 and C_{12} [27].

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References

[1] M. Ahel and W. Giger, Anal. Chem., 57 (1985) 1577.
[2] L.I. Nass (Editor), Encyclopedia of PVC, Vol. 1, Marcel Dekker, New York, 1976.
[3] G.A. Razuvaev and K.S. Minsker, Zh. Obschch. Khim. 28 (1985) 983.
[4] D.W. Mcheese, V. Zitko and C.D. Metcalfe, Chemosphere, 9 (1980) 79.
[5] W. Giger and E. Stephanou, Chemosphere, 10 (1981) 1253.
[6] E. Stephanou and W. Giger, Environ. Sci. Technol., 16 (1982) 800.
[7] M. Reinhard, N. Goodman and K.E. Mortelmans, Environ. Sci. Technol., 16 (1982) 351.
[8] A.W. Garrison and D.W. Hill, Am. Dyest. Rep., (February, 1972).
[9] W. Giger, P.H. Brunner and C. Schaffrev, Science (Washington,DC), 225 (1984) 623.
[10] V. Raverdino and P. Sassetti, J. Chromatogr., 153 (1978) 181.
[11] B.D. Bhatt, J.V. Prasad and G. Kalpana, J. Chromatogr. Sci., 30 (1992) 203.

[12] G.U. Zenkina and L.A. Tkacheva, Neftepererab. Neftekhim, 8 (1974) 34.
[13] K. Callmer, L.E. Edholm and B.E.F. Smith, J. Chromatogr., 136 (1977) 45.
[14] J.L. Oclowiz, Anal. Chem., 36 (1964) 2177.
[15] A. Marcomini, A. Di Corcia and S. Capri, J. Chromatogr., 644 (1993) 59.
[16] A.J. Sweetman, Water Research, 128 (1994) 343.
[17] A. Marcomini and W. Giger, Anal. Chem., 59 (1987) 1709.
[18] S. Terabe, Y. Miyashita, Y. Ishihama, O. Shibata, J. Chromatogr., 636 (1993) 47.
[19] S. Terabe, E.R. Barnhart, B.L. Karger, K. Hosoya, J. Chromatogr., 516 (1990) 23.
[20] S. Terabe, H. Ozaki and T. Anclo, J. Chromatogr., 332 (1992) 211.
[21] J. Snopek, I. Jelinek and M. Novotny, J. Chromatogr., 559 (1990) 215.
[22] T.E. Peterson, J. Chromatogr., 630 (1993) 353.
[23] H. Nishi and M. Matsuo, J. Liq. Chromatogr., 14 (1991) 973.
[24] M.J. Sepaniak, R.O. Code and B.K. Clark, J. Liq. Chromatogr., 15 (1992) 1023.
[25] S.G. Penn, D.M. Goodall and J.S. Loran, J. Chromatogr., 636 (1993) 149.
[26] Y.Y. Rawjee and G. Vigh, Anal. Chem., 66 (1994) 633.
[27] P.L. Jesbene, C. Bony, B. Desmazieves and J. Jacquier, J. Chromatogr., 608 (1992) 375.
[28] R. Roldan-Assad and P. Gareil, J. Chromatogr. A, 708 (1995) 339.
[29] H.B. Wan, W.G. Lan, M.K. Wong, Anal. Chim. Acta., 289 (1994) 371.
[30] D.W. Armstrong, A.M. Stalcup and J. Pitha, J. Chromatogr., 513 (1990) 181.
[31] D.S. Burgi and R.L. Chien, Anal. Chem., 63 (1991) 2042.