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Ionization of dichlorophenols for their analysis by capillary electrophoresis–mass spectrometry

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Abstract

In order to develop an advanced analytical method using capillary electrophoresis (CE) for non-volatile environmental pollutants such as endocrine disruptors, combination with mass spectrometry (MS) is necessary for their identification. We chose dichlorophenols (DCPs) as test samples because one of their isomers, 2,4-DCP, is suspected to have endocrine disrupting effects. A preliminary study on their separation by CE–MS was performed using a laboratory-made electrospray ionization (ESI) interface. For the effective ionization of 2,4-DCP at the ESI interface, applied voltage, assisted nitrogen-gas flow-rate, salts and their concentration, and organic solvents in the sample matrix were optimized according to the signal intensity. The intensity of other DCPs under the same conditions, however, was quite different. We tried to modify the atmospheric chemical pressure ionization (APCI) interface in order to reduce the flow-rate. The intensity of directly injected DCPs was not so different from one another using the modified prototype APCI interface.

Keywords: Positional isomers; Buffer composition; Electrospray ionisation; Chlorophenols; Endocrine disruptors

1. Introduction

Recently, several chemicals have been suspected of having endocrine disrupting effects [1]. The Environmental Agency of Japan arranged ‘Strategic Programs on Environmental Endocrine Disruptors ’98’ (SPEED ’98) in May, 1998 [2]. In the program, sixty-seven suspected chemical groups are listed. For the accurate assessment of human exposure to these chemicals, the development of easy analytical methods for them is very important. At present, gas chromatography–mass spectrometry (GC–MS) is the main analytical method.

However, non-volatile or thermally degradable chemicals cannot be analyzed directly by GC–MS. Capillary electrophoresis (CE) has a high separation efficiency and can be easily applied for the analysis of non-volatile or thermally degradable chemicals. The isomeric separation ability of CE is very useful in combination with MS as the detection method. Some papers have reported on CE–MS analyses of chemicals of environmental concern [3–9].

Dichlorophenol (DCP) has six positional isomers. One of them, 2,4-DCP, is listed in SPEED ’98. It is also one of the priority pollutants regulated by the US Environmental Protection Agency (EPA). There

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have been some reports on the separation of chlorophenol isomers by capillary zone electrophoresis (CZE) or micellar electrokinetic chromatography (MEKC) [10–14]. The separation of chlorophenols by commercially available CE–MS was reported by Jáuregui et al. [15]. They used an electrospray ionization (ESI) interface and monitored the isotropic ion ($m/z=163$) for detection of DCPs, because they used diethylmalonic acid as carrier electrolyte which produces an ion which interfered with the detection of the highest intensity ion of DCPs, $m/z=161$. In this paper, we investigated the ionization and separation of DCPs using other electrolytes that do not interfere with the detection of DCPs. An ESI interface was combined with CE–MS because ESI is most widely used for CE–MS [15–19]. The optimization of ionization conditions such as ESI applied voltage, nitrogen-gas flow-rate, salts and their concentration, organic solvents in sample solution for 2,4-DCP and preliminary separation of DCP isomers by CE–MS was investigated. We also made a prototype atmospheric pressure chemical ionization (APCI) interface for the application of CE–MS. The preliminary results of ionization were compared to those of ESI.

2. Experimental

2.1. Apparatus

A Hitachi M-8000 3DQMS mass spectrometer (Tokyo, Japan) was used. A Harvard Apparatus Model 11 syringe pump (MA, USA) was used for direct mode MS detection or for the delivery of sheath liquid. A high-voltage power supply for the ESI was a Matsusada HepLL-30N0.08 negative power supply (Shiga, Japan). CE voltage was applied by an Applied Biosystems Model 270A (CA, USA).

2.2. Ionization interface

Our laboratory-made interface was a co-axial sheath flow and gas type interface [19]. The interface is schematically shown in Fig. 1. It consists of two T-unions and two stainless steel tubes of different diameters. An 186 μm O.D.×50 μm I.D. fused-silica capillary (Polymicro Technologies, AZ, USA)

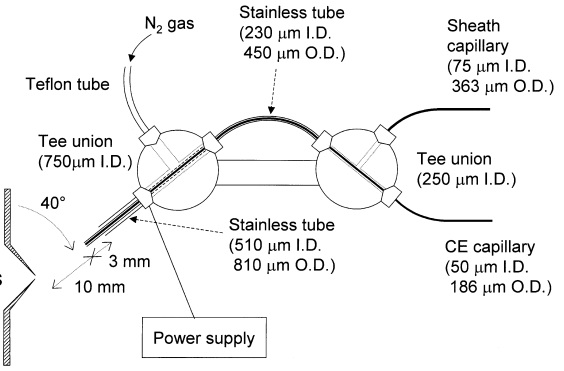

Fig. 1. Schematic outline of laboratory-made ESI interface.

of 54 cm total length was used as the CE capillary and was inserted into the interface. Two stainless tubes were used to deliver sheath liquid and nitrogen gas. These capillary and stainless tips were positioned as shown in Fig. 1. The angle and distance between the tips and the first skimmer of MS was set to those values for the effective transportation of the ionized sample [20].

A semi-micro APCI interface equipped M-8000 was modified as another interface. In the original interface, a stainless pipe, 375 μm I.D.×3 mm O.D. was set in an APCI chamber. A capillary tube of 100 μm I.D. was inserted into the stainless steel tube to deliver the eluent from HPLC. We replaced the stainless steel pipe with 2 mm I.D.×3 mm O.D., and insert the capillary and two stainless steel tubes into the pipe to make co-axial sheath and gas flow system in the same way as our ESI interface. The construction of the interface is shown in Fig. 2.

2.3. Reagents

Dichlorophenols were obtained from GL Sciences (Tokyo, Japan). Ammonium acetate, ammonium hydrogencarbonate and ammonia solution were obtained from Wako (Osaka, Japan). Methanol and acetonitrile were obtained from Kanto (Tokyo, Japan). Ethanol and isopropanol were obtained from Yoneyama (Osaka, Japan). All reagents were analytical grade and were used without further purification.

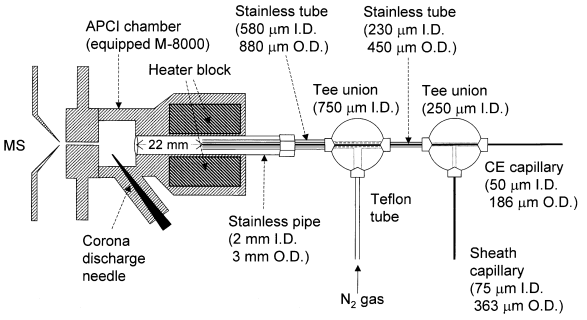

Fig. 2. Schematic outline of modified APCI interface.

2.4. Procedure

Stock solutions of DCPs (1000 mg/l) were prepared in methanol–water (20:80, v/v). Sample solutions were made by dilution the stock solutions with running buffer. Each concentration of solute was 100 mg/l unless otherwise described.

Optimization of ESI conditions was investigated using 2,4-DCP as a test sample. The sample solution (100 mg/l) was directly introduced into the ESI interface at 4 μl/min with a Harvard Apparatus syringe pump. The conditions were optimized to obtain highest signal intensity of DCP molecular ions. The molecular ion peaks appear at $m/z=161$, 163 and 165 at the intensity ratio 9:6:1 because DCP has two chlorine atoms. Therefore the total intensity of the range of $m/z=160$–166 was used for the comparison of signal intensity.

CE–MS was performed using the optimum conditions for MS detection. A mixture of six DCPs (each 50 mg/l) was used as a test sample. The sample solution was introduced by gravity, 5 cm×60 s and then a constant CE voltage of 15 kV was applied. After 1 min from the start of the run, ESI voltage was applied at the interface. The flow-rates of sheath liquid and the nitrogen gas were 4 μl/min and 0.6 l/min, respectively.

A preliminary investigation of the modified APCI interface was performed using the optimized conditions for ESI direct injection mode apart from the sample concentration and the flow-rate which were 1 mg/l and 10 μl/min, respectively. The corona discharge voltage and the temperature of the heater block were set at −3.5 kV and 350°C, respectively.

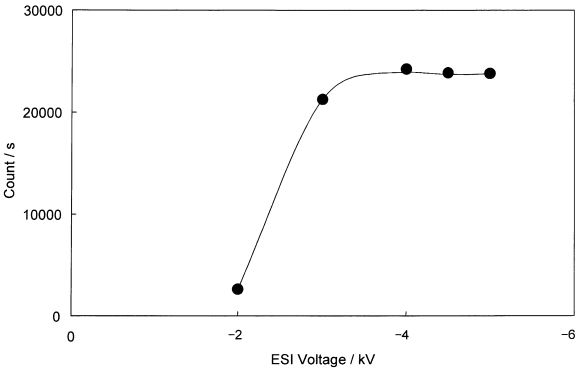

Fig. 3. Dependence of the signal intensity on ESI applied voltage. Conditions: sample, 2,4-dichlorophenol; concentration, 100 mg/l; sample matrix, 20 m*M* hydrogencarbonate–methanol (1:1); sample flow-rate, 4 μl/min; nitrogen gas flow-rate, 0.6 l/min.

3. Results and discussion

3.1. Optimization of ESI conditions

In order to investigate the effect of ESI applied voltage, we used 20 m*M* ammonium hydrogencarbonate–methanol (1:1) as the solvent. The result is shown in Fig. 3. The signal intensity reached a maximum below −4 kV, therefore we determined the optimum voltage was −4 kV. An unstable signal intensity was obtained below a nitrogen gas flow-rate

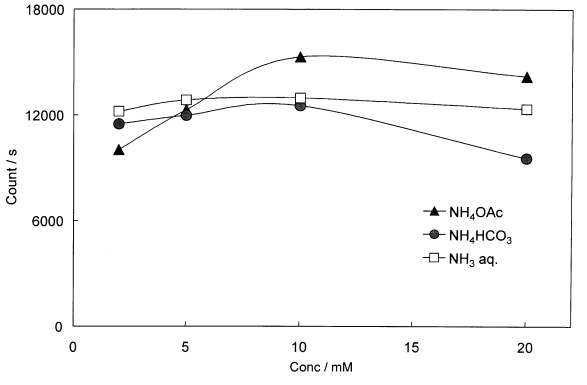

Fig. 4. Effect of the salt and their concentration on the signal intensity. Conditions: ESI voltage, −4 kV; other conditions as in Fig. 3.

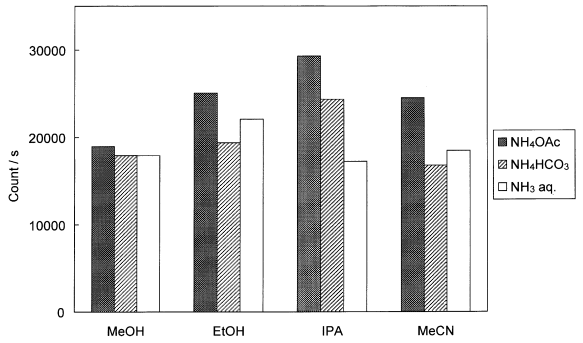

Fig. 5. Effect of the organic solvent on the signal intensity. Other conditions as in Fig. 3.

of 0.6 l/min. The flow-rate could not be raised moreover because of the laboratory-made interface.

We investigated the three kinds of salts, ammonium acetate, ammonium hydrogencarbonate and ammonium hydroxide (ammonia solution) as the CE buffer components. These salts were not mixed with one another and were used without pH adjustment. The effect of salts and their concentration in a mixture with methanol (1:1) is shown in Fig. 4. Ammonium acetate gave slightly higher intensity than that given with other salts at 10 m*M*. The optimum concentration of all salts was determined to 10 m*M*.

The effect of organic solvents was also investigated. Four organic solvents, methanol, ethanol, isopropanol and acetonitrile were used with 10 m*M* buffer (1:1). The result is shown in Fig. 5. The use of isopropanol gave the highest signal intensity for ammonium acetate or ammonium hydrogencarbonate. For ammonia solution, use of ethanol was the best. Some papers reported that isopropanol gave the best results among the solvents for negative ionization on ESI [7,9,15] but the reason was not clear.

3.2. CE–MS experiment

The use of an ammonium acetate–isopropanol (1:1) mixture was the most effective in the ionization of 2,4-DCP. However, the ammonium hydrogencarbonate solution (pH 8.1) gave better separation in CE than the ammonium acetate solution (pH 6.9)

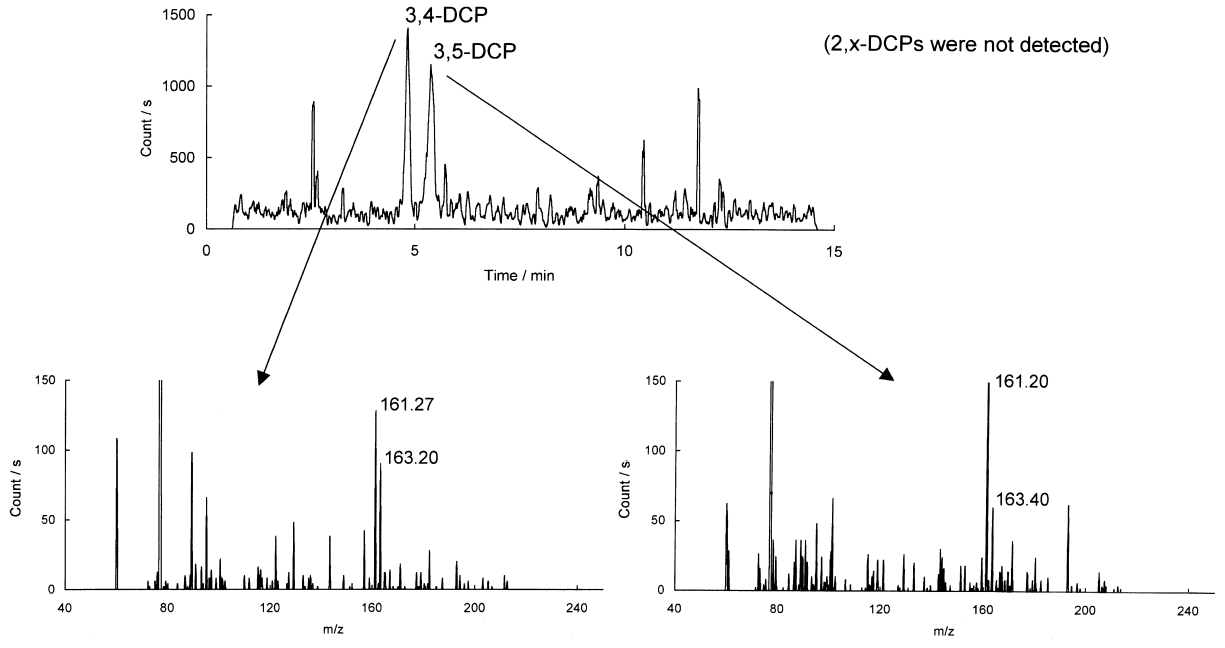

Fig. 6. Ion electropherogram and peak spectra of DCP isomers by CE–MS. Conditions: capillary, 50 μm I.D.×186 μm O.D., 54 cm in total length; sample concentration, 50 mg/l each; sample injection, 5 cm×60 s; separation solution, 10 m*M* ammonium hydrogencarbonate (pH 8.1); sheath liquid, a mixture of separation solution and isopropanol (1:1) at 4 μl/min; CE voltage, 15 kV; ESI voltage, −4 kV.

because the DCPs were more dissociated in the former. So we used ammonium hydrogencarbonate as separation solution for CE–MS.

The result of CE–MS is shown in Fig. 6. Two peaks were obtained and identified; 3,4- and 3,5-DCP. 2,3-, 2,4-, 2,5- and 2,6-DCP were not detected. Therefore, we checked their signal intensity in the direct mode. The result is shown in Fig. 7. The intensities of 2,*x*- and 3,*x*-isomers were quite different under the optimum ESI conditions. For the simultaneous determination of DCP isomers, an improvement of sensitivity especially for 2,*x*-isomers is necessary. From the data of Jáuregui et al. [15], there was not such a difference in the sensitivity of DCPs. We guess that the difference in the sensitivity of the isomers was due to the ESI capillary heating because the 2,*x*-isomers are more ionic than the 3,*x*-isomers at pH 8, therefore they are less vaporized on the ESI interface without heating. Their reported sensitivity, however, was not enough for the direct determination of DCPs in environmental waters, either.

3.3. Preliminary results obtained using the APCI interface

The ionization of DCPs using ESI seems not to be sufficient for CE–MS, therefore we tried to develop an APCI interface applicable to CE–MS. A semi-micro APCI interface equipped M-8000 was made for semi-micro HPLC, therefore the lowest flow-rate was 50 μl/min. We modified the interface as described in Section 2.2 and obtained a preliminary result on the direct injection mode. The result is shown in Fig. 8. The injection amount of APCI calculated from sample concentration and flow-rate was 1/40 compared with that of ESI. The signal intensity of 3,*x*-DCP, however, was almost the same as that obtained with ESI. The intensity of isomers was not so different compared with the results with ESI interface. The sensitivity of DCPs in the direct injection mode was improved by using APCI. The CE–MS analyses using modified APCI interface have not been successful yet because of the instability of the electrical contact in the interface. Therefore, we are promoting further modification of the interface, for example changing the diameter of capillary and tubes, to reduce the flow-rate and to maintain electrical contact. We are also trying the on-line concentration method of DCPs in CE to improve the sensitivity [21].

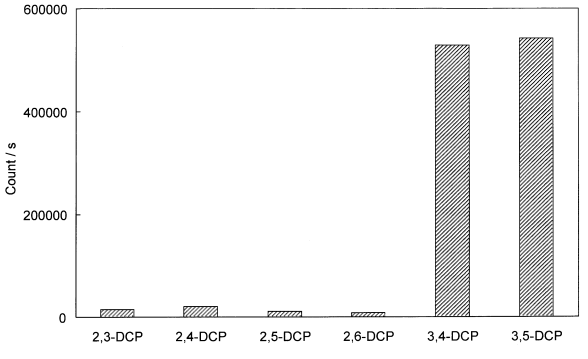

Fig. 7. Comparison of signal intensity of each DCP in the direct injection mode using the laboratory-made ESI interface. Conditions: DCP concentration, 100 mg/l. Other conditions as in Fig. 3.

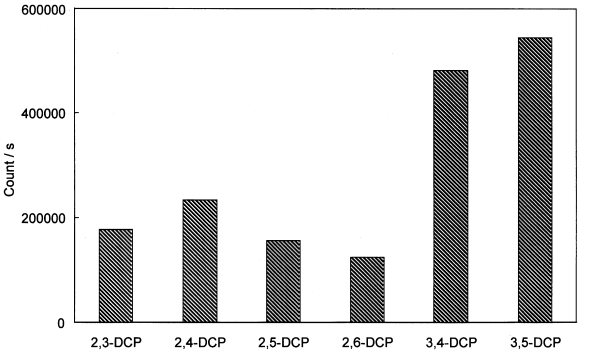

Fig. 8. Comparison of signal intensity of each DCP in the direct injection mode using modified APCI interface. Conditions: DCP concentration, 1 mg/l; flow-rate, 10 μl/min; other conditions as in Fig. 3.

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