Application of Polyamide Column to the Concentration and Fractional Analysis of Some Organic Compounds Responsible for Environmental Water Pollution

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We previously reported the efficiency of polyamide column in the concentration of polychlorodibenzo-*p*-dioxins (PCDDs) from environmental water samples. This study further applied the polyamide column to concentrate of the other organic compounds causing the pollution of waters: polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs) and chlorophenols (CPs) from their aqueous solutions.

The model compounds used were: benzo[*a*]pyrene, pyrene, anthracene and mono-trichloropyrenes (PAHs); KC300, KC400 and KC500 (PCBs); and 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol (CPs). The mixture of these compounds in much higher concentrations compared to their putative environmental existence were quantitatively adsorbed to the polyamide column. The fractional elution of the test compounds from the column was attained with ethanol (PCBs and polar PAHs), dichloromethane (nonpolar PAHs) and 5% acetic acid in dichloromethane (CPs plus PCDDs), in this order.

Each fraction could be analyzed by either high performance liquid chromatograph equipped with a fluorescence monitor in the case of PAHs or gas chromatograph with an electron capture detector for PCBs and CPs.

The polyamide column will be useful for the analysis of trace amounts of organic pollutants in environmental waters.

Key words — polycyclic aromatic hydrocarbons; polychlorobiphenyls; chlorophenols; environmental waters; polyamide column

Introduction

The analysis of toxic compounds in environmental waters requires the use of appropriate concentration procedures because of the extremely low concentrations. The column method is suitable for the treatment of a large volume of water samples. At present, columns packed with adsorbents such as Amberlite XAD-2, charcoal and polyurethane foam are popularly used.¹⁻³⁾ However, the recovery of some compounds like dioxins, polychlorobiphenyls (PCBs) and benzo[*a*]pyrene from these adsorbents is not satisfactory.

Polyamide is a high performance adsorbent for trace compounds from the viewpoints of adsorption capacity, range of compounds to be covered, physical and chemical stabilities, flow rate and price. The analyses of dyes and caffeine in food samples using polyamide powder have been reported.⁴⁻⁶⁾ However, there has been no report concerning the application to toxic organic compounds in environmental waters except for our previous one on dioxins (PCDDs).⁷⁾

In this study, the polyamide column was evaluated for its adsorption capacities for PCBs, polycyclic aromatic hydrocarbons (PAHs) and chlorophenols (CPs) and the conditions for fractional elutions of these compounds, aiming at its application to environmental water samples.

Materials and Methods

Reagents — Three PAHs, *i.e.*, pyrene, benzo[*a*]pyrene and anthracene, and 3 CPs, *i.e.*, 2, 4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP), were purchased from Wako Pure Chemical Industries, Ltd. KC300, KC400 and KC500 used as PCBs are the products of Kanegafuchi Chemical Industries, Ltd. The mixture of mono- — trichloropyrenes was synthesized by chlorinating pyrene according to the method of Colmsjö *et al.*⁸⁾

Polyamide Column — Polyamide (Wako Pure Chemical Industries, Ltd.), washed with elution solvents such as ethanol, dichloromethane and 5% acetic acid in dichloromethane(5% acetic acid/dichloromethane) and air-dried, was packed, in a water suspension, into a glass column 20 mm in diameter and 300 mm in length.

Equipment for Analysis — PAHs were analyzed using HPLC (LC6A, Shimadzu Co.) on a reverse phase column (Licrosorb RP18, Merck Co., Inc., 4 mm i.d. × 250 mm) with a fluorescence monitor (Ex.: 305 nm, Em.: 430 nm). Acetonitrile – water (7:3) was used in a mobile phase at a flow rate of 2 ml/min.

The analyses of PCBs and CPs were performed using a gas chromatograph (GC-9A, Shimadzu Co.) equipped with a ⁶³Ni electron capture detector (ECD). Nitrogen carrier gas was used at a flow rate of 5 ml/min.

Adsorption Experiment — Twenty ml of aqueous solution containing 5 μg each of anthracene, pyrene and benzo[*a*]pyrene was charged on the polyamide column. The column was washed with 100 ml of distilled water. The pass-through and washing were combined and extracted with *n*-hexane. After *n*-hexane was evaporated, the residue redissolved in acetonitrile was analyzed by HPLC. In parallel, the parental mixture was processed similarly.

The mixtures of 10 μg each of KC300, KC400 and KC500, and 10 μg each of TCP, TeCP and PCP were treated in the same manner except that the analysis was done using GC-ECD instead of HPLC.

Elution Condition — A series of solvents were screened for the fractional elution of PCBs, PAHs and CPs. PCBs and polar PAHs were easily eluted by ethanol, being separated from the majority of PAHs and CPs. Taking the *R_f* values and spot-shapes on TLC into

consideration, dichloromethane and 5% acetic acid/dichloromethane were selected as the best elution solvents for PAHs and CPs, respectively. To prevent environmental pollution with dichloromethane as much as possible, the solvent was used in a recycled fashion by employing fractional distillation.

Individual compounds in PAHs, PCBs or CPs retained on the polyamide column were eluted with the selected solvents. The eluate was fractionated into 20-ml portions, and these fractions were analyzed with the help of HPLC or GC-ECD as above described.

Results and Discussion

Adsorption Specificity

PAHs, PCBs and CPs were completely adsorbed to the polyamide columns as shown in Figs. 1, 2 and 3, respectively, even though samples were used at far higher concentrations than those in environmental waters. Based on these data, the polyamide column seems be a generally applicable high-affinity adsorbent for these toxic compounds.

Elution Conditions

In application of the polyamide column to the concentration of certain substances, the efficacy in the sample elution from the column is important as well as the quantita-

Fig. 1. HPLC Chromatograms of Parental PAH Sample and Polyamide Column Effluent

Column: RP-18, detector: fluorescence monitor (Ex.: 305nm, Em.: 430nm), mobile phase: acetonitrile-water (7 : 3), flow rate: 2ml/min.

tive adsorption. The elution profiles of pyrene, benzo[*a*]pyrene and anthracene are shown in Fig. 4A and mono-, di- and trichloropyrenes in Fig. 4B. The column was first

Fig. 2. Gas Chromatograms of Parental PCBs Sample and Polyamide Column Effluent
Column: SPB-5, 60m, column temp. : 120–190°C, detector: ECD, carrier gas: N₂, 5ml/min.

Fig. 3. Gas Chromatograms of Parental CP Sample and Polyamide Column Effluent
Column: SPB-5, 60m, column temp. : 120–190°C, detector: ECD, carrier gas: N₂, 5ml/min.

developed with 100 ml of ethanol followed by 200 ml of dichloromethane. To avoid the adverse effect of water remaining in the column following elution with dichloromethane, the elution with water-miscible ethanol preceded to the elution with dichloromethane.

Comparison of the elution profiles showed that anthracene, pyrene and benzo[*a*]-pyrene were eluted in this order and all three chlorinated pyrenes were eluted in between pyrene and benzo[*a*]pyrene.

The elution profiles of three PCB mixtures, KC300, 400 and 500, and tri- to pentachlorophenols are shown in Fig. 5. After these mixtures were completely eluted with 100 ml of ethanol, CPs were eluted with 300 ml of 5% acetic acid/dichloromethane. As in the case of PCDDs in the previous paper,⁷⁾ the elution of CPs was refractory to dichloromethane.

Recovery

Recoveries from the polyamide column of the compounds tested in this study are shown in Table 1, as well as that of PCDDs. The recovery of PAHs, PCBs and CPs was found to be almost quantitative, suggesting that the polyamide column might be applicable to the concentration and partial fractionation of organic pollutants in environmental water samples.

Based on these data, the analytical procedures for the organic compounds in environmental waters are proposed as depicted in Chart 1. After the adsorption of ingredient compounds in water samples, the elution should be started with ethanol for not only the recovery of PCBs and polar PAHs but

Fig. 4. Elution Profiles of PAHs on Polyamide Column with Ethanol and Dichloromethane

Fig. 5. Elution Profiles of PCBs and CPs on Polyamide Column with Ethanol and 5% AcOH/dichloromethane

Table 1. Recovery from Polyamide Column

Compounds	Recovery(%)
Benzo[<i>a</i>]pyrene	98
Pyrene	100
Anthracene	98
KC300	93
KC400	101
KC500	97
TCP	98
TeCP	102
PCP	100
PCDD ^{a)}	97

a) Nakahama, *Jpn. J. Toxicol. Environ. Health*, **39**, 469 (1993).

also the removal of water remaining in the columns. PAHs, PCBs and CPs can be concentrated simultaneously and partially fractionated by this method. If the analysis is limited to CPs and/or PCDDs, the step of elution with dichloromethane is conveniently omitted. In contrast, if PAHs are the target, both ethanol and dichloromethane fractions should be combined in advance of the GC-ECD analysis. PCDDs and CPs can be fractionated from one another since PCDDs are recovered in 1% acetic acid/dichloromethane and CPs in 5% acetic acid/dichloromethane.

The polyamide column was first introduced by one of the authors (NT) for

the efficient concentration of as low as picogram order of PCDDs in an environmental sample, since the conventional methods using polyurethane foam or XAD-2 could not attain the quantitative retention of PCDDs, which were hardly eluted from the activated charcoal.^{1-3,9)} The method based on the hydrogen bonds between intramolecular oxygens in PCDDs and amide groups in the adsorbent was superior to others in terms of adsorption capacity and recovery. Furthermore, the polyamide column was proven to be applicable to a wide range of compounds represented by PAHs in the present study; in these cases the adsorption to the column would be driven by the hydrophobic interac-

Chart 1. Proposed Procedures for Concentration and Partial Fractionation of Organic Compounds in Environmental Waters

tion and/or hydrogen bond.

Conclusion

Organic compounds causing the pollution of environmental waters could be concentrated simultaneously and partially fractionated using the polyamide column. It was elucidated that PAHs, PCBs and CPs in water could be quantitatively adsorbed to and differentially eluted from the polyamide column; the elutions of PCBs plus polar PAHs, other PAHs and CPs are achievable with ethanol, dichloromethane and 5% acetic acid/dichloromethane, respectively. The polyamide column method is, therefore, hopefully applicable to the analysis of various organic pollutants in environmental waters.

References

- 1) Utsumi H., Mitade C., Suzuki S., Nakazawa H., Fujita M., Hamada A., *Eisei Kagaku*, **35**, 273 (1989).
- 2) Onodera S., Yoshimatsu K., Saitoh H., Suzuki S., *Eisei Kagaku*, **35**, 1 (1989).
- 3) Rappe C., Kjeller L.-O., Andersson R., *Chemosphere*, **19**, 13 (1989).
- 4) Shyu J.F., Lee A.S., Hong J.D., *J. Food Drug Anal.*, **3**, 65 (1995).
- 5) Silva J.M.R.D., Rosec J.P., Bourzeix M., Heredia N., *J. Sci. Food Agri.*, **53**, 85 (1990).
- 6) Nakabayashi T., Masano M., *J. Jpn. Soc. Food Sci. Technol.*, **33**, 729 (1986).
- 7) Nakahama T., *Jpn. J. Toxicol. Environ. Health*, **39**, 469 (1993).
- 8) Colmsjö A., Rannig A., Rannig U., *Mutat. Res.*, **135**, 21 (1984).
- 9) Rossum P.V., Webb R.G., *J. Chromatogr.*, **150**, 381 (1978).