Retention Behavior of Halogenated Benzenes on a Stationary Phase Having a 2,4-Dibromophenoxy Group as the Halogen Recognition Functional Group

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Introduction
Organo-chlorinated compounds are widely used as raw materials for industrial and agricultural products, such as plastics, flame retardants, medicines and pesticides. However, many of these compounds are considered to be harmful to mammals. Polychlorinated biphenyls and organo-chlorinated pesticides, such as dichlorodiphenyltrichloroethane and hexachlorocyclohexanes, are bio concentrated in the food chain, and have long-term reproductive/health effects in wildlife and humans. An international meeting classified organo-chlorinated compounds as persistent organic pollutants (POPs) and recommended that their use be strictly limited. Subsequently, biodegradable organo-chlorinated pesticides were developed to protect the environment. However, continuous monitoring of even these biodegradable compounds is necessary to evaluate their risk to humans and wildlife.

Liquid chromatography–mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS) are commonly used to analyze residual pesticides. These methods have high selectivity and sensitivity but they require precleaning of the samples for best results. Excess matrix components cause the matrix effect, which is defined as “the combined effect of all components of the sample other than the analyte on the measurement of the quantity” in this analytical technology. However, there are presently no sample preparation procedures that can separate all the matrix components from the target pesticides, even when done by a skilled analyst.

We have developed new adsorbents for organo-chlorinated chemicals including residual pesticides that use the dispersion force created by halogen atoms. The adsorbents were synthesized by introducing halogenated phenoxy groups onto glycidyl groups on a porous glycidylmethacrylate resin surface. Halogen atoms such as chlorine, bromine and iodine have large atomic polarizabilities, causing halogenated molecules to strongly interact via London dispersion forces. Organochlorinated adsorbents strongly retain halogenated phenols in organic solvents, such as ethyl acetate, acetone and 1-propanol. These materials bind halogenated phenols by Debye interactions (dipole-induced dipole interactions), in which the affinity depends on the polarizability of the target chemical.

Tanaka and coworkers developed several silica stationary phases immobilized with halogenated aromatic rings to examine the dispersibility of heavy atoms. These columns were used to separate fullerene isomers, halogenated compounds and deuterium compounds. They found that the 3-(pentabromobenzyloxy)-propyl (PBB) group immobilizes silica and had a high specificity for halogenated benzenes.

Our new adsorbents mentioned above were not suitable for the solid-phase extraction of halogenated benzenes having no phenolic hydroxy group, although it was expected that they would also show high affinity for halogenated benzenes. It was estimated that their retention abilities based on the dispersion force might be lower than those of the adsorbents that were developed by Tanaka and coworkers. On the other hand, our adsorbents having halogenated phenoxy groups could show unique retention behavior based on multiple interactions including π–π interaction, CH/π hydrogen bonding and the Debye interaction. In particular, it was thought that the Debye interaction might be the source to produce novel specificity. Here, we evaluated the effect of a Debye interaction on the
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separation of halogenated benzenes by using an LC column packed with a resin that immobilizes the 2,4-dibromophenoxy group (DBP-resin).

Experimental

Reagents and chemicals
Most of the reagents for the synthesis of the DBP-resin were purchased from Wako Pure Chemical Industries (Osaka, Japan). Glycidylmethacrylate (GMA) and ethylenedimethacrylate (EGDM) (both from Tokyo Chemical Industries, Tokyo, Japan) were used as a functional monomer and a cross-linker, respectively. Butylacetate and 3-methylbutanol were chosen as inert diluents for pore generation. We selected 2,2-azobis(isobutyronitrile) as a polymerization initiator. Polyvinylalcohol (n=500) was used as a suspension stabilizer. Chromatographic properties were evaluated with four analytes purchased from Tokyo Chemical Industries: p-Xylene, 1,4-difluorobenzene, 1,4-dichlorobenzene and 1,4-dibromobenzene. Other analytes were purchased from Wako Pure Chemical Industries.

Liquid chromatography
The chromatographic column (4.6 mm i.d. × 150 mm) was prepared by a slurry packing technique. The LC system consisted of a GL Sciences (Tokyo, Japan) GL-7410 pump, a GL-7452A PDA detector, a GL-7420 auto sampler and a GL-7432 column oven. A SunShell C18 (4.6 mm i.d. × 150 mm, 2.6 μm) from ChromaNik Technologies (Osaka, Japan) was used for comparison with the DBP-resin in this experiment. Aqueous acetonitrile and aqueous methanol were used as the mobile phases, which are commonly used in reversed-phase mode. The separation factor α was calculated as follows;

\[ \alpha = \frac{k_{\text{solute1}}}{k_{\text{solute2}}} \]  \[ \text{(1)} \]

where \( k_{\text{solute}} \) is the retention factor of the solute.

Synthesis and characterization of the DBP-resin
A base resin for the LC stationary phase was prepared by a suspension copolymerization of GMA and EGDM according to our previous paper.10 The specific surface of the base resin classified as 10 μm was about 160 m2 g−1. Then, 2,4-dibromophenol was introduced onto the base resin by heating it in xylene at 116°C for 8 h in the presence of a catalyst, triphenylphosphine. Next, residual epoxy groups were converted to diol groups by reaction with sulfuric acid (0.01 mol L−1).

Results and Discussion

Retention of halogenated aromatics on the DBP-resin and ODS
We previously synthesized many adsorbents immobilized with brominated phenoxy groups.10 Adsorbents with DBP groups were better able to adsorb halogenated phenols per quantity of functional group than adsorbents with p-bromophenoxy or 2,4,6-tribromophenoxy groups. Consequently, the separation behaviors of halogenated solutes in reversed-phase liquid chromatography were examined on the DBP-resin and octadecylsilane (ODS) columns. in this study. We used 1,4-dihalogenated benzenes as probe solutes because their dipole moments are 0 D, which makes the dipole-dipole interaction between solutes free. To evaluate the specificity of such probes, non-halogenated benzene is necessary as a reference solute. Furthermore, it is preferable that the substituent used as a reference is a hydrophobic group that has a steric effect equal to the steric effect of halogen groups, so p-xylene was adopted.

Figure 1 shows the effects of organic modifier ratios in aqueous acetonitrile and aqueous methanol as the mobile phase on the separation factor α calculated from Eq. (1) on both stationary phases. The α values based on 1,4-dichlorobenzene and p-xylene were almost unity over a wide range of organic modifier ratios, and both benzenes were difficult to separate on ODS. The α values for 1,4-difluorobenzene/p-xylene increased slightly as the organic modifier ratio increased, but those for 1,4-dibromobenzene/p-xylene decreased. The α values on the
DBP-resin were larger than those on ODS for all halogenated benzenes. The curve plotted for the $\alpha$ values of 1,4-dibromobenzene/p-xylene had a relative minimum at 70 – 80% of organic solvent ratio, but those of 1,4-difluorobenzene/p-xylene had a relative maximum. Figure 2 shows chromatograms of p-xylene and 1,4-dichlorobenzene on the DBP resin and ODS at 50% of acetonitrile and 70% of methanol. The number of theoretical plates, $N$, of 1,4-dichlorobenzene on the DBP-resin was 1270 in the acetonitrile mobile phase, whereas that in the methanol mobile phase decreased to 860. In Fig. 2, the mutual separation of benzene and xylene was accomplished, but the $N$ values were low for particle diameter of 10 $\mu$m. These results might suggest that the secondary effect interaction such as the dispersion force or the $\pi$–$\pi$ interaction contributes to aromatic separation.

The separation behavior in reversed-phase liquid chromatography depends on the hydrophobicity of the solute. Halogenated benzenes were retained on both ODS and DBP-resins by partition to organic solvation layer on surface of the stationary phase.16 The elution order for 1,4-dichlorobenzene and p-xylene on ODS, which is shown in Fig. 2, was reversed, although their Log $P$ values were 3.44 and 3.15, respectively.13 The solubility parameter $\delta$ value17 of chlorobenzene, xylene, acetonitrile and methanol was 9.6, 8.8, 11.8 and 12.9, respectively. Further, the dispersion solubility parameter $\delta_D$ value17 of each solvent was 9.2, 8.8, 6.5 and 6.2, respectively. Solvents with similar $\delta$ and $\delta_D$ values are miscible. When the organic modifier ratio in the mobile phase is high, halogenated benzenes are thickly solvated by the organic modifiers and do not bind well to the resin. And the retention in the acetonitrile mobile phase was smaller than that in the methanol. Consequently, it was estimated that the retention selectivity between the functional group and solutes on the DBP-resin was governed by the dispersion force. However, the retention behavior on the DBP-resin and ODS cannot be explained only with $\delta_D$ values.

Hansen improved Hildebrands solubility parameters and introduced new solubility parameters based on total energy of vaporization.18 In his theory, the affinity between two chemicals is inversely proportional to the solubility parameter distance, $Ra$.

$$Ra = \left(4(\delta_D - \delta_D^0)^2 + (\delta_D - \delta_D^H)^2 + (\delta_D - \delta_D^H)^2\right)^{0.5}$$

where $\delta_D^0$, $\delta_D^H$ and $\delta_D^H$ are the dispersion solubility parameter, the polar solubility parameter and the hydrogen bonding parameter, respectively. These parameters of octadecene (as a substitute for the functional group of the ODS column), 1-bromo-4-ethoxybenzene (as a substitution for analogue of DBP-resin), p-xylene and 1,4-dichlorobenzene and Ra values calculated from Eq. (2) are summarized in Table 1. The stronger affinity of dichlorobenzene on the DBP-resin than that of p-xylene would explain the increase in $\alpha$ between 1,4-dichlorobenzene and p-xylene at organic modifier ratios above 80%. Although Ra values on the ODS column turn over, the decrease in $\alpha$.

| Table 1 | Dispersion solubility parameter, polar solubility parameter, hydrogen bonding parameter and solubility parameter distance (Ra) |
|---------|-------------------------------------------------------------------------------------------------------------------|
| Hansen solubility parameters | Ra (J cm$^{-1}$)$^{0.5}$ |
| $\delta_D$  | $\delta_P$  | $\delta_H$  | Octadecene (ODS) | Bromoethoxy benzene (DBP) |
| Octadecene | 16.4 | 0 | 0 | 16.4 | |
| 1-Bromo-4-ethoxy benzene | 19.5 | 7.7 | 5.3 | 19.5 | 7.7 | 5.3 |
| p-Xylene | 17.6 | 1 | 3.1 | 4.0 | 8.0 |
| 1,4-Dichlorobenzene | 19.7 | 5.6 | 2.7 | 9.1 | 3.4 |
between them was not seen due to their small retention factors. On the other hand, the Ra values of methanol for 1,4-dichlorobenzene and p-xylene were 22.7 and 22.8, respectively. Further, the Ra values of acetonitrile for 1,4-dichlorobenzene and p-xylene were 15.6 and 17.9, respectively. Although the Ra values for 1,4-dichlorobenzene of each solvent were almost equal to those for p-xylene, the DBP-resin showed clearly higher affinity for 1,4-dichlorobenzene than that for p-xylene.

The dispersion force acting on a compound acts on their halogen groups. The values of atomic polarizability expressing the disposability of atoms of fluoride, chlorine, and bromine are 0.557, 2.18, and 3.05 \( \times 10^{-24} \) cm\(^3\), respectively. The \( \alpha \) values of halogenated benzenes at high organic modifier ratios became large in this order on the DBP-resin. Therefore, dispersion forces appear to make a large contribution to the retention of halogenated benzenes on this column.

**Retention of halogenated aromatics on DBP-resin and PBB-silica**

Tanaka and coworkers developed several silica stationary phases immobilized with halogenated aromatic rings. They showed that the 3-(pentabromobenzyloxy)-propyl (PBB) group immobilizes silica (PBB-silica) and had a high specificity for halogenated benzenes based on the disposability. The separation factors \( \alpha \) value calculated from the retention factors of dihalogenated benzenes and p-xylene on the DBP-resin in this study were all larger than those reported for the PBB-silica in the previous study (Table 2). We previously concluded that the affinity of the DBP-resin toward halogenated phenols was explained by the Debye interaction, in which the affinity depends on the polarizability of the target solute. The selectivity for the halogenated phenols of the DBP-resin depends on the number of halogen substituents. This conclusion is supported by the finding that the DBP-resin has a larger orientation dipole moment than the PBB-silica.

On the contrary, the separation between p-xylene and toluene on the DBP-resin was the worst in three kinds of stationary phases. The contribution of the hydrophobic effect between the stationary phase and solutes on the DBP-resin might be lower than that on ODS or PBB-silica.

**Table 2**  Retention and separation factors for halogenated aromatics on the DBP-resin, ODS and PBB-silica

| Solutes         | DBP-resin | ODS | PBB-silica | Log \( P^a \) |
|-----------------|-----------|-----|------------|---------------|
|                 | \( k \)   | \( \alpha_{\text{chloro}} \) | \( \alpha_{\text{xylene}} \) | \( k \) | \( \alpha_{\text{chloro}} \) | \( \alpha_{\text{xylene}} \) | \( k \) | \( \alpha_{\text{chloro}} \) | \( \alpha_{\text{xylene}} \) |
| Benzene         | 1.10      | 1.60 | 0.56       | 1.22          | 0.56    | 2.13                          |
| Toluene         | 1.37      | 2.78 | 0.56       | 2.16          | 0.56    | 2.73                          |
| p-Xylene        | 1.68      | 4.95 | 1.78       | 3.86          | 1.79    | 3.15                          |
| Fluorobenzene   | 1.11      | 1.48 | 0.53       | 1.16          | 0.54    | 2.27                          |
| Chlorobenzene   | 1.71      | 2.65 | 0.95       | 2.29          | 1.06    | 2.84                          |
| Bromobenzene    | 2.09      | 3.11 | 1.12       | 2.99          | 1.38    | 2.99                          |
| Iodobenzene     | 2.70      | 3.96 | 1.42       | 4.38          | 2.03    | 3.25                          |
| 1,4-Difluorobenzene | 1.13  | 0.67 | 1.33       | 0.27          | 1.08    | 0.28                          |
| 1,4-Dichlorobenzene  | 2.76  | 1.64 | 4.33       | 0.87          | 4.44    | 1.15                          |
| 1,4-Dibromobenzene  | 4.17  | 2.48 | 5.98       | 1.21          | 7.67    | 1.99                          |
| 1,4-Diodobenzene  | 7.05  | 4.20 | 9.46       | 1.91          | —b       | 3.76                          |

Conditions are the same as those given in Fig. 1 except for mobile phase. Mobile phase: methanol/water = 70/30. a. Ref. 13, b. no data.

**Conclusions**

A stationary phase with dipole interaction was synthesized by introducing DBP groups onto the surface of a hydrophilic porous resin. The chromatographic properties for halogenated benzenes were evaluated on the column packed with the DBP-resin. The DBP-resin showed higher selectivity for halogenated benzenes than those on ODS or PBB-silica. It was considered that the Debye interaction caused the selectivity for halogenated benzenes to the DBP-resin. The retention of the solutes on the DBP-resin will ultimately depend on not only the Debye interaction shown in this study, but also multiple interactions including \( \pi-\pi \) interaction, CH/\( \pi \) hydrogen bonding and dispersion force.

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