❑ Summary

Study on the Creation of Novel Molecular Recognition Fields Specialized in an Electric Dipole Interaction

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Background and aims on this study

In the measurement of trace chemical compounds in the actual samples with large matrices, the separation and elimination of matrices in the sample solution is important as well as the effective extraction and concentration of the target solutes. Although various kinds of separation modes are used in high performance liquid chromatography (HPLC) and the solid phase extraction (SPE), the reversed phase (RP) partition mode is used most widely in a reason of its extensive use coverage. In the separation with RP mode, e.g., the separation using octadecyl silica (ODS) column, the separation will be accomplished by a difference of the hydrophobic affinity with stationary phase (ODS) and the solute. The separation behavior will be depends on water - octanol distribution constant (Log $P_{o/w}$) of a solute. A distribution constant is a value indicating the general characteristic of molecules called "solubility". In RP separation of the samples including complicated matrices, such as environment, foods and living body, the target solute will be often hardly separated from matrices having similar Log $P_{o/w}$. Although RP modes are widely used in spite of its poor molecular recognition ability, it is estimated that the spread of hyphenation technique with the high sensitive / high selective detector such as mass spectrometers is a main reason. The use of the hyphenation technique will allow the high sensitive / selective detection by monitoring at the specific mass number of the target solute, even if the separation by the column is a little poor. The use of the mass spectrometer can be called "high fusion of separation and the

detection", but it only behaves as the accomplished separation by depending upon the power of the mass spectrometer. The spread of such systems may contribute to the expansion of the separation analysis, but it seems that an isolation / purification by the separation which are the essence of the separation analysis were given up.

An ultimate aim of this study is performed to create the selective/specific molecular recognition ability by recurring to the root of the separation in the chromatography. To create unachievable unique separation mechanism by the conventional separation mode, the possibility of the novel separation field creation was pursued by developing the separation materials having weak interactions which have been overlooked as the secondary effect interaction. In this study, the author focused attention on an electric dipole interaction including the dispersion power and electron‐withdrawing to develop the separation materials having weak forces as main interactions, and developed several separation materials immobilized with halogenated phenoxy group, nitrophenoxy group and cyanophenoxy onto hydrophilic polymer matrix. The retention characteristics on the developed separation materials were evaluated using SPE and HPLC in detail, and the separation mechanism were elucidated. From these evaluation results, it was demonstrated that the usefulness of the utilization of weak power to produce selective / specific interaction. Furthermore, these separation materials were applied to the extraction / separation of pesticide and mold toxin in foods.

By utilizing the obtained knowledge in this study, the author aimed at "the production of the selectivity / specificity employing weak interaction" and "the development of the specific separation materials not to be affected by matrices".

Development of the solid phase extractants having a dispersion power as the main interaction and its retention properties for halogenated aromatic compounds

To examine the effects of weak intermolecular interactions on the SPE and chromatographic separation, we synthesized some novel stationary phases with a heavy atom effect layer by immobilizing halogenated aromatic rings and hydroxyl groups onto the surface of a hydrophilic base polymer. Using SPE cartridges

packed with the functionalized materials, we found that the heavy atom stationary phases could selectively retain halophenols in organic solvents, such as 1-propanol which blocks the hydrogen bonding, or acetonitrile which blocks the π-π interaction. The extraction efficiency of the materials toward the halophenols depended on the dipole moments of phenoxy groups present as functional groups. On the other hand, the extraction efficiency of solutes toward the functional group depended on their molar refractions, i.e., induced dipole moments. The retention of the solutes to the stationary phase ultimately depended on not only strong intermolecular interactions, but also the effects of weak interactions such as the dispersion force.

Evaluation retention properties of the dispersion force type separation material using HPLC

A stationary phase with dipole moment interaction was synthesized by immobilizing 2,4-dibromophenoxy (DBP) group onto a porous glycidylmethacrylate resin. The chromatographic properties for halogenated benzenes were evaluated on the DBP-resin by reversed phase mode. The separation efficiency of the DBP-resin for *p*-xylene and 1,4-dihalogenated benzenes in water-acetonitrile as a mobile phase was better than those of octadecyl-bonded silica (ODS) and pentabromobenzyloxy propyl-bonded silica. The DBP-resin showed some affinity for halogenated benzenes. Although the separation factors of 1,4-dichloro- and 1,4-dibromobenzenes for *p*-xylene on ODS decreased with increasing organic modifier ratio in the mobile phase, those on the DBP-resin showed minimum at around 80% organic modifier ratio. The result shows that the retention of the solutes on the DBP functional group depends on not only strong intermolecular interactions such as hydrogen bonding and/or hydrophobic interaction, but also weak interactions such as dispersion force.

Development of the separation materials having electron -withdrawing phenoxy group and its retention behavior for substituted aromatic compounds

The solid phase extractants immobilized with p-nitro phenoxy group and p-cyano phenoxy group which had a strong electron - withdrawing characteristics into were prepared based on a thought that the origin of the dispersion power on the separation materials having the halogenation phenoxy groups in the above studies was an electric dipole interaction. And the solid phase extraction properties for halogenated aromatic compounds and benzoic acids were investigated. The non-halogenated aromatic compounds were hardly trapped in these solid phase extractant, but the halogenated phenols were trapped strongly.

Applicable evaluation to the actual sample analyses of the extraction / separation materials having a weak force as the main interaction

Determination of Clopyralid in Whole Grain Using DBP-resin

A novel solid phase extractant that will demonstrate a dispersion force was synthesized to improve the extraction efficiency for halogenated solutes, including clopyralid (3,6-dichloro-2-pyridinecarboxylic acid), which is widely used as a herbicide. The 2,4-dibrominated phenoxy group was immobilized on the surface of a hydrophilic methacrylate resin. The solid phase extraction properties for the halogenated solutes were evaluated by solid phase extraction using a cartridge packed with the synthesized extractant. Clopyralid and its analogues, i.e. benzoic acid, 2,5-dichlorobenzoic acid, pyridine carboxylic acid and so on, were used as probes. This dispersion-force extractant could selectively entrap the halogenated aromatic carboxylic acids including clopyralid in every organic solvent used for the extraction of pesticide residues. The extractant was applied to the analysis of clopyralid in whole-grain samples. The extracts of the milled whole grain by water-acetone solution were passed through the extractant. Cropyralid trapped on

the extractant was eluted by a methanol-water solution containing diethylamine after washing the extractant with an organic solvent; then, the effluent was measured by the HPLC-UV method. The detection limit on the optimized method was 0.16 μg g^{-1} for clopyralid. The recoveries of clopyralid in four kinds of whole grain were good with 88~95% in triplicate.

Determination of Ochratoxin in Alcoholic Drinks Using pNP-resin

Novel solid phase extractants that will demonstrate a electron‐withdrawing characteristics were synthesized and their extraction properties on solid phase extraction was evaluated. The obtained *p*-Phenoxy type solid phase extractants having electron‐withdrawing group substituted at *p*- position on the phenoxy group were able to trap ochratoxin A (OTA) having an electric dipole moment. The obtained *p*-phenoxy type solid phase extractants having electron‐withdrawing group substituted at para position on the phenoxy group were able to trap ochratoxin A (OTA) having an electric dipole moment. Particularly, the *p*NP-resin having *p*-nitrophenoxy group produced high affinity for OTA. The *p*NP-resin was applied to the solid phase extraction of OTA in alcoholic drinks. As a result, high purified efficiency of OTA was obtained, and the peaks based on polyphenols that will interfere to the determination of OTA were not observed on the chromatograms of the eluates from wine and beer by utilizing only *p*NP-resin packed cartridges.

Conclusions

In this study, the creation of the novel separation fields having molecular recognition abilities which are specialized in electric dipole interaction was aimed.

At first, the DBP-resin immobilized with 2,4-dibromophenoxy group onto hydrophilic base resin was developed, and production of the interaction based on the dispersion force and affinity for halogenated aromatic compound on the DBP resin were elucidated by detailed studies. Although the non-halogenated aromatic compounds were hardly trapped on the DBP-resin, the halogenated phenols were clearly trapped. And the DBP-resin was applied to the preparation of pesticide analysis in foods and allowed the determination of trace clopyralid in whole grain.

Furthermore, based on a thought that electric dipole interaction included the essence of the dispersion power, the solid phase extractant immobilized with a nitrophenoxy group and cyanophenoxy group which show strong electron‐withdrawing interaction was prepared. The extraction properties for the aromatic compounds on these solid phase extractants were investigated. The non-halogenated aromatic compounds were scarcely trapped on these solid phase extractants, on the other hand, the halogenated aromatic compound were strongly trapped. The nitrophenoxy type extractant was applied to the preparation of ochratoxin which has clear dipole moment, and trace ochratoxin in alcoholic drinks was determined with no interferences from matrices.

From these findings in this study, it was recognized that a novel/unique selectivity that could not be obtained in the conventional separation mechanism will be created by utilizing the weak power such as the dispersion power and electron‐withdrawing interaction.

This study was accomplished by the following propositions.

" Is it possible to create the selective / specific separation field by only utilizing the weak molecular interaction ? "

Unfortunately, the ultimate solution could not be obtained in this study, but practical "a novel separation field based on electric dipole interaction" could be established. And the basic design concept of the separation fields based on the weak molecular interaction, i.e. dispersion force and electron‐withdrawing force was demonstrated. In future, the author will challenge the unsolved following assignments by utilizing the knowledge provided in this study.

- Elucidation of the influence on retention properties by interaction/solvation of solvent as the eluent/mobile phase
- Elucidation of "the matrix effect" particularly the hydrogen bonding with polar compounds and optimization of the matrix resin
- Creation of the practical selective separation field by hybrid/fusion with multiple weak interaction

Publications in relation to this dissertation thesis

1. Determination of Clopyralid in Whole Grain Using Solid Phase Extractant Immobilizing with 2,4-Dibromophenoxy Group.

   Toshio MIWA, Shiho SUZUKI, Isao SAITOH, Yoshinori INOUE, and Atsushi YAMAMOTO.

   Bunseki Kagaku, **61**, 673 – 677 (2012).

2. Retention of Halogenated Solutes on Stationary Phases Containing Heavy Atoms.

   Toshio MIWA, Atsushi YAMAMOTO, Mitsuru SAITO, and Yoshinori INOUE.

   Molecules, **18**, 5163 – 5171 (2013).

3. Retention behavior of halogenated benzenes on a stationary phase having 2,4-dibromophenoxy group as the halogen recognition functional group.

   Toshio MIWA, Shiho SUZUKI, Rina TAKAI, Ryohei YAMAMOTO, Yoshinori INOUE, and Atsushi YAMAMOTO.

   Analytical Sciences, *submitted*.