別紙様式５(Attached Form 5)

学位論文要旨 Abstract of Thesis

所属専攻 Field: Applied Chemistry and Biochemistry 専攻(Field)
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Title of Thesis
Preparation and evaluation of functional polymer-grafted porous silica for molecular shape recognitive HPLC
（分子形状認識 HPLC のための機能性ポリマーグラフト化多孔質シリカの調製と評価）

Abstract（within 1600 words）

High performance liquid chromatography (HPLC) is playing an increasingly important role in various fields such as research, medicine, food, and manufacturing due to its universal applicability and excellent measurement accuracy. Stationary phase is the core part of the development of HPLC system. In order to meet the diverse separation requirements, it is very important for the development of high-performance liquid chromatography to vigorously develop various new stationary phases. Silica-based stationary phases still occupy the dominant position in HPLC, and this thesis is about the preparation of polymer-modified silica-based stationary phases. Various stationary phases with different separation properties and capabilities can be achieved by immobilizing organic polymers on the surface of silica. Analytes exhibit different retention behavior on stationary phases mainly because of their different interactions with the stationary phase. Separation and identification of isomers is very challenging in reversed-phase liquid chromatography separations and solving this problem is very important in food science and environmental disciplines because sometimes they have similar polarities and differ only in molecular structure. In this thesis, novel polymer grafted silica stationary phases have been prepared, which can separate common analytes (polycyclic aromatic hydrocarbons and nucleosides/ nucleobases, etc) and different aromatic isomers in reversed-phase liquid chromatography (RPLC), hydrophilic interaction chromatography (HILIC) and ion exchange chromatography (IEC). The thesis contents are divided into four chapters as follow:

In chapter 1, we first discuss the development of HPLC systems and introduce three types of stationary phase carrier materials. Then four single HPLC separation modes are introduced, including reversed-phase, normal-phase, hydrophilic interaction, ion-exchange, and size-exclusion chromatography. To better understand the separation and selection capabilities of immobilized relative analytes, the retention mechanisms of RPLC and HILIC were investigated, respectively, and the retention mechanisms present in shape-selective chromatography were highlighted. Subsequently, the development of reversed-phase and hydrophilic columns is introduced, and the structures of various stationary phases are listed. When two or more interaction mechanisms are present on the column, the stationary phase has a mixed chromatographic retention mode, which can improve the separation selectivity of complex real-world samples to some extent. After understanding the development of mixed-mode stationary phases, it was found that the pairwise combinations between HILIC, RPLC and IEC constitute most of the mixed-mode stationary phases, and the mixed-mode stationary phases are classified by these combinations. At the same time, four mixed-mode stationary phases and their retention mechanisms were introduced, and it was found that mixed-mode columns can provide multiple interaction mechanisms between solutes and stationary phases compared with columns with only a single interaction mechanism.

In chapter 2, a highly ordered surface polymer-bonded silica stationary phase was successfully prepared by introducing chiral glutamine-derived lipids (G) onto the surface of poly(4-vinylpyridine)-bonded silica gel. The first step is to bond (4-vinylpyridine) to the surface of silica by polymerization, and then introduce glutamine lipids with brominated groups (BrG) to the surface of silica by quaternization. The resulting materials were characterized by elemental analysis (EA), Fourier transform infrared spectroscopy, thermogravimetric analysis and scanning electron microscopy. According to the calculation of elemental analysis, the total amount of organic phase on the stationary phase surface and the degree of quaternization of pyridine functional groups were about 38 and 32.5, respectively. The materials were characterized by differential scanning calorimetry analysis of methanol suspension. The G part can also maintain a highly ordered structure on the silica surface at the phase transition temperature. The Sil-VP$_{15}$ column and the octadecylated silica commercially available column simultaneously separated PAHs under the same conditions, and it was found that the Sil-VP$_{15}$ stationary phases exhibited excellent selectivity. At the same time, it has good selectivity for geometric isomers and positional isomers. also exhibited excellent separation selectivity for phenol derivatives and successfully separated biologically relevant molecules containing phenolic groups, including steroids. The results show that due to the co-action of the carbonyl and alkyl chains of the highly ordered glutamine derivatives on the silica surface, the carbonyl-π interaction and the hydrophobic interaction of the alkyl chains are generated between the analyte and the stationary phase, resulting in the Sil-VP_{15} stationary phase has excellent shape selectivity.

In chapter 3, two zwitterionic polymer-coated silica stationary phases are described, where they consist of different side chains (phosphonate and carboxylate) anions and the same pyridinium cation, respectively in the hydrophilic interaction liquid chromatography and ion-exchange chromatography mixed mode. These two new columns firstly grafted 4-vinylpyridine onto the silica surface by polymerization, and then quaternized with (3-Bromopropyl) phosphonic acid (Sil-VP^+PA^-) and 3-Bromopropionic acid (Sil-VP^+CA^-), respectively. The two columns possess negatively charged carboxylate and phosphonate groups as well as positively charged pyridinium groups. The materials were characterized by Brunauer-Emmett-Teller (BET), elemental analysis, thermogravimetric analysis, Fourier transform infrared spectroscopy and Zeta potential. The retention properties of different types of compounds on the two packed columns were evaluated by the pH of the eluent and salt concentration. To investigate and compare the separation of different analytes, namely, disubstituted benzene isomers, phenols and aromatic acids, nucleosides/nucleobases, and sulfonamide drugs in HILIC and IEC modes between two zwitterionic columns and commercial column, the results indicate that the separation of analytes on the stationary phase is based on the retention mechanism of hydrophilic interaction as well as ion exchange interaction. And from the separation results, it was found that the Sil-VP+PA- stationary phase showed excellent separation ability, which may be due to the introduction of phosphonate on the silica surface to improve the selectivity of the stationary phase.

In chapter 4, a novel phenolic resin-coated silica stationary phase is prepared through the copolymerization of 1,3,5-trimethylhexahydro-1,3,5-triazine as well as 1,5-dihydroxynaphthalene in a simple solvent on APS- amino-modified silica and then followed by calcination at 200 °C. The products are determined by thermogravimetric analysis, Fourier-transform infrared spectroscopy, scanning electron microscope and elemental analysis. The new column has the ability to separate different types of analytes in the mixed-mode chromatography. For better retention behavior of the stationary phase, the relationship between log k and log Po/w of common hydrophobic analytes on the Sil-NP-h stationary phase was investigated. The retention of the compound is much stronger than that of alkylbenzenes. The retention of base nucleosides was further investigated by changing the content of acetonitrile, and it was proved that the stationary phase also had a typical HILIC retention mode. Under hydrophilic interaction liquid

chromatography conditions, sulfonamides and nucleosides/nucleobases were completely separated with good resolution even without buffer salts in the eluent. In reversed-phase liquid chromatography, the column exhibits good separation of positional isomers, alkylbenzenes, and polycyclic aromatic hydrocarbons (PAHs). On the other hand, the selectivity and retention of the new column for PAHs are better than those of alkylbenzenes, which may be affected by the π-π forces between the PAH analytes and the naphthalene rings on the silica surface. The stationary phase can simultaneously separate hydrophobic and hydrophilic compounds, and when the proportion of organic phase in the mobile phase is changed, the elution order on the stationary phase is reversed. As a result, new stationary phases can be used in new mixed-mode columns, and retention and selectivity can be adjusted very flexibly by changing chromatographic conditions.

In chapter 5, We summarized all the studies covered in this paper. Four polymer coated silica-based stationary phases exhibit different separation capabilities and retention mechanisms due to the introduction of different functional groups in each of them. The good separation capability of the four packed columns for some compounds allows them to be further applied to the separation of real samples.