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RESEARCH PAPER

Preparation and Evaluation of a C_{18}-Sulfonic Group Dual Modification Chromatographic Stationary Phase

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Abstract: A C_{18}-sulfonic group dual modification chromatographic stationary phase was prepared by one-pot reaction to modify the surface of silica with two modifier of octadecyltrichlorosilane (OTS) and trimethoxysilylpropanethiol (MPS), and then oxidize the thiol group. Under the optimized reaction conditions, the stationary phase with a mole ratio of 3:7 between C_{18} and sulfonic group was obtained. The morphology and feature of the stationary phase were characterized by scanning electronic microscopy, elemental analysis and infrared spectra. The chromatographic properties of the prepared stationary phase were systematically investigated in different separation modes. Five alkyl benzenes and three nucleosides were separated successfully by reversed phase chromatography and hydrophilic interaction chromatography, respectively. The bovine serum albumin digest was also well separated on the stationary phase. The results indicated that the prepared mixed-mode stationary phase could provide multiple separation mechanism and had potential advantages in separating complex samples and adjusting the selectivity.

Key Words: Mixed-mode chromatographic stationary phase; Reversed phase chromatography; Hydrophilic interaction chromatography; 3-Mercaptopropyltrimethoxysilane

1 Introduction

With the rapid development of pharmacy, proteomics and metabonomics, the tested samples are more and more complicated, making higher demand on separating efficiency of liquid chromatography. From the initial irregular silica to fully porous silica, microballoons of metal oxides and monolithic polymer column, and so on, the separating mediums of liquid chromatography have been changed. For different analyses, an increasing number of liquid chromatographic packing materials with special structures have been developed, which provide alternative options for development of chromatographic separation methods.

Bonding new model functional group, bi-functional groups such as polyethylene glycol[1], polyacrylamide[2], humic acid[3], peptide[4] and amino acid[5,6] on the surface of silica gel as well as applying them to separation of complex samples were attracted considerable interest in this field. A silica-based reversed phase/strong anion exchange mixed-mode stationary phase was synthesized with 3-chloropropyltrichlorosilane and octadecyltrichlorosilane as modifiers, and further used to extract aristolochic acids by solid phase extraction[7]. With a new type of reversed phase/weak anion exchange column, ten vitamins of seven water-soluble and three fat-soluble were baseline separated with a single run[8]. A weak anion exchange/hydrophilic dual-function mixed-mode chromatographic stationary phase was synthesized from activated silica with 3-glycidyloxypropyltrimethoxysilane, sodium azide and 1-diethylamino-2-propyne by Zhao *et al*[9] for the separation of nine kinds of tested proteins with a new

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method of off-line two-dimensional chromatography using only a single column. In addition, a reversed phase/weak cation exchange mixed-mode chromatographic stationary phase bonding by octyl-carboxylic group was used to separate three kinds of cationic surfactants and six antidepressants[10]. Through the "thiol-ene" click chemistry[11], a series of hydrophilic interaction chromatographic stationary phases were prepared and applied to the separation of polar substances and two-dimensional liquid chromatography. As for reversed phase and ion exchange mixed stationary phase, the retention characteristic of solutes in the mode of electro chromatography was systematically studied in our previous work[12], which laid a firm foundation for the stationary phase preparation. In practice, a stationary phase modified in multiple modes on its surface could provide multi sites interaction of different separation mechanisms, which has an advantage in selectivity of separating complex samples.

In this study, octadecyltrichlorosilane and trimethoxysilylpropanethiol were simultaneously bonded on the surface of silica. Then, a dual modification chromatographic stationary phase was prepared by oxidizing thiol group. The stationary phase with mixed retention mechanisms was further employed to study on the chromatographic behavior of different modes.

2 Experimental

2.1 Instruments and reagents

Chromatographic experiments were carried out by using an Elite 1201 HPLC system, including two P1201 pumps, a UV1201 UV-vis detector and an EC2006 chromatography workstation supported by Dalian Elite Analytical Instruments Co., Ltd. (Dalian, China). The products from the preparation of stationary phase were characterized by Tensor 27 infrared spectrometer (Bruker, Germany) and their analyses of elements were performed on Vario EL organic element analyzer (Elementar, Germany). Microspheres morphology was observed by JSM-7800F field emission scanning electron microscope (JEOL, Japan). Solution pH value was measured by pH meter (Mettler Toledo).

Spherical silica (5 μm, 169 m^2 g^{-1}, 120 Å) was purchased from ThermoFisher Scientific (MA, USA). Octadecyltrichlorosilane (OTS), trimethoxysilylpropanethiol (MPS), trypsin, bovine serum albumin (BSA), dithiothreitol (DTT), uridine, inosine and guanosine were purchased from J&K Scientific Ltd. (Beijing, China). Iodoracetamide (IAA) was bought from Amresco (TX, USA). Toluene, ethyl benzene, propyl benzene, butylbenzene, amylbenzene, hydrochloric acid (38%), hydrogen peroxide (30%), dichloromethane, sulfuric acid (98%), ammonium acetate, triethylamine, urea and NH_4HCO_3 were purchased from Kemiou Chemical Regent Co., Ltd. (Tianjin, China). All above chemical reagents were of analysis grade. Methonal and acetonitrile were HPLC grade and purchased from Tedia (OH, USA).

2.2 Preparation of C_{18}-sulfonic group dual modification chromatographic stationary phase

The bonding rates of OTS and MPS were investigated at different mole ratios between OTS and MPS that were of 1:1, 2:1, 4:1. Finally, the mole ratio of 1:1 was the best choice for the following experiments and the specific steps were as follows. Typically, 3 g of silica, 50 mL of hydrochloric acid and 20 mL of deionized water were mixed and refluxed with continuous stirring for 8 h at 110 °C. Then the reaction solution was filtrated and washed 5 times with 500 mL of deionized water. The activated silica (AS) was gotten after dried at 120 ºC in vacuum overnight. Subsequently, the AS was dropped in 50 mL of toluene, 1.0 mL of OTS, 0.5 mL of MPS and 50 μL of trimethylamine and refluxed with continuous stirring for 24 h at 100 ºC under nitrogen protection. Then the reaction solution was filtrated and washed successively with 100 mL of dichloromethane, 50 mL of methanol, 100 mL of deionized water and 50 mL of methanol before drying at 80 ºC in vacuum overnight to achieve C_{18}-mercapto silica (octadecyl-mercapto silica, OMS). Subsequently, 15 mL of methanol, 50 mL of hydrogen peroxide and 20 μL of sulfuric acid were added in OMS. The mixed solution was then stirred for 20 h at 25 ºC and filtrated. Finally, the products, called C_{18}-sulfonic silica (octadecyl-sulfonic silica, OSS), were washed with 100 mL of deionized water and 100 mL of methanol before drying at 80 ºC overnight. The synthetic procedures of OSS are shown in Fig.1.

2.3 Packing of chromatographic columns

Approximately 2.2 g of stationary phase was used to prepare and sonicate slurry with the technique of high pressure and equivalent density homogenization. Then the slurry was put into packing tank and then displaced to pack columns (150 mm × 4.6 mm i.d.) for the subsequent evaluation.

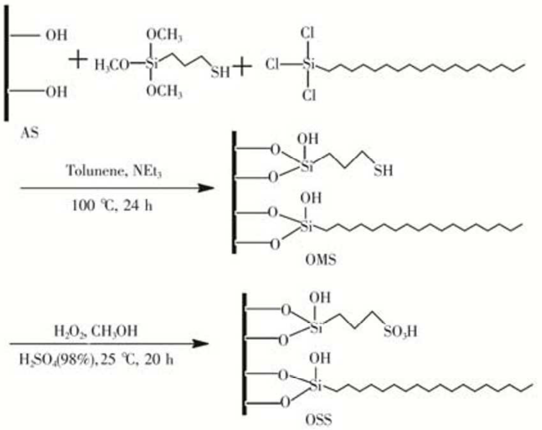

Fig.1 Schematic diagram of synthesis of octadecy-sulfonic silica (OSS)