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Poly(4-vinylpyridine) as a reagent with silanol-masking effect for silica and its specific selectivity for PAHs and dinitropyrenes in a reversed phase

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Abstract

This paper demonstrates that poly(4-vinylpyridine) is applicable as an effective masking reagent for silica to reduce undesirable side effects due to silanol groups. It also shows that this chemical modification brings about unique retention behaviors absolutely different from conventional ODS, which appear in molecular-shape selectivity for polycyclic aromatic hydrocarbons and in selectivity for position isomerism, especially for electron-withdrawing substitution compounds. Separation of 1,6- and 1,8-dinirtopyrenes as carcinogens is also described.

Keywords: Polymeric organic phase; Column liquid chromatography; Molecular-shape selectivity; Dipole–dipole interaction; π–π interaction; Dinitropyrene

1. Introduction

To analyze basic compounds with HPLC is very important because many pharmaceutical and bio-related materials often include amino groups. Especially, an RP-HPLC method using C_{18}-bonded silicas is very powerful and convenient for this purpose, and thus has been widely used around the world. However, serious problems have been noted; specifically peak-tailing and poor reproducibility through residual silanol groups on silica surfaces. To eliminate these undesirable properties, many end-capping reagents and methods have been developed. The most widely used reagents for this purpose include end-capping with trimethylchlorosilane [1,2] and hexamethyldisilazane [3] for silanol groups. These become more useful by coupling with high temperature treatment. Hydrosilation is also a very useful method for end-capping [4,5]. Alternatively, *n*-alkyl-bonded silica with embedded polar functional groups has been attracting attention as an another approach to improve peak shape and reproducibility. It have been reported that these phases having amide or carbamate groups, showed a better peak shape for basic compounds compared with conventional *n*-alkyl-bonded phases [6,7]. This is often explained by the hydration layer on silica surface or the competitive interaction between its polar functional groups and silanols on silica [8].

In this paper, we wish to introduce a unique method to reduce undesirable silanol effect against basic compounds in HPLC. This method is based on the fact that a masking effect is brought about by poly(4-vinylpyridine) (Fig. 1) as a weak basic polymer which provides exclusion effect for basic compounds. In addition, we focus on polycyclic aromatic hydrocarbons (PAHs) and dinitroarenes because pharmaceuticals often possess aromatic groups and their geometrical and structural isomers often show extremely different action in the human body. Especially, 1,6- and 1,8-dinitropyrenes are targeted in this work because they are not only universally involved in diesel emissions but also recognized as one of the most hazardous human carcinogens [9,10]. Therefore, this paper also describes how poly(4-vinylpyridine)

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as an organic phase yields exceptionally high selectivity for PAHs, especially for electron-withdrawing substitution compounds.

2. Experimental

2.1. Preparation of VP_n and grafting onto silica

Poly(4-vinylpyridine), VP_n, where n is the average degree of polymerization, with a terminal reactive group at the one side was prepared by modification of the telomerization method [11,12] of 4-vinylpyridine with 3-mercaptopropyl trimethoxysilane. The typical synthetic procedure is as follows: ten ml of 4-vinylpyridine and 0.91 ml of 3-mercaptopropyl trimethoxysilane were mixed and then N_2 gas was bubbled for 20 min at room temperature. After addition of 0.1 g of AIBN, the mixture was stirred with bubbling N_2 gas at 60 °C for 6 h. The yellow solid obtained was collected and dissolved in 20 ml of chloroform. The solution was poured into 200 ml of hexane to precipitate as pale yellow powders. Similar precipitation was repeated three times and the collected powders were successively washed with *n*-hexane and dried in vacuo. Yield: 9.9 g, 91%. The average degree of polymerization was estimated by NMR spectroscopy to be 21.7. NMR data: δ_H (400 MHz; $CDCl_3$; Me_4Si) 0.7 (9 H, s, Si–OCH_3), 8.0–8.8 (4.34 H, m, 3- and 5-positions of pyridyl group).

The resultant VP_n was grafted onto silica by using the terminal trimethoxysilyl group: 4.0 g of porous silica (YMC 120-S5, diameter 5 μm, average pore size 12.6 nm, specific surface area 333 $cm^2\,g^{-1}$) and 4.0 g of VP_n were mixed in 40 ml of chloroform and then was stirred gently at 60 °C for 1 week. The silica was collected and washed successively with chloroform, an acetic acid–methanol mixture and methanol. The recovery was 4.95 g and the amount of immobilization was determined by elemental analysis to be 27.1 wt.%.

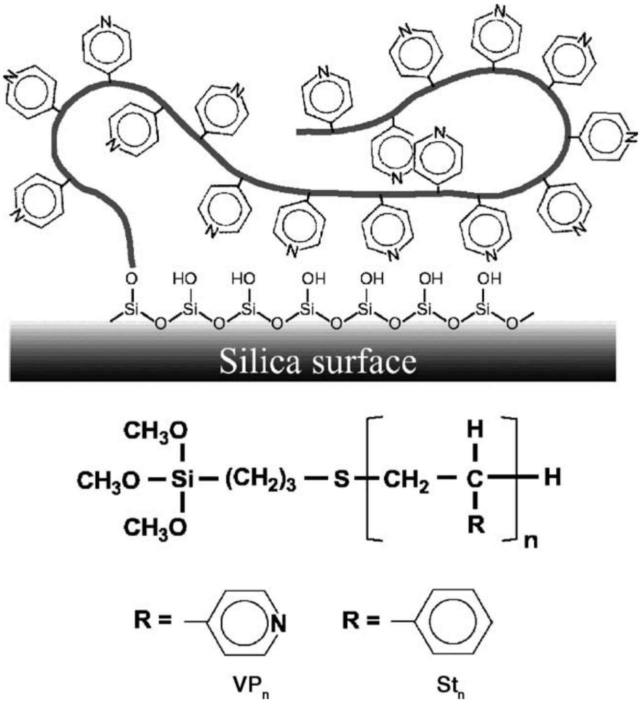

Fig. 1. Schematic illustration of VP_n-grafted silica with the chemical structures of VP_n and St_n as polymeric organic phases.

Poly(styrene)-grafted silica (Sil-St_n) was prepared according to the similar procedure [13]. The average degree of polymerization and the amount of immobilization on silica were 24.0 and 18.2 wt%, respectively.

2.2. Measurements

The VP_n-grafted silica (Sil-VP_n, n = 21) without any further end-capping procedure was packed into a stainless-steel column (4.6 mm i.d. × 250 mm) using a hexanol–chloroform (1:1) mixture and the liquid chromatographic property was examined using methanol-water as a mobile phase. The chromatograph included a JASCO 980 PU pump, a JASCO photodiode array detector (UVIDEC-100-IV) and a column heater (Sugai U-620 Type VP50). The solutes were dissolved in methanol with ultrasonication (45 W, 38 kHz) for 10 min and then filtered through a membrane filter (0.5 μm). Five microliter of the sample solution was injected through a Reodyne Model 7125 injector. Chromatograph was obtained at flowrate 1.0 ml min^{-1}. The retention factor (k') was determined by $(t_r - t_0)/t_0$ where t_r and t_0 are retention time of samples and methanol, respectively. The separation factor (α) was given by the ratio of retention time.

Poly(styrene)-grafted silica (Sil-St_n) packed into a stainless-steel column (4.6 mm i.d. × 250 mm). ODS-5FJ (4.6 i.d. × 250 mm, Masis Inc., Japan) was used as a reference column.

Water–1-octanol partition coefficient ($\log P$) was determined by retention factor with octadecylated silica, ODS (Inertsil ODS, 4.6 mm i.d. × 250 mm, GL Science Co. Ltd.): $\log P = 3.759 + 4.207 \log k$ ($r = 0.99997$) [14]. Log Ps and dipoles of di-substituted benzenes were estimated by CAChe MOPAC with the AM1 option.

The structures of PAHs were estimated by HyperChem Ver 5.1 with molecular mechanics (until the energy changes were below 0.001 kcal mol^{-1}) and following semi-empirical AM1 method.

3. Results and discussion

3.1. Retention behaviors for basic compounds

A typical chromatographic behavior of poly(4-vinylpyridine)-grafted silica (Sil-VP_n) appeared in separation for a mixture of pyridine and phenol. As shown in Fig. 2, Sil-VP_n showed a very small retention factor ($k = 0.18$) for pyridine with a good asymmetric factor ($A_s = 1.21$), while ODS-5FJ as a reference column showed a poor A_s value