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A Bidenate Amide Bonded Stationary Phase for HPLC

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Abstract: A novel bonded stationary phase, bidenate amide bonded stationary phase (BABSP), was prepared by reacting YWG silica gel firstly with 3-aminopropyltrimethoxysilane, then with diacid chloride. Hydrophobicity, selectivity, and especially silanophilic activity of BABSP were evaluated. The stationary phase exhibited good column efficiency and peak shape for separation of some basic solutes.

Keywords: High performance liquid chromatography, stationary phase, silanophilic activity, hydrophobicity.

Reversed-phase high performance liquid chromatography (RP-HPLC) is currently one of the most widely used chromatographic methods. The incomplete reaction of the surface silanol group on silica gel with a silylating reagent and the formation of new silanol when bi- or tri-functional modifiers are utilized, may plague the area of RP-HPLC. These residual silanol groups on silica surface may cause severe band tailing, low plate numbers and low resolution for separating polar samples, especially basic solutes. Acceptable separations have been achieved using silica gel endcapped with short-chain alkyl silanes[1] and silica gel with sterically protecting groups[2]. Recently, the stationary phase possessing a ligand with an internal polar functional group[3-5] and the stationary phase prepared with bidenate silanes[6] were used to solve this problem satisfactorily.

In this paper, a novel silica gel bonded phase, bidenate amide bonded stationary phase(BABSP), is described which contains internal polar amide group with bidenate structure. BABSP exhibits good column efficiency and peak shape for the separation of some basic solutes.

Experimental

Preparation of NH_2-silica gel

To a suspension of 3.5 g of pretreated YWG silica gel (Qingdao Oceanorgraphic Chemical Plant, 7μm) in 70 mL of toluene, 2 mL of 3-aminopropyltrimethoxysilane (Fluka, Switzerland) was added. The mixture was stirred and refluxed for 8 h under a dry nitrogen atmosphere. After cooling and the solid was filtrated, washed with toluene

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and methanol in turn, dried in vacuum to give 3.91 g of NH_2-silica gel (elemental analysis, found: C 6.22, N 2.39, H 1.95).

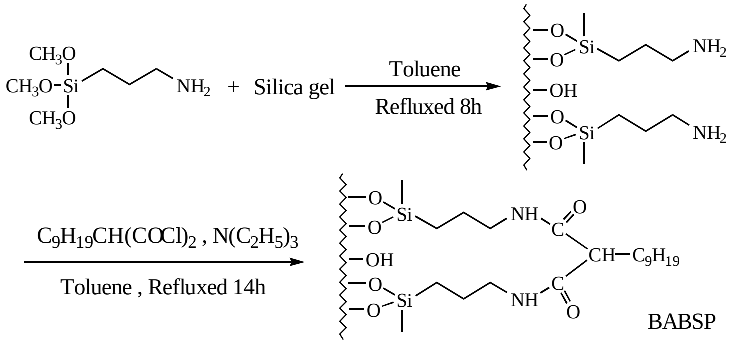
Preparation of 2-nonyl propane diacid chloride

2-Nonyl propane diacid prepared according to the reference[7] was reacted with $SOCl_2$ to give 2-nonyl propane diacid chloride.

Preparation of BABSP

3.00 g of NH_2-silica gel prepared above was suspended in 100 mL of toluene, 2 mL of triethylamine and 3 mL of 2-nonyl propane diacid chloride were added. The mixture was stirred and refluxed for 14 h. After filtration the residue was washed several times with benzene, methanol-water (1:1,v/v), methanol, and ethyl ether in turn, dried in vacuum overnight to give 3.30 g of BABSP (found: C 12.92, N 2.17 and H 2.55).

Scheme 1 The synthetic route of BABSP



The infrared spectra of BABSP were acquired on an FT-IR spectrophotometer (Nicolet MX-1E spectrophotometer, KBr, cm^{-1}). The characteristic absorption was 2930，2858，1641，1544，1468，1444，1412.

Column packing

The columns (stainless steel, 300×3.9 mm I.D.) were packed with the balanced density slurry packing technique on a Chemco Model CPP-085 packer.

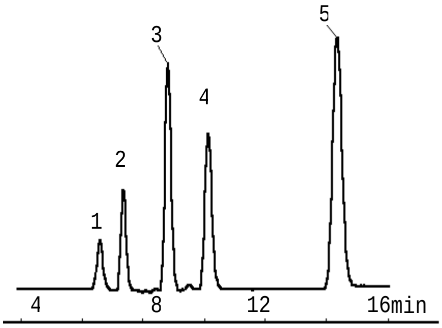
Chromatographic evaluation

All measurements were completed on a chromatographic system consisting of Waters 6000A pump, Rheodyne Model 7725i injector, M481 UV/visible detector and Qianpu HW chromatographic workstation. Methanol-water was used as mobile phase with detection wavelength at UV 254 nm and column temperature being 30°C.

Results and Discussion

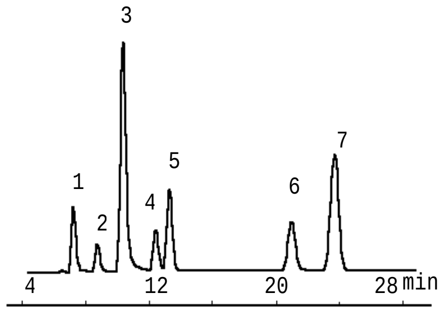
Hydrophobic retention of hydrocarbons on stationary phase is mainly dependent on surface coverage with alkyl groups. From carbon content of stationary phase it could be expected that BABSP should possess comparable hydrophobicity. **Figure 1** and **Table 1** exhibit the results of chromatographic separation of some aromatic hydrocarbons on BABSP. When methanol-water (80/20, v/v) was used as a binary mobile phase, the relative retentions (hydrophobic selectivity) α of toluene/benzene and biphenyl/naphthalene were found to be 1.35 and 1.29, respectively. This result showed that the hydrophobicity and selectivity of BABSP phase are acceptable.

Figure 1 Chromatogram of some aromatic hydrocarbons on BABSP



The conditions are the same as those in **Table 1**.
Peak: 1.Benzene; 2.Toluene; 3.Naphthalene; 4.Biphenyl; 5.Anthracene

Figure 2 Chromatogram of acidic, basic and neutral compounds on BABSP



The conditions are the same as those in **Table 2**.
Peak: 1.Thiourea; 2.Pyridine; 3.Aniline; 4.*o*-Toluidine; 5.Phenol; 6.Benzene; 7.N,N-Dimethylaniline

Table 1 Chromatographic data of some aromatic hydrocarbons on BABSP*

Compound	Benzene	Toluene	Naphthalene	Biphenyl	Anthracene
Capacity factor , k$'$	0.52	0.70	1.03	1.33	2.31
Column efficiency, n/m	33000	35000	36000	38000	38000
Asymmetry	1.10	1.08	1.06	1.04	1.03

* Mobile phase : methanol-water(80/20, v/v); flow rate:0.6 mL/min; temperature: 30°C; detection :UV 254 nm; dead time: 4.34 min.

Figure 2 and **Table 2** show the results of the separation of acidic, neutral and basic compounds, among them pyridine, aniline, *o*-toluidine and N,N-dimethylaniline were used to evaluated the silanophilic activity of BABSP. The basic compounds were separated on BABSP by using methanol-water (55:45, v/v) as mobile phase described as the reference[8] for evaluation the silanolphilic activity of BABSP. The results revealed good chromatographic peak shape for the basic compounds, and the asymmetry factors of pyridine, aniline, *o*-toluidine and N, N-dimethylaniline are 1.12, 1.06, 1.04 and 0.92, respectively. The fact, that aniline was eluted before phenol, demonstrated the activity of silanol being neglected[8]. The excellent chromatographic performance of BABSP are due to the existence of internal polar functional group and the bidenate structure which coordinately weaken the interaction between basic analytes and the residual silanol groups on the silica surface[9].

Table 2 Chromatographic data of some acidic, basic and neural compounds on BABSP [a]

Compound	Thiourea[b]	pyridine	Aniline	*o*-Toluidine	Phenol	Benzene	N,N-Dimethylaniline
Capacity factor, k′	0	0.21	0.44	0.73	0.85	1.92	2.30
Column efficiency n/m		18000	18000	26000	29000	34000	34000
Asymmetry		1.12	1.06	1.04	1.07	0.99	0.92

a. Mobile phase : methanol-water(55/45, v/v); flow rate:0.4 mL/min; temperature: 30°C; detection :UV 254 nm. b. dead time: 7.18 min.

After purging the column with 3000 column volumes of the mobile phase the capacity factors k′ and the column efficiencies of the samples on BABSP were not found obvious change under above chromatographic conditions.

References

1. H. H. Freiser, M. P. Nowlan, D. L. Gooding, *J. Liq. Chromatogr.*, **1989**, *12*, 827.
2. J. J. Kirkland, J. W. Henderson, *J. Chromatogr. Sci.*, **1994**, *32*, 473.
3. J. E. O’Gara, B. A. Alden, T. H. Walter, *et al.*, *Anal. Chem.*, **1995**, *67*, 3809.
4. Y. Q. Jing, Y. D. Long, T. B. Huang, *Chem. J. of Chinese Univ.*, **2002**, *23*, 210.
5. X. J. Huang, Y. Liu, R. Z. Cong, X. L. Liu, J. D. Wang, *Chin. J. chromatogr.*, **2001**,*19*,293
6. J. J. Kirkland, J. B. Adams, M. A. Straten, H. A. Claessens, *Anal. Chem.*, **1998**, *70*, 4344.
7. M. J. Kates, J. H. Schauble, *J. Org. Chem.*, **1996**, *61*, 4164.
8. H. Engelardt, M. Arangie, T. Lorbert, *LC-GC Asia Pacific*, **1998**, *1*, 36.
9. B. J. Feibush, *J. Liq. Chromatogr. Rel. Technol.*, **1996**, *19*, 2315.

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