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Dependence of cyano bonded phase hydrolytic stability on ligand structure and solution pH

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Abstract

As part of our program to develop more stable cyano (CN) high-performance liquid chromatography (HPLC) column packings, we have evaluated hydrolytic stability as a function of ligand connectivity, chain length, and side group steric protection and the pH of the mobile phase. Three accelerated tests were used to evaluate stability: (1) A non-HPLC screening test measuring carbon loss in refluxing MeOH-100 m*M* KH_2PO_4 pH 4.5 (1:1, v/v) solution; (2) a continuous flow HPLC test measuring capacity factor maintenance in 1% trifluoroacetic acid in water (pH 1.02) at 80°C; and (3) a continuous flow HPLC test measuring column efficiency maintenance in 50 m*M* triethylamine in water (pH 10.00) at 50°C. The stability of the CN phases was found to be dependent on both ligand chemical structure and the pH of the test conditions. The starting screen test of intermediate pH was least able to differentiate the CN phases based on structure, because two different degradation mechanisms appear to offset each other (acid induced siloxane bond cleavage vs. base induced silica dissolution). A trifunctional and a sterically protected CN phase were notably stable under the acidic test conditions, but had poor stability under basic conditions. Conversely, chain extension afforded poor stability under acidic conditions, but did afford improved stability at higher pH. In total, the data indicate that good CN column stability can be achieved by using a trifunctional or a sterically protected phase in acidic mobile phases. However, as mobile phases of intermediate or higher pH are employed, shorter column lifetimes can be expected due to an accelerated dissolution of the underlying silica substrate. Materials were also compared chromatographically using a mixture of non-polar, polar, and basic analytes under reversed-phase conditions.

Keywords: Ligand structure; Hydrolytic stability; Stationary phase, LC; Cyano bonded phase

1. Introduction

Although reversed-phase columns are used approximately 51% of the time for analytical high-performance liquid chromatography (HPLC), normal-phase columns continue to be in demand (16% usage) for applications where reversed-phases are unsatisfactory [1]. Cyano (CN) columns remain a popular choice for both reversed-phase and normal-phase separations, despite their history of poor chemical and mechanical stability. CN columns typically are ascribed poor retention time stability in low pH mobile phases due to acid induced ligand hydrolysis from the silica support [2]. In addition, stability in high pH mobile phases can suffer due to

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dissolution of the silica skeleton, where the short chain ligand provides little protection.

Cyano HPLC bonded phases started out from GC column bonded phase technology, where siloxane polymers were coated onto oxide particles. One of the first transitions to CN bonded phases as they are known today came in a report by Kirkland and DeStefano in 1970, where (2-cyanoethyl)siloxane polymer was reacted with silica surface silanols to form a siloxane bond between the surface and ligand silicon atoms [3]. Since that time, a plethora of CN phases have been reported in the literature for study and use in HPLC, where the majority of these phases employ mono-, bi-, and tri-functional (3-cyanopropyl)methylsiloxane bonding chemistries [4,5]. A notable exception is the work of Kirkland and Glajch, who reported a (3-cyanopropyl)-diisopropylsiloxane bonded phase with improved hydrolytic stability imparted by steric protection of the siloxane bond with the large isopropyl side groups [6].

In the present report, hydrolytic stability results are presented for a series of CN bonded phases, where the effects of ligand functionality, chain length, side-group steric encumbrance and solution pH are compared. An accelerated stability test was used to identify lead CN bonded phases for subsequent testing under continuous flow HPLC conditions in both low and high pH mobile phases.

2. Experimental

2.1. General

Retention factor and high pH stability data were acquired on a modular HPLC system consisting of a 600E solvent delivery system, a 490E Programmable Multiwavelength Detector, and a 717 Plus Autosampler, with a thermostated water bath for column temperature control (50.0±0.1°C, RTE-111D, NESLAB Instruments, Inc., Portsmouth, NH, USA). The system was controlled by Millennium^{32} Software 2.15.01. Low pH stability data were acquired on either a 616 LC System, a 717 Plus Autosampler, a 2487 Dual Wavelength Absorbance Detector, an In-Line Degasser, and a Temperature Control Module (80.0±1.0°C) or an Alliance 2690 Separations Module, a 486 Tunable Absorbance Detector and a Temperature Control Module (80.0±1.0°C). Both low pH systems were controlled by Millennium^{32} Software 3.05.01 (unless otherwise noted, all from Waters Corporation, Milford, MA, USA). NMR, IR, and elemental analyses data were measured as described previously [7].

All solvents were HPLC grade (J.T. Baker, Phillipsburg, NJ, USA). (3-Cyanopropyl)dimethylchlorosilane, (3-cyanopropyl)trichlorosilane, and (3-cyanopropyl)diisopropyl-chlorosilane were purchased from United Chemical Technologies, Inc. (Bristol, PA, USA). Syntheses have been reported for (5-cyanopentyl)dimethylchlorosilane [4] and 3-(dimethylsilyl)propyl *N*-(2-cyanoethyl)-*N*-methylcarbamate [8]. (3-Cyanopropyl)diphenylchlorosilane was prepared from the reaction of allyl cyanide (Aldrich, Milwaukee, WI, USA) with diphenylchlorosilane (United Chemical Technologies, Inc., Bristol, PA, USA) under standard hydrosilation conditions using Speier's catalyst (Aldrich, Milwaukee, WI, USA) [9]. ^{1}H and ^{13}C NMR spectra were consistent with the assigned structures. A single lot of silica was used for all of the silane bonding reactions, which were run in the normal manner [8,10]. The unbonded silica (Waters Corporation, Milford, MA, USA) had the following properties: average particle diameter, 5.0 μm; specific surface area, 339 m^2/g; pore volume, 0.91 cm^3/g; mean pore diameter, 96 Å; metal content, Fe<1 μg/g, Na 4 μg/g, Al<1 μg/g.

2.2. Bonded phase characterization

The chemical representation of each bonded phase is shown in Fig. 1. The ligand surface concentration was calculated from the base silica surface area and the percentage carbon of the bonded phase [11]. Data are shown below in Table 1. The dentation ratio of mono/bi/tridentate for trifunctional bonded phase **2** was determined to be 3/45/52 by ^{29}Si CP/MAS NMR spectroscopy. Bonded phases **2**, **4**, and **5** were compared by HPLC using a mixture of non-polar, polar, and basic analytes in a MeOH-20 m*M* KH_2PO_4/K_2HPO_4 pH 7.00 (1:1, v/v) mobile phase at 23.4°C. Dihydroxyacetone was used as the void volume marker. Tabulated results are found in Table 2. The materials were packed into 3.9×150 or 4.6×

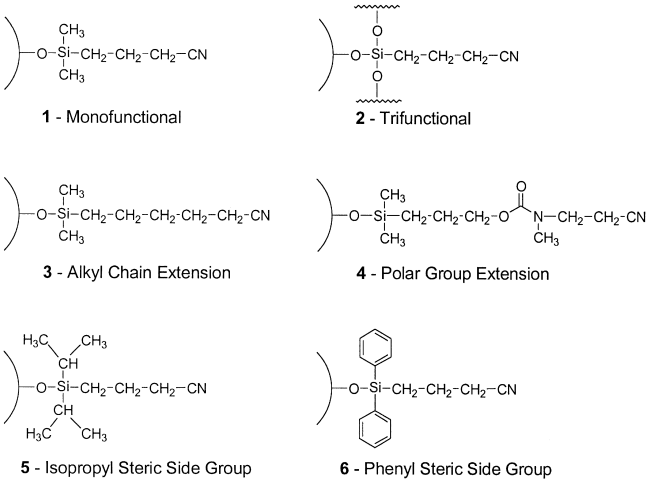

Fig. 1. Chemical structures of cyano bonded phases **1**–**6**.

Table 1
Percentage carbon and surface concentration values for CN phases **1**–**6**

CN phase	%C	Surface concentration (μmol/m^2)
Mono – **1**	7.84	3.75
Tri – **2**	6.50	4.91
Chain Ext. – **3**	9.82	3.60
Polar Ext. – **4**	11.00	3.43
Isopropyl – **5**	7.06	1.95
Phenyl – **6**	10.91	1.96

75 mm stainless steel columns using conventional high pressure slurry techniques [12].

2.3. Reflux stability test

A 1.5 g sample of bonded phase was refluxed in 50 ml of MeOH-100 m*M* KH_2PO_4 pH 4.5 (1:1, v/v) solution. At 2, 4, 7, and 23 h, a 10-ml sample of the dispersion was taken, and the solvents were separated by vacuum filtration. Each sample was subsequently washed with a 25-ml aliquot of methanol, acetone-water (1:1, v/v), and acetone. After drying in a vacuum oven for 16 h at 80°C, each sample was measured for percentage carbon (%C) by combustion analysis. Combustion analysis samples were run in duplicate, and the average value is reported here.

Table 2
Chromatographic data for CN phases **2**, **4** and **5**

Chromatographic data	Tri – **2**	Polar Ext. – **4**	Isopropyl – **5**
Retention factor *k*			
Butylparaben	0.30	1.01	3.27
Toluene	0.47	1.06	1.46
Diisobutylphthalate	0.75	1.49	5.12
Acenaphthene	1.26	3.48	10.70
Propranolol	4.33	1.55	9.58
Doxepin	7.48	2.38	16.56

2.4. Low pH column stability test

Hydrolytic stability in a low pH mobile phase at 80°C was measured using the following protocol. (1) The column (4.6×75 mm) was equilibrated to temperature for 2 h running at 1.0 ml/min with acetonitrile. At the end of the equilibration time, acetone was injected to measure the void volume. (2) The column was then equilibrated at 1.0 ml/min in acetonitrile:water (1:1, v/v), and acenaphthene (with uracil as a void volume marker) was injected to obtain a retention factor. (3) The column was equilibrated for 1 h in 1% trifluoroacetic acid in water (pH 1.02) at 1.4 ml/min. An injection of methyl paraben and ethyl paraben was made with a 90-min runtime, and then an injection of benzene and toluene was made, also with a 90-min runtime. (4) Any hydrolyzed bonded phase was then eluted using 1% trifluoroacetic acid in acetonitrile at 3.0 ml/min for 24 min. (5) Steps 3 and 4 were repeated

until the retention times for the compounds in step three were reduced to less than 50% of their original value. After every 10 cycles of steps 3 and 4, steps 1 and 2 were repeated.

2.5. High pH column stability test

Hydrolytic stability in a high pH mobile phase was measured using the following protocol. (1) The plate number, *N*, (5 sigma method) was measured for a test analyte, acenaphthene. Mobile phase conditions were MeOH-20 m*M* KH_2PO_4/K_2HPO_4 pH 7.00 (1:1, v/v) at a flow of 1.0 ml/min and a column temperature of 50.0°C. (2) The column (3.9×150 mm) was purged over to and run for 1 h in a 50 m*M* triethylamine pH 10.00 mobile phase at a flow of 2.0 ml/min and a column temperature of 50.0°C. (3) In 1 or 2 h increments, the column was purged with 100% water (10 min at 2.0 ml/min) and then purged with 100% methanol (10 min at 2.0 ml/min). (4) Next, the column was purged over to and equilibrated in the mobile phase of step 1 above, and *N* for acenaphthene was measured. (5) The process was then repeated starting at step 2. The experiment was continued until *N* decreased by 75% or until the column back pressure increased to greater than 6000 p.s.i.

3. Results and discussion

3.1. CN bonded phase selection

Six CN bonded phases were chosen for study where the ligand functionality (mono vs. tri), chain length (C_3 vs. C_5 vs. C_5 with an embedded carbamate group), and side group size (dimethyl vs. diisopropyl vs. diphenyl) were systematically changed (see Fig. 1 and Table 1). Functionality or dentation [13], chain length [14,15], and large steric side groups [16] are all known to afford improved ligand hydrolytic stability in reversed-phase systems. The same batch of silica was used for all bonded phases to remove the effect of substrate on stability, and none of the CN bonded materials were end capped.

Bonded phase **1**, a (3-cyanopropyl)-dimethylsiloxane ligand, served as the benchmark material for relative measurements of stability. An increase in ligand dentation was achieved by preparation of bonded phase **2**, a (3-cyanopropyl)trisiloxane ligand, where the chain length was held constant and only ca. 3% of the ligands were monodentate. Chain length was increased by substituting a pentyl group for the propyl spacer, bonded phase **3**, where the side groups were held as methyl moities. Further extension using only methylene groups was not pursued, since it was expected that the phase would become too hydrophobic and lose cyano-like attributes. Instead, extension was provided by insertion of an N-methylcarbamate functional group between the C_3 and C_4 methylenes of the cyanopentyl group to afford bonded phase **4**. Insertion of a carbamate group within an alkyl chain has been shown to result in reduced retention times for basic compounds and to give highly stable retention times in highly aqueous mobile phases [7,17–19]. Indeed, oven dried bonded phase **4** wets rapidly upon gentle mixing with pure water. The steric size of the side groups was increased by making (3-cyanopropyl)diisopropyl bonded phase **5** and (3-cyanopropyl)diphenyl bonded phase **6**. Silyl ethers with sterically large groups have been used extensively in organic synthesis to protect alcohols [20], and both isopropyl and phenyl groups are significantly larger than the methyl group, with steric energies or A Values of 1.70 ($-CH_3$), 2.15 [$-CH(CH_3)_2$], and 3.0 ($-C_6H_5$) kcal/mol [21].

3.2. Reflux stability results

In an effort to reduce the amount of equipment time required to screen for stability, an accelerated hydrolysis test was used where the bonded phase was heated at 55°C in a MeOH-100 m*M* KH_2PO_4 pH 4.5 (1:1, v/v) solution. The use of 50% organic solvent ensures complete wetting of all the CN substrates. Phosphate does not buffer at pH 4.5, but the elevated salt concentration and an elevated temperature were used to accelerate ligand removal. The %C remaining after reflux vs. the %C of the original bonded phase was interpreted as a measure of ligand loss. For reference under these conditions, a dimethyloctyl bonded phase (surface concentration 3.48 $\mu mol/m^2$) did not wet completely and lost no carbon after 23 h.

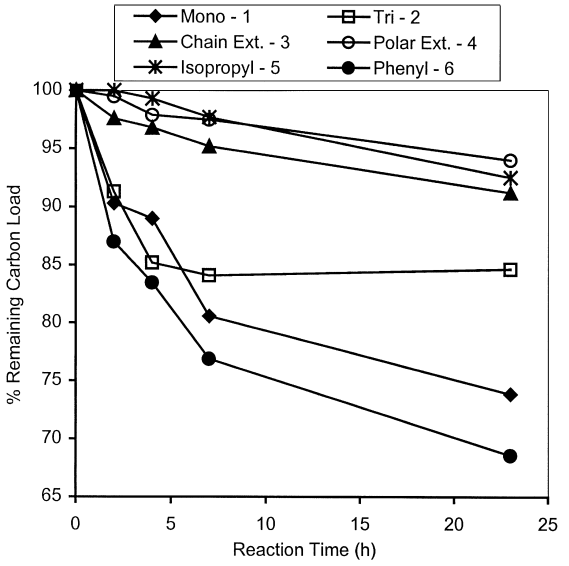

Fig. 2. Accelerated stability test results. Percentage remaining %C of bonded phase vs. reaction time at 65°C in MeOH-100 m*M* KH_2PO_4 pH 4.5 (1:1, v/v) solution.

A dimethylbutyl bonded phase (surface concentration 3.69 μmol/m^2) had limited wetting, and 98% of its carbon content remained after a 23-h test period. Accelerated stability test results for the CN phases **1**–**6** are plotted in Fig. 2.

Unsurprisingly, monofunctional phase **1** was less stable than its trifunctional counterpart **2**. The trifunctional phase **2** lost most of its carbon in the first 4 h, and then leveled off. For chain extension, moving from CN propyl phase **1** to the CN pentyl phase **3** led to a significant increase in stability. Further chain length extension with the carbamate group in phase **4** yielded only a minor improvement in stability. These results are consistent with previous work by Hetem and coworkers in the stability of reversed-phase alkyl bonded phases [14]. For side group protection, diisopropyl bonded phase **5** showed significantly improved stability vs. the less bulky dimethyl phase **1** as expected from the work of Kirkland, Glajch, and Farlee [16]. Diphenyl CN phase **6** was less stable than both phases **1** and **5**, despite the phenyl ring being the largest steric group. This result was contrary to what is known in silyl ether protecting group chemistry [20], and a concise explanation remains unavailable at this time.

3.3. Low and high pH stability under continuous flow conditions

With the exception of CN diphenyl phase **6**, the accelerated stability results were consistent with literature precedence. As a next step, CN phases **1**, **2**, **4**, and **5** were chosen for further testing in a 1% trifluoracetic acid (TFA) mobile phase at 80°C under continuous flow HPLC conditions (see Experimental section). As shown in Fig. 3, monofunctional phase **1** and chain extended monofunctional phase **4** both degraded rapidly under these test conditions. Trifunctional phase **2** and steric protected phase **5** showed significantly improved stability through 75 h of continuous flow testing. A failed seal lead to termination of phase **5** testing, but trifunctional phase **2** maintained 50% of its retention through 100 h. Both **2** and **5** lasted longer than an end capped dimethyloctyl bonded phase (3.52 μmol/m^2) which maintained 50% of its retention to 25 h.

The clear differentiation of phases **1**, **2**, **4** and **5** and the end capped dimethyloctyl phase using a low pH HPLC test suggested the reflux stability test had a component of a high pH hydrolysis mechanism, where the silica substrate can be dissolved and the siloxane ligand can be cleaved [14]. CN phases **1**, **2**, **4**, **5** and the end capped dimethyloctyl phase were

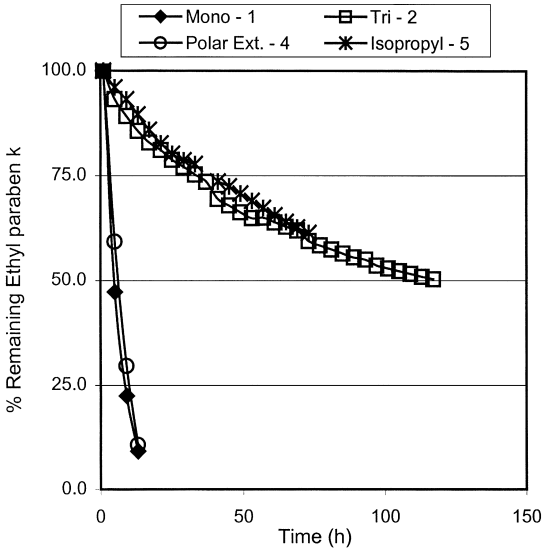

Fig. 3. Percentage remaining ethylparaben k vs. running time in 1% TFA (pH 1.02) at 80°C (see Experimental section for details).

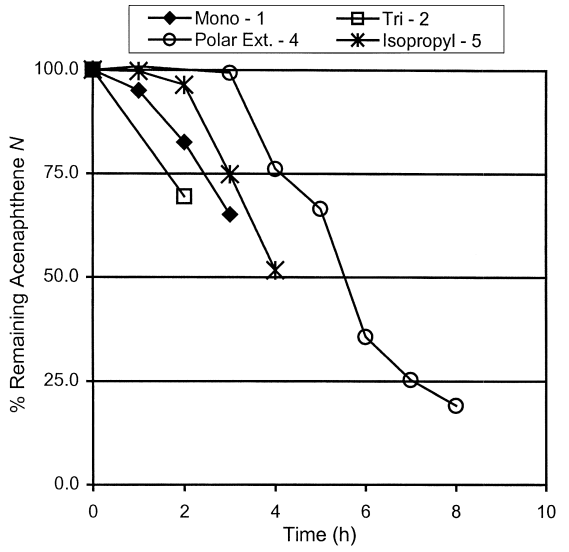

Fig. 4. Percentage remaining acenaphthene *N* vs. running time in 50 m*M* triethylamine (pH 10.00) at 50.0°C (see Experimental section for details).

next tested for stability in a 50 m*M* triethylamine pH 10.00 mobile phase at 50.0°C (see Experimental section). Column pressures at the start of the test were 1600 p.s.i. As shown in Fig. 4, trifunctional phase **2** had the shortest lifetime and developed a pressure exceeding 6000 p.s.i. after only 2 h. Monofunctional phase **1** lasted only slightly longer up to 3 h and then developed a pressure exceeding 6000 p.s.i. Sterically protected phase **5** maintained good efficiency to 2 h and then degraded rapidly until 4 h when the column pressure exceeded 6000 p.s.i. Polar chain extended phase **4** maintained its efficiency to 3 h and then degraded through 8 h to less than 25% of its original efficiency, however, the column pressure did not increase significantly. As a reference, the dimethyloctyl phase lasted 13 h before 50% of the column efficiency was lost.

*3.4. Chromatographic characterization of phases **2**, **4** and **5***

Phases **2**, **4** and **5** represented the most stable ligand from the functionality, chain length, and side group sets. The three materials were characterized using a mixture of non-polar, polar, and basic analytes in a MeOH-20 m*M* KH_2PO_4/K_2HPO_4 pH 7.00 (1:1, v/v) mobile phase. As listed in Table 2, each bonded phase yielded significantly different retention profiles for the test mixture. In brief, non-polar and polar analyte retention decreased in going from phase **5** to **4** to **2**, respectively. Basic compounds were less retained in going from phase **5** to **2** to **4**, respectively. This switch in order for the basic analytes was not unexpected since embedded carbamate groups have been shown to inhibit basic analyte interaction with surface silanols [7,19]. In addition, phases **2** and **5** are expected to have larger populations of unreacted silanols due to the population of mono- and bi-dentate ligands in phase **2**, and the lower ligand surface concentration of phase **5**, that results from the steric encumbrance of the isopropyl groups.

4. Conclusions

The study described here suggests that CN column stability is dependent on both ligand chemical structure and solution pH. The starting screen test of intermediate pH 4.5 was least able to differentiate the CN phases based on structure, because two different degradation mechanisms appear to offset each other (acid induced siloxane bond cleavage vs. base induced silica dissolution). A trifunctional and a sterically protected CN phase were notably more stable under the acidic test conditions, whereas monofunctional dimethylsilyl phases **1** and **4** and even a dimethyloctyl phase all were less stable. Conversely, the dimethyloctyl phase and the chain extended CN phase **4** afforded better stability at higher pH over the short chain phases, presumably due to a combination of increased chain length (vs. **2** and **5**) and higher surface concentration (vs. **5**), attributes which better protect the underlying silica substrate from dissolution [14,15]. The data indicate that improved CN column stability can be achieved by using trifunctional or sterically protected phases in acidic mobile phases. As mobile phases of intermediate or higher pH are employed, shorter column lifetimes can be expected due to an accelerated dissolution of the underlying silica substrate.

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