

CHROM. 23 084

Characterization of cyano bonded silica phases from solid-phase extraction columns

Correlation of surface chemistry with chromatographic behavior

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(First received October 31st, 1990; revised manuscript received January 15th, 1991)

ABSTRACT

Cyano bonded phases from six solid-phase extraction cartridge vendors (identified as A to F) have been characterized by elemental analysis, chromatography of probe molecules, and infrared and solid-state nuclear magnetic resonance spectroscopy. The results indicate that cyano solid-phase extraction cartridges from different vendors possess diverse surface properties presumably arising from differences in bonding chemistry and manufacturing methods. For example, nuclear magnetic resonance and infrared spectra indicate that vendors E and F use mono- and difunctional cyanopropyl silane, respectively, while all others use trifunctional. A wide range of hydrophobicities (determined using the alkylarylketone retention index scale) was observed among these bonded phases, determined primarily by degree of cyano group loading, whether the product has been endcapped or not and ratio of cyano functional group to endcapping reagent. Products from vendors A and D showed the presence of carboxyl groups, possibly arising from hydrolysis of cyano groups during silane bonding, thus imparting them with ion-exchange character. One would expect that extraction properties of cyano cartridges from different vendors may be very different. These results help explain the difficulty in transfer of procedures from cyano cartridges of one vendor to another and lend insight into the possible origin of lot-to-lot variability of cyano bonded phase from a single vendor.

INTRODUCTION

Solid-phase extraction has become an important step in the analysis of complex biological, environmental and industrial matrices. The end user has a wide range of surface chemistries to choose from. Although C₁₈ is the most often used surface chemistry, there is an increasing trend to use less hydrophobic surfaces [1] that require weaker solvents to elute retained analytes. The cyano bonded phase is being increasingly used as a short alkyl chain alternative to C₁₈.

De Smet and Massart [2] have been extending their pioneering work on the development of expert high-performance liquid chromatography (HPLC) systems using cyano columns to solid-phase extraction processes. Musch and Massart [3] have chosen a cyano solid-phase extraction cartridge for the extraction of basic drugs from plasma prior to analysis on a cyano HPLC column. They [3] have shown that the cyano solid-phase extraction cartridge has enough hydrophobicity to retain basic drugs with carbon number ≥ 11 . Extension of this work has shown [4] that neutral and acidic drugs with carbon number > 10 and < 25 can also be extracted on a cyano solid phase extraction column. It has also been used to extract cyclosporin [5] and/or its metabolites [6], β -blockers [7] and catecholamines [8] from many biological matrices.

In spite of the many advantages, cyano solid-phase extraction columns have not found widespread acceptance. This is perhaps because of lot-to-lot variability in performance often experienced by users. Experience in our laboratory indicated that the methods used to routinely analyze our bonded phases did not reveal the lot-to-lot variability observed in sensitive solid-phase extraction methods. Therefore, we initiated a study to not only understand the basis of retention of analytes on the cyano bonded phase but also develop methods to monitor the various surface characteristics that affect retention.

Considerable work has been done to understand the basis of retention in normal- and reversed-phase chromatography with cyanopropyl silica in HPLC columns which can also be extended to the cyano bonded phase present in solid-phase extraction columns. A number of conclusions have been reached. In the normal-phase mode, the cyano column acts essentially as a deactivated silica surface resulting in the interaction of analytes with residual silanols [9]. If the polarity of the solvent in the normal phase mode is increased, the effect from the silanols is suppressed leaving the cyano groups as the principal adsorption site [10]. In the reversed-phase mode, the cyano column acts like a short chain alkyl silica [11]. The primary interaction of the analyte with the bonded phase is through the cyano groups whose concentration (or surface coverage) determines the hydrophobicity of the surface. The residual silanols also play a key role in determining the overall adsorptive property.

Differences in silica and bonding chemistry may explain the differences in the properties of HPLC columns with the same principal functional group from different vendors. Smith and Miller [12] compared the performance of cyano HPLC columns from three vendors. They obtained retention indices (based on the alkylarylketone scale [13]) for a series of test compounds in water-methanol medium. Although the carbon loading for the three columns prepared by using monofunctional silane were comparable (3.5, 3.9 and 4.4% C), the retention indices for *p*-cresol and 2-phenylethanol on the CPS-Hypersil column were considerably higher than that for the Spherisorb CN and Ultrasphere CN columns. The retention indices for the CPS-Hypersil cyano column were comparable to those obtained for ODS-Hypersil. The difference among brands was larger than that observed for the same test compounds on different brands of ODS silicas [14]. This suggests that carbon loading alone cannot be used to explain the hydrophobic nature of cyano bonded phases. Such chromatographic evaluations only reveal that columns from different vendors exhibit differences in retention behavior but does not explain why. Spectroscopic analysis of the bonded phases should reveal the nature of the chemical species, the relative population of primary and endcapping siloxane groups and their functionalities.

Infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy have been extensively used to characterize chemically modified silica surfaces. Diffuse reflectance (DR) Fourier transform (FT) infrared spectroscopy was used to study the hydrolytic stability of cyano bonded silica samples [13]. The characteristic infrared bands from the cyano, amide and carboxyl groups can be used to determine the functional group purity of cyano bonded phases used in solid phase extraction columns from different vendors. Solid-state ^{13}C and ^{29}Si NMR spectroscopy is being increasingly used to understand the nature of the alkyl chain, confirm the presence of an endcapping agent, and also determine the functionality of the silane used to modify the silica surface. Recent publications have shown that chromatographic evaluations combined with spectroscopic data result in more complete understanding of the modified surface. Solid-state NMR studies on a series of commercially available C_{18} columns provided data regarding surface structure, silane loading and endcapping confirmation that agreed well with the chromatographic retention behavior method using large polycyclic aromatic hydrocarbons as probe molecules [16]. Two independent groups used solid-state NMR spectroscopy and extensive leaching followed by chromatography of probe molecules to show that trifunctional octyl [17] and octadecyl [18] silanes are bound more strongly to silica than di- or monofunctional silanes. This confirms earlier data obtained by HF-digestion followed by GC analysis of bonded phases [19].

This paper describes the use of elemental analysis, chromatography of probe molecules and IR and NMR spectroscopy to characterize cyano bonded phases from commercially available solid-phase extraction cartridges.

EXPERIMENTAL

Reagents

The cyano solid-phase extraction columns from J. T. Baker, Burdick & Jackson, Fisher, Supelco, Analytichem and Waters were purchased. The plastic housing of each column was cut open and the bonded phase recovered. A micro riffler was used to obtain a representative sample. Each sample was identified by a letter code A to F chosen at random. "Baker analyzed"-grade reagents such as potassium chloride, salts, triethylamine, HPLC-grade solvents and water were used. Acetophenone, valerophenone, octanophenone and dopamine were purchased from Aldrich.

Instrumentation

The cyano bonded phase being tested was packed dry into HPLC columns (250 \times 4.6 mm I.D.). The column was equilibrated with methanol, water and then with the mobile phase being used.

A Varian HPLC pump (Model 9010), variable-wavelength detector (Model 9050) and an integrator (Model 4400) were used. Absorbance was measured at 254 nm while the mobile phase was being pumped at a flow-rate of 1 ml/min. The back pressure for each column was monitored.

To determine the hydrophobic nature of the cyano bonded phase, the test compounds used were a set of alkylarylketones such as acetophenone, valerophenone and octanophenone. They were diluted (usually 0.25–0.5 μl in 1 ml of methanol–water, 50:50) and injected onto the cyano column (20- μl loop). The void volume of

the column was established by injecting acetone. The average retention time for each test compound was obtained from at least three injections from which the capacity factors were calculated. The process was repeated for three columns packed with cyano bonded phase B (chosen because it had the highest percent nitrogen value) to obtain the average capacity factors which were plotted against carbon number ($\times 100$) for each test compound. The regression equation obtained from this plot was used to calculate the retention index for the test compounds for other cyano columns.

To determine the ion-exchange properties of the cyano phases, a 4 mM solution of dopamine (pK_a 8.8) in 0.1 M phosphate buffer at pH 6.0 was used. To overcome severe peak tailing due to interaction of dopamine with residual silanols, 1% triethylamine was added to the mobile phase. To obtain reasonable retention times on all the cyano columns by reducing hydrophobic interactions, the mobile phase used was buffer-methanol (80:20). Capacity factors (k') were obtained from the measured retention time for dopamine for each column.

DR-FT-IR spectra were recorded using a Nicolet 740 spectrometer (Madison, WI, U.S.A.). A 10% dispersion of the bonded phase in finely ground KCl was filled in the sample cup of a Harrick diffuse reflectance accessory (Ossining, NY, U.S.A.). The sample spectrum, recorded at 8 cm^{-1} resolution, was ratioed to that of pure KCl and converted to Kubelka-Munk units. The area of the cyano and other bands of interest were ratioed to that of the silica reference band [20].

Solid-state NMR spectra of ^{13}C at 37.74 MHz and of ^{29}Si at 29.81 MHz were obtained on a Nicolet NT 150 spectrometer that has been modified for cross polarization magic angle spinning (CP-MAS) experiments. Approximately 0.6-g samples were spun at 3.5–4.0 kHz using Delrin rotors based on the design of Wind *et al.* [21]. In both ^{13}C and ^{29}Si experiments, rf field strengths were matched at 50 kHz. For ^{13}C cross-polarization, a contact time of 2 ms was used. A 5-ms contact time was used in the ^{29}Si measurements. In neither case were the observed intensities corrected for distortions that can arise from non-uniform cross-polarization dynamics. Therefore, the relative intensities within a spectrum are semiquantitative.

RESULTS AND DISCUSSION

Bonded phase variability has been a continuing concern throughout the development of HPLC and its companion technique, solid-phase extraction. Of the widely used bonded phases, the cyano phase has the unenviable reputation of being the most variable and especially prone to reproducibility problems. In spite of this, a number of solid-phase extraction applications have been routinely performed using Bakerbond cyano solid-phase extraction columns with no apparent problems. However, a particular production lot (A39081) that appeared normal by our routine analytical procedures, failed to perform for some of these applications. Table I gives the elemental analysis data for some recent lots of cyano bonded phase along with that for lot A39081. Clearly, from elemental analysis or C/N ratio, one cannot conclude why this lot should behave differently from others. This prompted us to investigate various aspects of surface structure that determine analyte retention on a cyano bonded phase and the actual surface composition of cyano bonded phases using a number of techniques. As only a limited amount of the material from lot A39081 was available, we chose to use a later lot for an in-depth analysis. We also decided to investigate cyano

TABLE I
ELEMENTAL ANALYSIS DATA FOR SOME LOTS OF CYANO PHASES FROM J.T. BAKER

Lot No.	%CHN	C/N Ratio
A39081	9.6%C, 1.8%H, 2-3%N	4.17
A48117	10.8%C, 1.9%H, 2.4%N	4.50
C04125	9.2%C, 1.5%H, 2.5%N	3.68
C07102	10.9%C, 2.0%H, 2.6%N	4.19
D04106	9.8%C, 1.7%H, 2.3%N	4.26

bonded phases from solid-phase extraction cartridges from Analytichem, Burdick & Jackson, Fisher, Supelco and Waters to extend the study to better understand the basis for retention differences. These vendors were thought to be representative of a variety of cyano bonded phase types. In the discussions that follow, each sample is identified by a letter code (A to F) chosen at random to represent each vendor. The identity of each sample (vendor and lot number) is available to the reader upon request.

Chemical characterization

Table II gives the chemical analysis data for the various cyano bonded phases. Products A and B form a group with the highest carbon loading while the rest have comparable but lower silane loading. The C/N ratio for these products vary over a wide range.

Chromatographic characterization

The retention indices of three alkylarylketones were determined as described in the Experimental section to establish the hydrophobic properties of the different cyano bonded phases. Fig. 1 shows the average (from three columns) $\log k'$ values obtained for acetophenone, valerophenone and octanophenone plotted against the number of carbon atoms in these molecules ($\times 100$) for the cyano bonded phase from vendor B. The regression equation calculated from this data was used to determine the retention index (calculated carbon number $\times 100$) for all the cyano bonded phases after determining k' values for the three alkylarylketones. The data are given in Table III. A plot of the theoretical carbon numbers ($\times 100$) against the experimentally determined values is shown in Fig. 2. If the bonded phase is more hydrophobic in a reversed-phase mode than that from vendor B, the regression line should fall above that of B and *vice versa*. It is clear that the cyano phase from E has the most hydrophobic surface even though it has lower %C than B. Products A and B have comparable hydrophobicity while that from vendor D is the least hydrophobic. This behavior cannot be explained simply based on carbon and nitrogen content if cyano groups are responsible for the hydrophobic character.

Dopamine was used as a probe molecule to determine the ion-exchange properties of the cyano phases. The mobile phase pH was fixed at 6.0 to ensure that dopamine (pK_a 8.8) was present as a positively charged species capable of interacting with acidic groups. Severe peak tailing was observed for all the columns due to the interaction of dopamine with residual silanols. This was overcome by adding 1% triethyl-

TABLE II
COMPARATIVE CHEMICAL AND SPECTROSCOPIC DATA FOR CYANO BONDED PHASES

Vendor code	%CHN	C/N Ratio	IR CN band ratio	Peak positions (ppm) in the	²⁹ Si NMR spectrum	
					¹³ C NMR spectrum	²⁹ Si NMR spectrum
A	9.05C, 1.86H, 0.66N	13.71	0.15	-0.1, 12.9, 19.2, 36.8, 50.9, 176.4	15, -56, -66, -100, -110	
B	9.18C, 1.53H, 2.52N	3.64	1.23	-0.2, 12.7, 19.1, 50.7, 120.5	15, -56, -66, -100, -110	
C	7.06C, 1.42H, 1.60N	4.41	0.74	-0.2, 12.4, 19.7, 51.1, 121.6	15, -57, -66, -100, -110	
D	6.88C, 1.34H, 1.61N	4.27	0.82	-, 12.4, 19.7, 36.6, 50.8, 120.3	-45, -55, -66, -100, -110	
E	7.24C, 1.64H, 1.16N	6.24	0.43	-0.4, 16.1, 19.7, 118.9	15, -100, -110	
F	6.66C, 1.18H, 1.34N	4.97	0.38	-0.4, 16.1, 19.8, 50.6, 118.9	15, -9, -16, -100, -110	

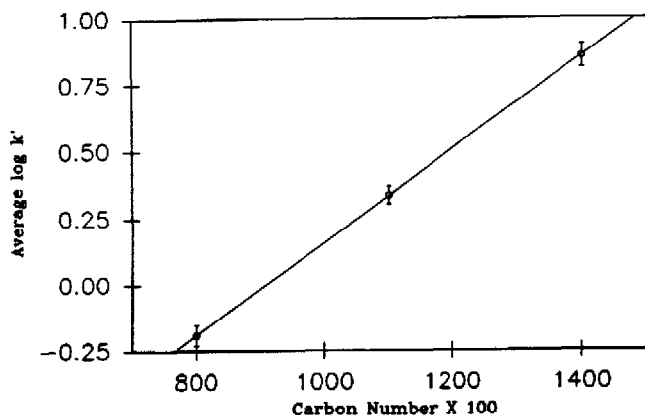


Fig. 1. Plot of average $\log k'$ values *versus* carbon number ($\times 100$) of acetophenone (800), valerophenone (1100) and octanophenone (1400). Slope: $1.74 \cdot 10^{-3}$; intercept: -1.587 ; correlation coefficient: 0.999. Error bars represent 3σ .

amine to the mobile phase. Under these conditions, dopamine was not expected to be retained on the column unless the surface had ion-exchange properties. Capacity factors given in Table III indicates that samples C, E and F did not have ion-exchange properties. The ion-exchange capacity (based on k' for dopamine) for other phases decreased in the order $A > D > B$.

The back pressure was generally 5–10 atm for all the columns during chromatographic evaluations, except for the sample from vendor E which gave a back pressure of 90–100 atm. When the bonded phase was suspended in methanol, the supernatant layer was turbid indicating the presence of fine silica particles. After decanting this layer several times to remove most of the fine silica, the column back pressure was 8–10 atm while the k' values for the test compounds were unchanged.

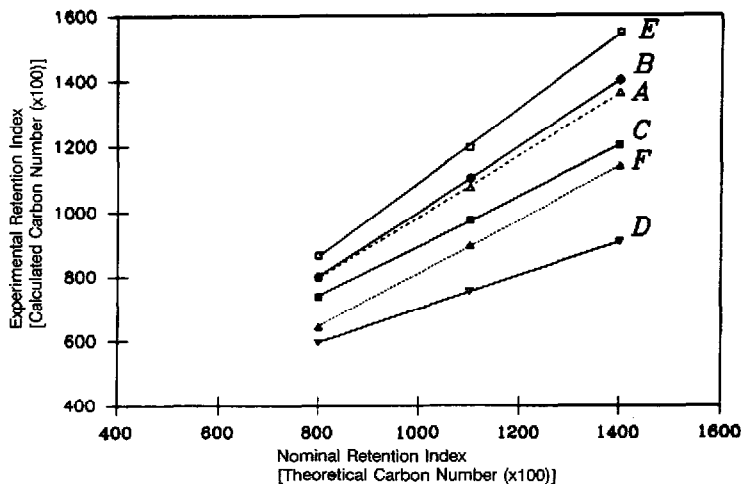


Fig. 2. Plot of theoretical carbon number ($\times 100$) *versus* the number calculated from regression equation from Fig. 1 for samples A to F.

TABLE III
COMPARATIVE CHROMATOGRAPHIC DATA FOR CYANO BONDED PHASES

Vendor code	Retention index for			Capacity factor for Dopamine
	Aceto	Valero	Octano	
A	799	1073	1362	0.97
B	800	1101	1400	0.15
C	738	975	1201	0.02
D	557	754	907	0.24
E	866	1196	1547	0.01
F	645	896	1138	0.01
Theoretical	800	1100	1400	

Spectroscopic characterization

Fig. 3 shows the DR-FT-IR spectrum of the cyano bonded phase B. It shows the expected cyano group stretch band at 2252 cm^{-1} and the silica substrate Si-O-Si combination band at 1871 cm^{-1} . The ratio of the cyano to silica band area for each sample was obtained. This ratio was used to compare the relative concentration of the cyano groups. Table II lists the ratios obtained, which follows the same trend as the %N data. Trace levels of amide groups, produced by the hydrolysis of cyano groups in the presence of hydrochloric acid (which is a by-product of the reaction with chlorosilanes) and water give rise to two bands at 1666 and 1624 cm^{-1} (amide I and II bands, respectively) shown in Fig. 3. IR spectra of samples A and D (spectra A and B in Fig. 4) showed carboxyl carbonyl bands at 1720 cm^{-1} , presumably produced by further hydrolysis of the amide groups, in addition to the amide and cyano bands. Fig. 4 also shows the IR spectra of samples from (C) vendor B and (D) J.T. Baker (lot

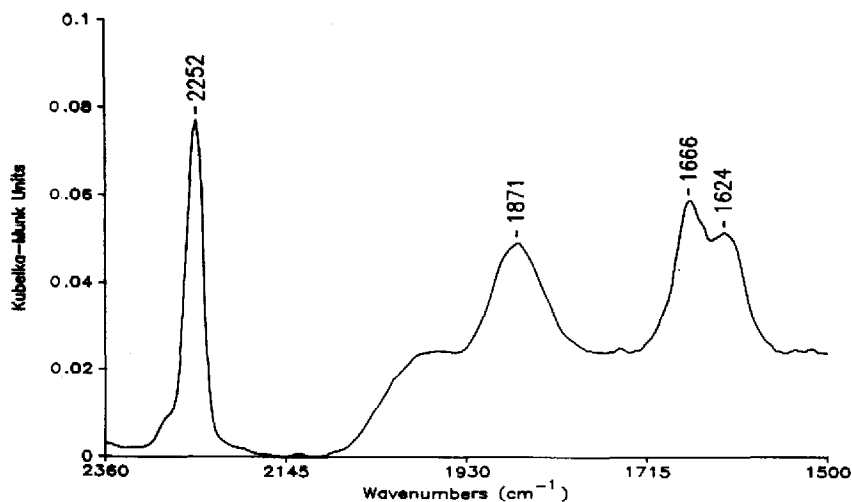


Fig. 3. DR-FT-IR spectrum of cyano bonded phase from vendor B. See text for band assignments.

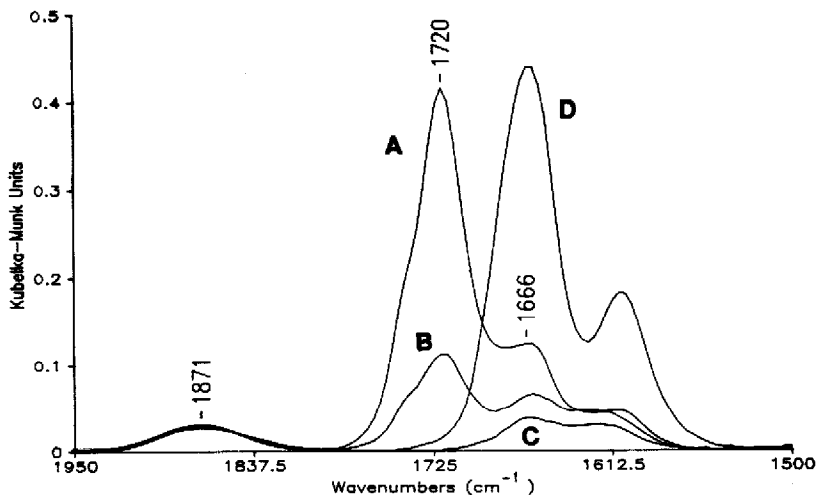


Fig. 4. IR spectra of cyano bonded phase samples from vendors (A) A, (B) D, (C) B and (D) J.T. Baker (lot No. A39081).

No. A39081). These spectra have been normalized to the silica band area for easy comparison of carbonyl band intensities. Spectra of samples from other vendors showed carbonyl band intensities similar to spectrum C.

Fig. 5 shows the solid-state ^{13}C NMR spectra of cyano bonded phases A, B and D as spectra A, B and C, respectively. The solid-state ^{13}C NMR spectrum of the cyano bonded phase C was very similar to that from vendor B and hence is not shown. A resonance located at about 0 ppm is from methyl carbon directly attached to silicon such as the trimethyl silyl group. Resonances at 12.4 and 19.2 ppm arise from methylene carbon while that at 121.5 ppm is from the cyano carbon. A peak at 50 ppm has been attributed [22] to methoxy carbon, presumably produced during the washing of the bonded phase with methanol. In addition to the aliphatic, cyano and methoxy carbon resonances, the spectrum of a sample from vendor D (C in Fig. 5) shows a peak at 36.7 ppm which has been attributed to the carbon adjacent to an amide carbon. The carboxyl carbonyl carbon peak should occur at 176 ppm and is barely visible above the instrumental noise. The absence of a peak at 0 ppm indicates that the sample does not have methyl groups directly attached to silicon as would be expected from an endcapped product or from a monofunctional silane. The solid-state ^{13}C NMR spectrum of the cyano bonded phase A (A in Fig. 5) shows, in addition to the aliphatic and methoxy carbon resonances, two intense peaks at 36.7 and 176.4 ppm. They have been attributed to the amide and carboxyl carbonyl carbons, respectively. The NMR spectrum shows virtually no cyano carbon peak, which is confirmed by the low cyano band area ratio in the infrared spectrum and by the low analysis for nitrogen. The peak at 0 ppm suggests that either a monofunctional silane has been used or the product has been endcapped.

The alkyl carbon resonances of samples A to D all occur at the same position (Table II) indicating that the silane with the same alkyl chain structure has probably been used. Readily available silanes containing the cyano groups are the cyanopropyl

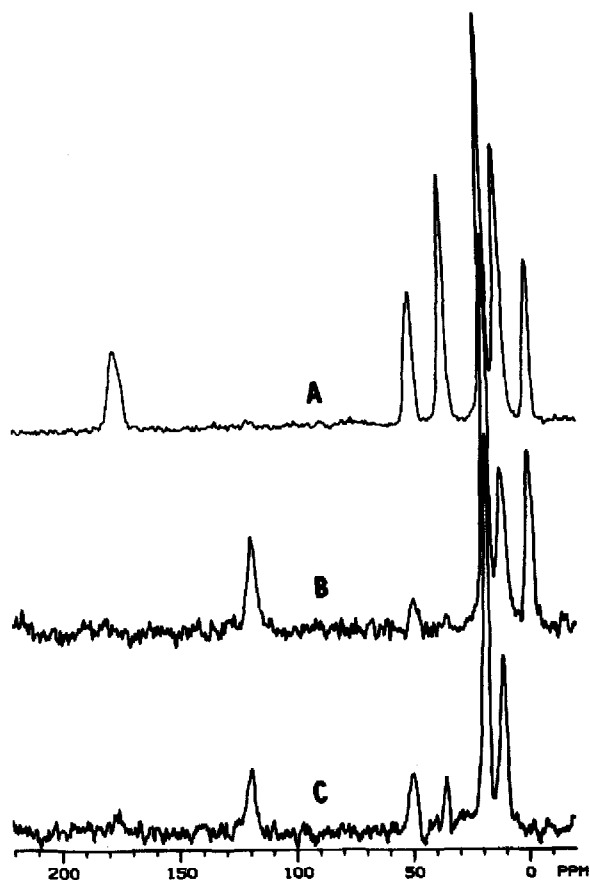


Fig. 5. Solid-state ^{13}C NMR spectra of cyano bonded phases (A) A, (B) B and (C) D. See text for band assignments.

or cyanoethyl trichloro (or trimethoxy) silanes. A ^{13}C NMR spectrum of cyanopropyltrichloro silane, used by J.T. Baker to make its product showed four peaks. A resonance at 18.9 ppm was assigned to the methylene carbon adjacent to silicon while the peaks at 19.2 and 23.2 ppm arise from the α - and β -methylene carbons, respectively: the cyano carbon resonance occurs at 118.5 ppm. Based on these assignments, the peak at 12.4 ppm in the solid-state ^{13}C NMR spectrum of the bonded phases (Fig. 5) is attributed to the methylene carbon adjacent to silicon while the peak at 19.7 ppm is a combination of peaks from the α - and β -methylene carbons. Curves B and C in Fig. 5 confirm the presence of cyano groups in samples B and D, respectively.

Solid-state ^{13}C NMR spectra of samples E and F are shown as spectrum A and B, respectively, in Fig. 6. The aliphatic carbon resonances are at 16.1 and 19.8 ppm. Both samples have a peak at 118.9 ppm from the cyano group. The resonance at 0 ppm can arise from any siloxane surface that has one or more methyl groups attached

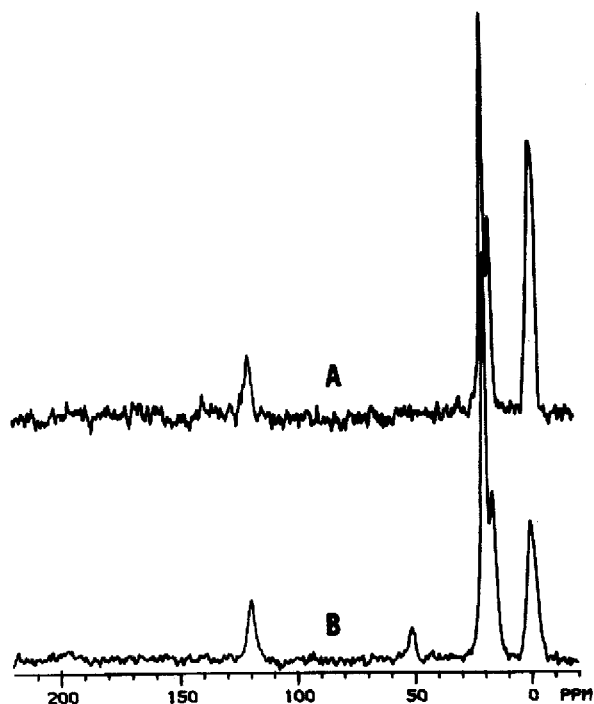


Fig. 6. Solid-state ^{13}C NMR spectra of cyano bonded phases (A) E and (B) F. See text for band assignments.

at 0 ppm arising from $\equiv\text{SiO}-\text{Si}(\text{CH}_3)_x\text{R}$ (where $x = 2$ or 3) is higher for a sample from vendor E compared to that from vendor F. This may suggest that sample E is more thoroughly endcapped or that vendor F may have used a different silane. Table II gives the positions of the observed resonances in the ^{13}C NMR spectra of all the samples.

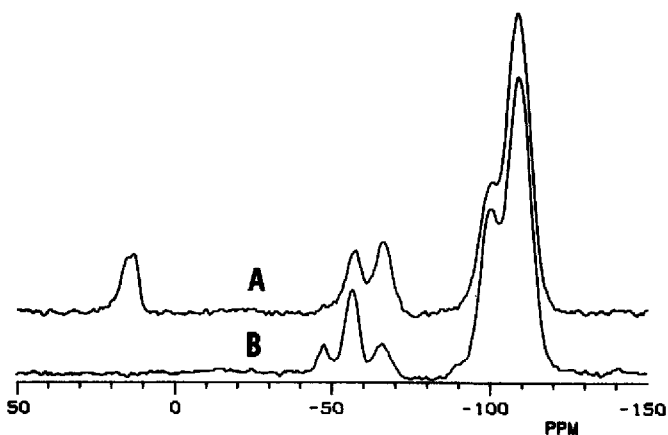


Fig. 7. Solid-state ^{29}Si NMR spectra of cyano bonded phases (A) B and (B) D. See text for band assignments.

TABLE IV
STRUCTURAL ELEMENTS AT THE SURFACE OF BONDED PHASES

Monofunctional	Difunctional	Trifunctional
$\begin{array}{c} \text{CH}_3 \\ \\ \text{-O-Si-CH}_2\text{R} \\ \\ \text{CH}_3 \end{array}$ <p><i>a</i></p>	$\begin{array}{c} \text{CH}_3 \\ \\ \text{-O-Si-CH}_2\text{R} \\ \\ \text{OH} \end{array}$ <p><i>c</i></p>	$\begin{array}{c} \text{OH} \\ \\ \text{-O-Si-CH}_2\text{R} \\ \\ \text{OH} \end{array}$ <p><i>e</i></p>
$\begin{array}{c} \text{CH}_3 \\ \\ \text{-O-Si-CH}_3 \\ \\ \text{CH}_3 \end{array}$ <p><i>b</i></p>	$\begin{array}{c} \text{-O} \quad \text{CH}_3 \\ \quad \diagdown \quad / \\ \quad \text{Si} \\ \quad / \quad \diagdown \\ \text{-O} \quad \text{CH}_2\text{R} \end{array}$ <p><i>d</i></p>	$\begin{array}{c} \text{-O} \quad \text{CH}_2\text{R} \\ \quad \diagdown \quad / \\ \quad \text{Si} \\ \quad / \quad \diagdown \\ \text{-O} \quad \text{X} \end{array}$ <p><i>f</i></p>
	$\begin{array}{c} \text{X} \\ \\ \text{-O-Si-CH}_2\text{R} \quad g \\ \\ \text{-OH} \quad \text{O} \\ \quad \\ \text{-O-Si-} \quad \text{H}_2\text{R} \quad h \\ \quad \\ \text{-OH} \quad \text{O} \\ \\ \text{-O-Si-CH}_2\text{R} \quad g \\ \\ \text{X} \end{array}$	

Fig. 7 shows the solid-state ^{29}Si NMR spectra of cyano bonded phases (A) B and (B) D. The ^{29}Si NMR spectrum of sample A was very similar to B and hence is not shown. However, peak positions are listed in Table II for all the samples. Table IV gives the structural elements that give rise to different peaks. The resonance at 15 ppm arises from Si atoms with two or three methyl groups directly attached (such as the trimethylsilane) as shown by *a* or *b* in Table IV. A resonance at -55 ppm is assigned to the silicon atom with the environment shown as *f* or *g*, and the peak at -66 ppm is assigned to the silicon atom with environment shown as *h*. The other resonances at -100 and -110 ppm arise from the silicon atoms in the silica matrix. These data confirm that the cyano bonded phase B is made using a trifunctional silane, as it is bound to silica with di- and trisiloxane bonds, and that it has been endcapped.

The solid-state ^{29}Si NMR spectrum of sample D (B in Fig. 7) has no resonance at 15 ppm, confirming the absence of an endcapping agent. The presence of three peaks in the -45 to -66 ppm region confirms the use of a trifunctional silane. The relative intensity of the three peaks indicates that the cyano silane is bound to silica with more disiloxane bonds than trisiloxane bonds. It even has a resonance at -48 ppm attributed to a silicon atom with only one siloxane bond and two free silanols as shown by *e* in Table IV.

The ^{29}Si NMR spectrum of sample E has only one resonance at 15 ppm (A in Fig. 8) arising from silicon atoms with environment shown as *a* + *b* in Table IV. As

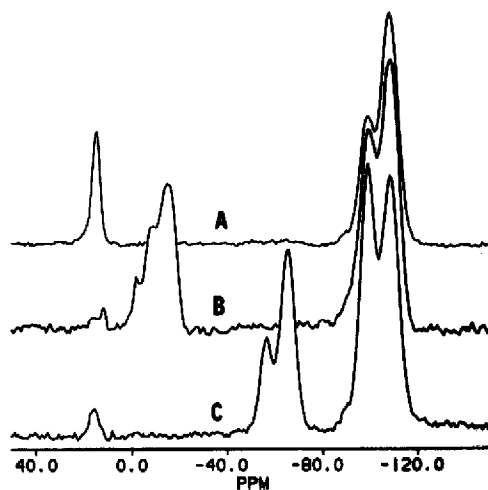


Fig. 8. Solid-state ^{29}Si NMR spectra of cyano bonded phases (A) E (monofunctional), (B) F (difunctional) and (C) C (trifunctional).

there are no peaks in the -45 to -65 ppm region, it has no silicon atoms with one or more siloxane bonds with other than methyl groups attached to them. This implies that a monofunctional cyano silane has been used. It does not confirm whether the sample has been endcapped or not. The ^{29}Si NMR spectrum of sample F (B in Fig. 8) has two peaks which can be attributed to silicon atoms with environment shown as *c* (-9.0 ppm) and *d* (-15.9 ppm). This suggests the use of a difunctional cyano silane for the primary bonding. From the intensity of the peak at 15 ppm, one can conclude that the product has been very lightly endcapped. For comparison, curve C in Fig. 8 is the ^{29}Si NMR spectrum of sample C which is identical to that from vendor B. Both samples have been made with a trifunctional silane and have been endcapped.

CONCLUSIONS

The retention of analytes (such as basic drugs) on a cyano bonded phase is achieved by a combination of hydrophobic, hydrophilic and ion-exchange mechanisms. The relative contribution of each of these mechanisms is affected by the relative population of cyanopropyl and endcapping silanes, presence of residual silanols and other ionizable groups. The six cyano bonded phases compared here have widely different hydrophobic and ion-exchange properties. They can be classified into groups depending on the nature of the bonding chemistry. Vendors of samples A to D all apparently use a trifunctional silane with cyanopropyl groups. The C/N ratio for this silane is 3.4. Vendor E uses a monofunctional silane, most probably functionalized with cyanopropyldimethyl groups. The C/N ratio for this compound is 5.1. Vendor F apparently uses a difunctional silane. If it is cyanopropylmethylsilane, the C/N ratio will be 4.3. The extent of deviation from the theoretical C/N ratio can be used to determine the relative population of the cyano and endcapping silanes if it is known that no cyano group hydrolysis has occurred. Based on the above assump-

TABLE V
COMPARATIVE SUMMARY OF CYANO PHASES

Vendor code	Cyano silane functionality	End cap	Remarks
A	Tri	Yes	High amide/carboxyl content
B	Tri	Yes	High cyano group content and purity
C	Tri	Yes	High cyano group purity
D	Tri	No	Amide/carboxyl groups present
E	Mono	Yes	Higher level of silica fines
F	Di	Yes	Lightly endcapped

tions and the experimental results, the following conclusions can be drawn about each cyano bonded phase (also summarized in Table V).

Sample A

This product has high carbon loading which is comparable to that from vendor B. It has the lowest cyano group content and hence the highest C/N ratio. Most of the cyano groups appear to have been hydrolyzed to amide and carboxyl groups giving the bonded phase the highest ion-exchange properties. The alkylarylketones used to determine the hydrophobicity are not retained by ion-exchange mechanisms and hence are not affected by the presence of these ionizable groups. The product is made with a trifunctional silane and has been endcapped. The silane is bound to silica with mostly di- and trisiloxane bonds.

Sample B

This cyano product has the highest carbon loading, cyano group content and functional group purity. It is also made with a trifunctional silane and has been endcapped. The C/N ratio is the closest to theoretical value for a trifunctional silane, from which it can be concluded that only 7% of the carbon loading is from the endcapping agent. The silane is bound with only di- and trisiloxane bonds.

Sample C

This product belongs to a set of bonded phases with lower carbon content, about 7% carbon, even though it is made with a trifunctional silane. It has been endcapped and thoroughly cured to bond the silane with di- and trisiloxane bonds. It has good cyano functional group purity. The C/N ratio indicates that about 29% of the carbon loading is from the endcapping agent. The lower population of cyanopropyl groups on the surface makes it less hydrophobic than B but more hydrophobic than D and F.

Sample D

Carbon loading is relatively low for a product that is made with a trifunctional silane. Failure to endcap has left residual silanols making it the least hydrophobic phase. Some of the cyano groups have been hydrolyzed to amide and carboxyl groups thus increasing the C/N ratio and heterogeneity in surface characteristics.

Sample E

This cyano phase is made with a monofunctional silane leading to only 7% carbon loading. From the C/N ratio, one can conclude that 22% of the carbon loading is from the endcapping agent. The relatively higher population of trimethylsilyl groups renders this product to be the most hydrophobic in the reversed-phase mode. However, the retention properties of this product for basic drugs may be quite different from one that has predominantly cyanopropyl groups. It has high levels of fine silica particles possibly restricting the flow during solid-phase extraction.

Sample F

This product is made with a difunctional silane and has been lightly endcapped. These facts explain why the relative intensity of the peak at 0 ppm in the ^{13}C NMR spectrum (Fig. 6, curve B) is much lower than that for sample E (curve A). From the C/N ratio, one can conclude that only 12% of the carbon loading arises from the endcapping agent. However, the presence of silicon atoms with residual silanols makes this product only slightly more hydrophobic than that from vendor D.

General

This study expands the results of earlier studies [2,3,9–12] in demonstrating that a number of variables determine the surface properties of the cyano bonded phase. Although chromatographic evaluations reveal differences in the overall retention properties [12–14], chemical and spectroscopic data support and explain why these differences arise. Failure to control particle size distribution in the final product can result in contamination of samples with finely divided silica and prevent reproducible flow-rates during solid-phase extraction. The functionality and functional group purity of the cyano silane present on the silica surface markedly affects the hydrophobic properties of the final bonded phase. Failure to cure or endcap leave the often undesirable silanols which are known to interact strongly with basic compounds. Improper conditions during the manufacturing process that permit the hydrolysis of cyano groups to amide/carboxyl groups produces a surface with ion-exchange properties. It was concluded from this study that the performance problems found with the J.T. Baker cyano phase (lot No. A39081) apparently occurred because it had a relatively high concentration of amide groups resulting from the first step in the hydrolysis of cyano groups (curve D in Fig. 4). A detailed study [15] of the hydrolytic stability of the cyano group has revealed reaction conditions that lead to hydrolysis. As a result of these studies, cyano bonded phases now meet strict specifications for their cyano and amide group content determined by FT-IR spectrometry.

The above study supports the perception that cyano bonded silica phases contained in solid phase extraction columns from different vendors possess widely varying properties. It is, hence, not surprising that transfer of extraction procedures from one vendor's product to another is often difficult. Understanding the actual surface composition should aid both in development of and in modification of solid phase extraction procedures if it is necessary to transfer to another vendor's product. It should be emphasized that the results described here apply only to specific lots of product from each vendor. Deviation from the theoretical surface structure observed for some of the products could have occurred during aberrations in the manufacturing process for a particular lot.

ACKNOWLEDGEMENT

The authors are grateful to the Colorado State University Regional NMR Center funded by National Science Foundation Grant No. CHE 78-18581.

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