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STUDIES ON THE STABILITY OF *n*-ALKYL-BONDED SILICA GELS UNDER BASIC pH CONDITIONS

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SUMMARY

Mobile phase and stationary phase parameters that influence stability of silica-based reversed phases are examined under high pH conditions. Mobile phases containing 0.1 *M* NaOH and variable methanol content were cycled through columns packed with various alkyl-bonded phases. Chromatography of small molecules and %C analysis were used to probe changing chromatographic performance as phase hydrolysis proceeded. It was evident that the rate of bonded phase hydrolysis was increased when the 0.1 *M* NaOH solution contained a high amount of organic solvent, especially when followed by a high organic-containing acidic wash. C_4 bonded phases were less stable than C_{18} bonded phases. In the latter case, low bonded ligand coverage or the lack of endcapping also reduced stability against base hydrolysis. Reversed phases bonded to acid-washed silica showed greater base stability than those bonded to non-treated silica. The use of a silica precolumn did not serve to increase system usefulness. However, the use of soluble sodium silicate in the NaOH solution increased column lifetime for both short (*i.e.* C_4) and long (*i.e.* C_{18}) *n*-alkyl chain reversed phases.

INTRODUCTION

Recent advances in synthesis and characterization of *n*-alkyl-bonded silicas for reversed-phase liquid chromatography (RPLC) have resulted in improved reproducibility and stability of these packings. While a number of reports are concerned with the use of silica-based column packings in mobile phases of pH *ca.* 2–7[1–4], several groups have been concerned with packing stability in the region of eluent pH > 7. Various approaches to this problem have included the use of metal oxide doped silica[5,6], polymer coatings on silica[7–9], alumina or polymers as alternative stationary phase supports[10–12], as well as mobile phase additives including organic amines or aluminum salts[13,14]. A frequently employed approach is the use of a short precolumn loosely packed with silica gel or the bonded phase placed between the pump and injector to allow equilibration of the mobile phase with silica[15].

A distinction should be made between the use of basic pH eluent conditions in order to achieve the separation as opposed to a column cleaning step. The former case may employ relatively dilute basic conditions (*e.g.* 10–100 m*M* triethylamine or am-

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monium acetate) for longer periods of time, while column washing may require a more concentrated base (*e.g.* 0.1 *M* NaOH) for a relatively short time (*ca.* five column volumes). Apparently, the use of 0.1 *M* NaOH as a column regeneration solution stems from the conventional biochemical practices on agarose and dextran-type gels[16]. Several advantages of this 0.1 *M* NaOH solution include cost-effective disinfectant action for pyrogen removal, safe presence in the final product, and easy disposal. Interestingly, although so-called base-stabilized reversed phases are commercially available, the problem of packing stability under basic conditions can be of greater concern for more hydrophilic bonded phases, *i.e.* ion-exchange and size-exclusion packings[17–19]. Moreover, acidic solutions of high organic content are frequently promoted as cleaning recipes for silica-based reversed phases[17,20]. Thus, column stability across a wide pH range is often given as a general reason for the use of polymer-based stationary phases[21].

It is well known that silica-based packings can be unstable in basic pH systems. The solubility of silica in water at room temperature is roughly 100–150 ppm over the pH range 2–9[22]. From pH 9–10.7, there is an increase in silica solubility to *ca.* 1000 ppm, owing to the formation of silicate ion and monomeric $Si(OH)_4$. At high pH conditions, the solubility of silica decreases from *ca.* 75 ppm in 25% aqueous methanol to *ca.* 15 ppm in 75% aqueous methanol. The reduction in silica solubility with increase in organic modifier content may explain in part the successful use of silica columns for polar solute separation using alkaline (*e.g.* ammonium acetate or ammonium nitrate) mobile phases rich in organic modifier (*e.g.* methanol)[23,24]. Impurities in the silica or organic coatings on the silica can provide altered solubility depending on the type of impurity or coating and its distribution on the surface[22].

In this paper, we describe several results of our studies on the stability of *n*-alkyl-bonded silicas at high pH. We will show that base stability of C_{18} reversed phases in 0.1 *M* NaOH is a function of organic modifier content in the mobile phase as well as stationary phase variables, including bonded alkyl chain length, coverage, endcapping and acid-washing of the base silica gel. Lastly, we will show that column lifetime can be extended by the use of soluble sodium silicate in the mobile phase. Taken together, these data can serve to improve the knowledge base concerning the reliability of silica-based reversed-phase systems under basic pH solution conditions.

EXPERIMENTAL

Equipment and materials

The liquid chromatograph was composed of a Series 410 four-solvent delivery liquid chromatography (LC) pump, a Model ISS-100 autosampler with injector, and a Model LC-95 variable-wavelength UV detector (Perkin-Elmer, Norwalk, CT, U.S.A.). Data were collected on a C-R6A Chromatopac integrator (Shimadzu, Columbia, MD, U.S.A.) and processed using a Nelson Analytical (Cupertino, CA, U.S.A.) Model 2600 chromatography software package on an IBM-PC AT (Boca Raton, FL, U.S.A.).

Alkylsilanes were obtained from Petrarch Systems (Bristol, PA, U.S.A.). IMPAQ® RG2010Si, RG2010-C18, RG2020-C18 and RG2010-C4 silicas and bonded phases (nominal 200 Å pore diameter, 10 μm or 20 μm diameter packings) were obtained from The PQ Corporation (Conshohocken, PA, U.S.A.). The physical and

TABLE I

CHARACTERISTICS OF SILICA GELS USED

	RG2010 Si	*RG2020 Si*
Pore volume (ml/g)[a]	1.34	1.37
Surface area (m^2/g)[a]	246	257
Median pore diameter (Å)[a]	198	196
Particle size properties		
D_v, 50 (μm)[b]	8.92	17.15
D_v, $50/D_p$, 50[b]	1.22	1.14
D_v, $10/D_v$, 90[b]	1.72	1.66
Metals, anions (ppm)[c]		
Al	77	85
Ca	<25	<25
Fe	54	51
Na	30	36
SO_4^{2-}	<25	<25
Cl^-	<25	<25
pH[d]	5.1	5.2
Loss on drying[e]	5.6	4.6

[a] Porosimetry determined by nitrogen sorption (BET method).

[b] Particle-size analysis by Coulter counter. $D_{v,50}$ = 50% point on the cumulative volume (mass) distribution of particle size; $D_{p,50}$ = 50% point on the cumulative population (frequency) distribution of particle size.

[c] Cation analysis by HF digestion of silica followed by flame atomic absorption spectroscopy for Na and inductively coupled plasma spectroscopy for Al, Ca, Fe and Mg determination. Anion analysis was by ion chromatography of water extracts of the silica.

[d] pH measured of a 10% slurry of silica in water.

e Loss on drying determined by the weight change of the silica after heating at 105°C for 2 h.

chemical characteristics of these silica gels are shown in Table I. The bonded packings utilize monomeric *n*-alkylsilane chemistry with endcapping on acid-washed silica. In certain experiments to study stationary phase variables, *n*-alkyl-bonded silicas were synthesized using a modified procedure of Kinkel and Unger[25]. Elemental analyses were determined on a Perkin-Elmer Model 2400 CHN analyzer with a precision of *ca.* 1% R.S.D.

Column hardware was obtained from Extrudehone (Irwin, PA, U.S.A.) and Valco Instruments (Houston, TX, U.S.A.). Small molecule solutes were obtained from Sigma (St. Louis, MO, U.S.A.) and Aldrich (Milwaukee, WI, U.S.A.) and used as received. Orthophosphoric acid was a "Baker Analyzed" grade reagent (J. T. Baker, Phillipsburg, NJ, U.S.A.), while monobasic and dibasic sodium phosphate were obtained from Sigma. Urea was from Bio-Rad (Richmond, CA, U.S.A.). Sodium hydroxide, HPLC-grade methanol and isopropanol (IPA) were from E. M. Science (Cherry Hill, NJ, U.S.A.). Sodium silicate "N" Clear was obtained from PQ Corporation. HPLC-grade water was prepared in-house.

Chromatographic procedures

The stationary phases were packed into 15 × 0.46 cm I.D. columns using standard slurry procedures with methanol as the driving solvent. Methanol–water

and IPA–water mobile phases were prepared by adding the correct volumes of organic solvent and water following by dissolution of the appropriate acid or buffer salt. pH was adjusted as desired by use of the appropriate acid or base.

The multi-solvent LC pump and autosampler were programmed to automate column testing with repeated exposure of the column to alkaline mobile phases. In the typical experiment, an initial evaluation was performed on the freshly packed reversed-phase column using a standard test mixture of uracil, N-acetylprocainamide (NAP), caffeine and phenol[2] in a standard mobile phase (*i.e.* solvent A) of methanol–water (20:80) containing 0.1% (*ca.* 15 m*M*) orthophosphoric acid. This analysis lasted 20 min. Keeping the flow-rate constant at 1 ml/min, the pump then delivered a 5-min linear gradient to reach 100% solvent B consisting of 0.1 *M* NaOH in methanol–water (80:20 or 20:80, see Table III). Solvent B was then pumped isocratically for 15 min and was followed by a 5-min linear gradient to 100% solvent C consisting of 0.1 *M* or 25 m*M* HNO_3 in aqueous IPA. After isocratic operation of solvent C for 15 min to wash away any hydrolyzed hydrocarbon chains from the packing, the LC pump then delivered a 5-min linear gradient back to 100% solvent A. After equilibration of the column in solvent A for 15 min, the autosampler made an injection of the standard test mixture for reevaluation of solute retention. This solvent program

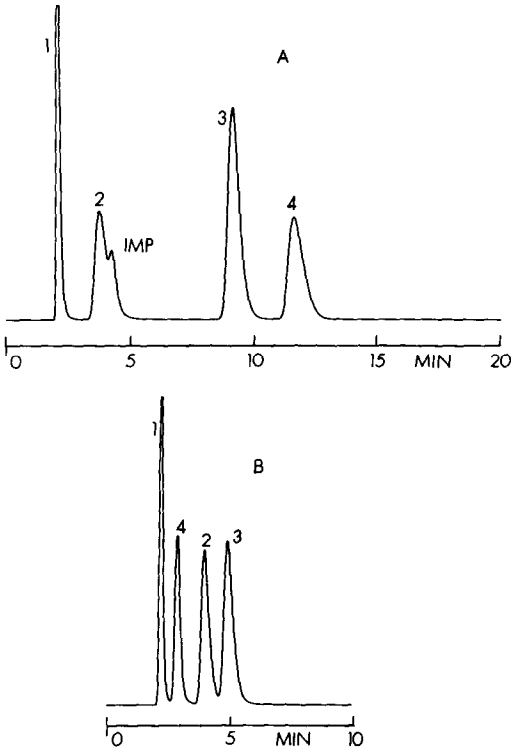

Fig. 1. Chromatography of uracil (1), NAP (2), caffeine (3) and phenol (4) on C_{18} columns. The solutes were chromatographed in a mobile phase of 20% (v/v) aqueous methanol containing 15 m*M* H_3PO_4 at 1.0 ml/min and 25°C. (A) Bonded silica containing 3.41 μmol/m^2 C_{18} groups. (B) Bonded silica containing 1.97 μmol/m^2 C_{18} groups. IMP = Impurity.

could be recycled as many times as needed. At the end of the study, the columns were washed with IPA and sacrificed to obtain elemental analysis data.

RESULTS AND DISCUSSION

The aim of this paper was to evaluate the effects of basic pH eluents on *n*-alkyl phases bonded to silica gel. Our first experiments involved the development of a small molecule test mixture to characterize the reversed-phase columns prior to treatment with a NaOH solution. Fig. 1 compares chromatograms of the solute mixture uracil, NAP, caffeine and phenol from a fully bonded (*i.e.* 3.41 μmol/m^2) C_{18} packing and from a partially bonded (*i.e.* 1.97 μmol/m^2) C_{18} packing, both based on high purity 10-μm granular silica gel having 200-Å pores (Table I). Note that altered selectivity for NAP, caffeine and phenol is obtained on the two C_{18} bonded phases. Table II shows the results of alterations in the methanol–water (20:80) mobile phase to investigate the retention behavior of these solutes on the fully bonded C_{18} reversed phase. Decreases in mobile phase pH or increases in ionic strength caused a decrease in k' or band sharpening of NAP, suggesting a largely ionic interaction of this solute with the surface (*i.e.* with acidic silanols). The NAP solute is also retained relatively longer on the partially bonded C_{18} packing, which is known to have accessible silanols. Caffeine retention is largely independent of pH, but is reduced upon addition of urea (or acetonitrile) to the mobile phase. This result suggests that caffeine is mainly sensitive to hydrogen bonding with the surface silanols[26]. Lastly, the solute phenol can be used to gauge the hydrophobicity of the bonded phase except at high pH (pK_a of phenol is *ca.* 10[27]). The results obtained for caffeine and phenol for the partially bonded C_{18} reversed phase in Fig. 1B support the above explanation.

In the next sections of this paper, we will examine the role of mobile phase components and stationary phase variables on column stability under high pH conditions. Lastly, we will briefly examine the use of soluble sodium silicate as an aid to extended column lifetime. Taken together, the results provide a perspective on parameters that control the stability of silica-based *n*-alkyl reversed phases at high pH.

Effect of mobile phase variables

In this study, we examined mobile phase parameters of the basic pH solution that influence the stability of *n*-alkyl-bonded silicas. Several 15 × 0.46 cm I.D. columns of a fully bonded C_{18} reversed phase based on 10-μm granular silica of 200 Å pore diameter were packed and evaluated using the standard 20 min separation of uracil, NAP, caffeine and phenol. Subsequent to this result, the mobile phase was changed to 0.1 *M* NaOH in aqueous methanol as outlined in Experimental. A final wash of the column with acidic aqueous IPA was programmed before return to the standard conditions for reevaluation of the column.

Table III summarizes the variables studied in this set of experiments including NaOH concentration, and the effects of methanol and IPA content in the wash solutions and reports initial and final %C and k' results. Interestingly, losses in %C do not correlate well with observed changes in k'. For example, the C_{18} packing of experiment 3 shows a loss of 1.76%C with reduction in k' for NAP, caffeine and phenol, while in experiment 5, a loss in selectivity for caffeine and phenol is observed with roughly the same *ca.* 10% loss in bonded carbon. Note that experiment 7 in-

TABLE II

RETENTION BEHAVIOR (k' VALUES) OF N-ACETYLPROCAINAMIDE (NAP), CAFFEINE AND PHENOL ON A HIGH-COVERAGE C_{18} BONDED SILICA

Mobile phase, methanol–water (20:80) with additives as indicated; flow-rate, 1.0 ml/min; detection at 254 nm, 0.2 a.u.f.s.; temperature, 25°C; column: 3.41 μmol/m^2 C_{18} groups on 10 μm silica of 200 Å pores; column dimensions, 15 × 0.46 cm. I.D. pH values measured in hydro-organic mixture.

Solute	*Mobile phase additives*								
	None	*15 mM H_3PO_4, pH 2.3*	*15 mM NaH_2PO_4, pH 5.0*	*15 mM NaH_2PO_4, pH 7.0*	*15 mM Na_2HPO_4, pH 9.2*	*0.1 M NaOH, pH 13*	*15 mM H_3PO_4, 1 M urea*	*1 mM NaCl*	*10 mM NaCl*
NAP	–[a]	0.60	0.94	1.91	–[a]	–[a]	0.58	1.26[b]	1.40
Caffeine	3.45	3.51	3.51	3.54	3.68	3.90	2.79	3.61	3.61
Phenol	4.35	4.23	4.23	4.25	4.13	0.42	3.96	4.38	4.40

[a] No elution observed.
[b] Eluted with tailing.

dicates bonded phase hydrolysis is not due to the acidic aqueous IPA as observed previously[2]. It is likely that chromatographic performance is more sensitive to phase hydrolysis than %C measurements. Moreover, the entire column was sacrificed to provide a sample for %C, which may represent an "averaging" of the result. More study is required.

Fig. 2 presents a plot of caffeine–phenol selectivity change as a function of base-catalyzed hydrolysis of the C_{18} bonded silica surface, while Fig. 3 shows chromatograms from the C_{18} column through 12 cycles of the 0.1 *M* NaOH wash conditions causing the greatest degradation, experiment 5 (see Table III). Typically, the change in chromatography occurs as a loss of k' for phenol and a k' increase for caffeine, *i.e.* a loss in caffeine/phenol selectivity. We also frequently noticed an increase in operating pressure of the LC system, often enough (*i.e.* >6000 p.s.i.) to shut down the HPLC solvent pump. Interestingly, NAP retention was not often affected in these experiments. Fig. 2 indicates that the most deleterious condition results from washing the C_{18} bonded silica with a basic solution high in organic modifier content. One may speculate that the organic modifier solvates the hydrophobic surface and permits access of OH^-; however, it is not possible at this time to identify the point of hydrolytic cleavage on the packing. The increased IPA content (80%) of the acidic solution decreased the C_{18} column stability only when this solution was used in conjunction with the 80% aqueous methanol containing 0.1 *M* NaOH. This result suggests that the high IPA content is needed for elution of the cleaved stationary phase ligands. In fact, washing the columns at the end of experiments 1 and 2 with IPA–water (80:20) containing 25 m*M* HNO_3 did not change retention of caffeine or phenol.

In separate experiments, we found that 20% aqueous methanol did not wet the C_{18} bonded silica upon suspension of the bonded phase with this mobile phase in a test tube. Chromatography of the small molecules using this mobile phase on an originally "dry" column resulted in one peak eluting at the V_0 position. After passage of methanol (either during the column packing operation or as a separate step) and return to the 20% aqueous methanol, retention and separation of the small molecule probes occurs. This phenomenon probably relates to the presence of a solvent layer enriched in methanol sorbed to the C_{18} bonded surface[28]. Thus, if NaOH is chosen to sanitize a column, one caveat is to minimize the organic content of the basic solution where possible, such that the desired cleaning action is still obtained and column lifetime is maximized.

Effect of stationary phase variables

We next examined in greater detail the role of stationary phase parameters on the stability of the column to basic pH conditions. Table IV lists the characteristics of several packings synthesized for this study, while Fig. 4 plots caffeine–phenol selectivity as a function of passage of 0.1 *M* NaOH in 80% aqueous methanol. With the exception of the partially bonded C_{18} packing (experiment 4) and the C_4 reversed phase (experiment 5) based on 10-μm silica of 200 Å pore diameter, the remaining bonded phases were synthesized on 20-μm silica with 200 Å pores with similar surface chemistry to the 10-μm material (see Table I). In each of the experiments in Table IV, we varied some aspect of the bonding chemistry ranging from a fully bonded material with endcapping on a acid-washed silica (experiment 1) to a partial bonding with no

TABLE III

INFLUENCE OF MOBILE PHASE PARAMETERS ON BASE STABILITY OF C_{18} BONDED SILICA

Experiment No.	*Hydrolytic solvent*	*Wash solvent*	*Vol. of each solution passed (ml)*	*Final %C*[a]	*Final k' values*[b]		
					NAP	*Caffeine*	*Phenol*
1	Methanol–water (20:80), 0.1 *M* NaOH	IPA–water (40:60) 0.1 *M* HNO_3	90	16.17	0.48	3.26	4.27
2	Methanol–water (20:80), 0.2 *M* NaOH	IPA–water (40:60) 0.2 *M* HNO_3	60	15.45	0.44	3.24	4.19
3	Methanol–water (20:80), 0.1 *M* NaOH	IPA–water (80:20), 25 m*M* HNO_3	165	15.09	0.44	3.16	4.06
4	Methanol–water (80:20), 0.1 *M* NaOH	IPA–water (40:60), 0.1 *M* HNO_3	165	15.57	0.41	3.73	4.0
5	Methanol–water (80:20), 0.1 *M* NaOH	IPA–water (80:20), 25 m*M* HNO_3	165	15.32	0.44	4.36	4.36
6	Methanol–water (80:20), 0.1 *M* NaOH	None	165	15.46	0.40	3.56	3.98
7	None	IPA–water (80:20), 0.1 *M* HNO_3	165	16.51	0.51	3.14	4.65

[a] The freshly synthesized C_{18} bonded phase on 10 μm silica of 200 Å pore diameter showed 16.85% C by elemental analysis.

[b] Intitial k' values were 0.71, 3.23 and 4.53 for NAP, caffeine and phenol, respectively.

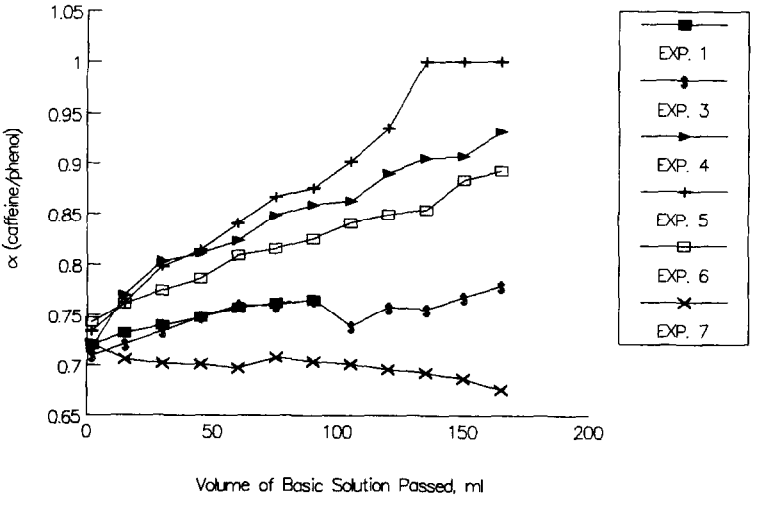

Fig. 2. Caffeine–phenol selectivity on several C_{18} columns as a function of 0.1 *M* NaOH solution passed through the column. Caffeine and phenol *k'* values were measured on each column using the conditions of Fig. 1. See Table III and text for the conditions of experiments 1–7.

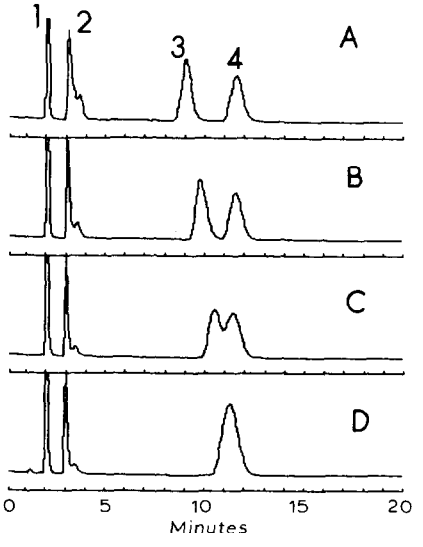

Fig. 3. Chromatography of uracil (1), NAP (2), caffeine (3) and phenol (4) on a C_{18} column through twelve cycles of 0.1 *M* NaOH aqueous methanol solution passed. The solutes were chromatographed on the C_{18} column using the conditions of Fig. 1. See Experimental, Table III, and experiment 5 in Fig. 2 for description of the 0.1 *M* NaOH treatment. (A) Initial evaluation; operating pressure was 150 p.s.i. (B) Column evaluation after cycle 4 of treatment, equivalent to passage of 60 ml each of 0.1 *M* NaOH in 80% aqueous methanol and 25 m*M* HNO_3 in 80% aqueous IPA through the column. (C) Column evaluation after cycle 7, equivalent to 105 ml of each of the wash solvents in (B) passed through the column. (D) Column evaluation after cycle 11, equivalent to 165 ml of each of the wash solutions in (B) passed through the column. Operating pressure was 180 p.s.i.

TABLE IV

INFLUENCE OF STATIONARY PHASE PROPERTIES ON BASE STABILITY OF *n*-ALKYL-BONDED SILICAS[a]

Experiment No.	*Bonded phase*	*Basic solution passed (ml)*[b]	*Initial %C*	*Final %C*	*NAP*		*Caffeine*		*Phenol*	
					Final k'	*Initial k'*	*Final k'*	*Initial k'*	*Final k'*	*Initial k'*
1	C_{18}, endcapped (acid-washed)	75	16.33	14.29	0.74	0.83	3.83	3.21	4.46	4.47
2	C_{18}, endcapped (non-acid-washed)	75	17.16	14.70	0.88	1.08	4.07	3.22	4.07	4.46
3	C_{18}, non-endcap (non-acid-washed)	75	16.87	12.71	0.84	1.10	4.96	3.37	4.24	4.38
4	C_{18}, non-endcap[c] (non-acid-washed)	15	10.11	7.39	0.84[d]	0.78	1.31[d]	1.21	0.32[d]	0.29
5	C_4, endcapped[c] (acid-washed)	45	5.97	4.88	0.27	0.43	2.09	1.94	2.09	2.63

[a] Except as noted, all bonded phases were monomeric *n*-alkylsilyl groups bonded to 20 μm granular silica gel having 200 Å diameter pores.

[b] The basic solution employed in this study was methanol–water (80:20) containing 0.1 *M* NaOH followed by a rinse with 25 m*M* HNO_3 in IPA–water (80:20).

[c] These bonded phases (C_{18} or C_4) were monomeric *n*-alkylsilyl groups bonded to 10 μm silica of 200 Å pore diameter. The C_{18} packing was a partially bonded phase.

[d] High operating pressure terminated this experiment after only one cycle.

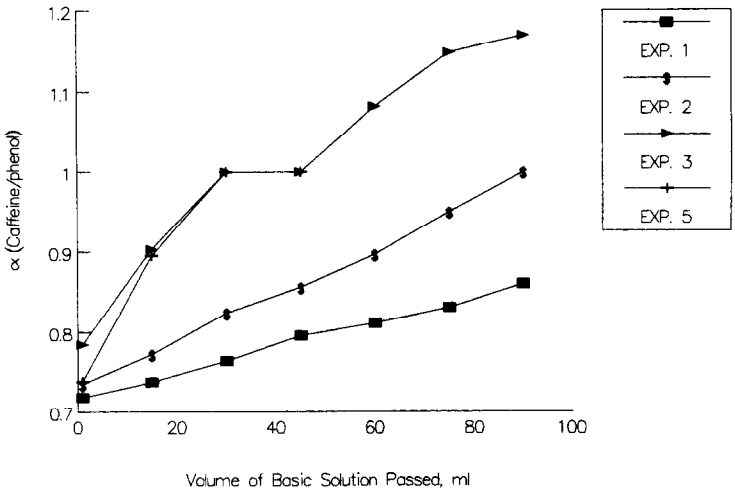

Fig. 4. Caffeine–phenol selectivity as a function of passage of 0.1 *M* NaOH solutions through columns of various stationary phase chemistries. The solutes were chromatographed under the conditions of Fig. 1. See Table IV and text for the conditions of experiments 1–5.

endcap or acid-washing (experiment 4). Finally, a C_4 bonded phase was synthesized with endcapping on the acid-washed silica as indicated in experiment 5. Each of the packings was exposed to the most deleterious conditions of Table III, *i.e.* the 0.1 *M* NaOH in 80% aqueous methanol followed by the 25 m*M* HNO_3 in 80% aqueous IPA wash. In these experiments, a rough correlation was observed between %C loss in the packing and loss of caffeine–phenol selectivity. Thus, the C_{18} column of experiment 1 showed the greatest resistance to hydrolysis corresponding to a *ca.* 13% loss of %C, while the partially bonded C_{18} column of experiment 4 deteriorated (*i.e.* excessive operating pressure) within one cycle with a *ca.* 27% loss of bonded carbon. Fig. 4 indicates that full C_{18} coverage with endcapping on a acid-washed silica provides the greatest resistance to base hydrolysis. Interestingly, inverted elution order of caffeine and phenol was obtained on the non-endcapped (non-acid-washed) C_{18} column (experiment 3) by the fourth cycle (Fig. 4). This column also showed a *ca.* 25% loss in bonded carbon by the end of the experiment. Lastly, the short chain C_4 column (experiment 5) deteriorated within two cycles and high column back-pressure had shut off the LC system by the fourth cycle. Thus, for the same bonded phase chemistry and the same basic pH eluent conditions, the C_4 column appears less stable relative to the C_{18} column. This result agrees with column stability under acidic conditions, in that a more hydrophobic surface shows greater stability to hydrolysis[2]. In the next section, we briefly examine stability of the columns against base hydrolysis using soluble sodium silicate.

Use of sodium silicate

In the past, workers have used precolumns containing silica or bonded phase to presaturate the mobile phase with stationary phase such that the analytical column is not degraded[15]. In a single experiment, we dry-packed a 5 × 0.46 cm I.D. precolumn with 10-μm silica of 200 Å pore diameter and used it in the system in conjunction with

TABLE V

INFLUENCE OF SOLUBLE SODIUM SILICATE ON THE BASE STABILITY OF *n*-ALKYL-BONDED SILICAS[a]

Experiment No.	*Bonded phase*	*Basic solution passed (ml)*	*Initial %C*	*Final %C*	*NAP*		*Caffeine*		*Phenol*	
					Final k'	*Initial k'*	*Final k'*	*Initial k'*	*Final k'*	*Initial k'*
1	C_{18}	165[b]	16.85	15.32	0.44	0.92	4.36	3.28	4.36	4.59
2	C_{18}	165[c]	16.85	15.88	0.44	0.74	3.59	3.28	4.39	4.60
3	C_4	45[b,d]	5.97	4.88	0.27	0.43	2.09	1.94	2.09	2.63
4	C_4	120[c,e]	5.97	5.45	0.25	0.42	2.26	2.13	2.26	2.90

[a] All reversed phases were monomeric *n*-alkyl groups on 200 Å, 10-μm silica.

[b] The basic solution passed was methanol–water (80:20) containing 0.1 *M* NaOH followed by a rinse with IPA–water (80:20) containing 25 m*M* HNO_3.

[c] The basic solution passed was a silicate-saturated 0.1 *M* NaOH in methanol–water (80:20) followed by a rinse with 25 m*M* HNO_3 in IPA–water (80:20), see text.

[d] Excessive operating pressure shut this experiment down in the fourth cycle.

[e] Excessive operating pressure shut this experiment down in the ninth cycle.

a freshly packed 15 × 0.46 cm I.D. column of the fully bonded C_{18} (endcapped, acid-washed) bonded phase based on the same silica. The wash solutions employed were the 0.1 *M* NaOH in 80% aqueous methanol followed by the 25 m*M* HNO_3 in 80% aqueous IPA.

Initially, the operating pressure of the chromatograph was *ca.* 150 p.s.i. By the fourth cycle, the instrument had shut down owing to the 5000 p.s.i. back-pressure of the precolumn. The analytical column showed only *ca.* 180 p.s.i. Note that in the absence of the precolumn we could achieve all eleven of the cycles on this C_{18} packing (see experiment 5 in Fig. 2 and Fig. 3) with initial and final operating pressures of 150 and 180 p.s.i. respectively.

We next examined the use of a commercially available sodium silicate solution in the 0.1 *M* NaOH in 80% aqueous methanol for the purpose of pre-saturating this solution with silica. This solution has a weight ratio of 3.22 SiO_2/Na_2O with 8.9% Na_2O and a pH of 11.3[29]. We diluted this solution by *ca.* 400 times with water to obtain concentrations of *ca.* 12 m*M* SiO_2 (*ca.* 1000 ppm silicate) and *ca.* 7.2 m*M* NaOH. We then dissolved additional NaOH (*ca.* 20 g/l of solution) into this solution to raise the NaOH concentration to 0.5 *M* such that the mixture of 200 ml of this solution with the 800 ml of methanol would bring the final NaOH concentration to 0.1 *M*. Upon addition of the methanol, silica was observed to precipitate from solution, which was not unexpected as the literature indicates a *ca.* 15 ppm solubility of silica in this system[22]. After stirring, the solution was filtered and placed in the chromatograph. All of the solutions and dilutions were done in polyethylene containers to avoid dissolution of silica from the walls of glass solvent bottles. As with use of any mobile phase containing salt, compatibility with other solvents used in gradients is mandatory. We did not notice any precipitation of silica when aliquots of the 25 m*M* HNO_3 in 80% aqueous IPA were contacted with the silica-saturated 0.1 M NaOH in 80% aqueous methanol. Table V and Fig. 5 present the results of substitution of this

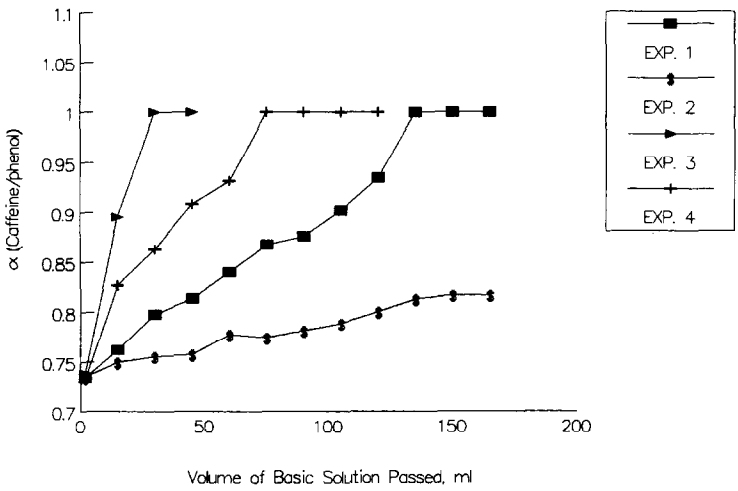

Fig. 5. Caffeine–phenol selectivity on C_{18} and C_4 columns as a function of 0.1 *M* NaOH solution passed through the column with and without silicate-saturation of the basic solution. The solutes were chromatographed under the conditions of Fig. 1. See Table V and text for the conditions of experiments 1–4.

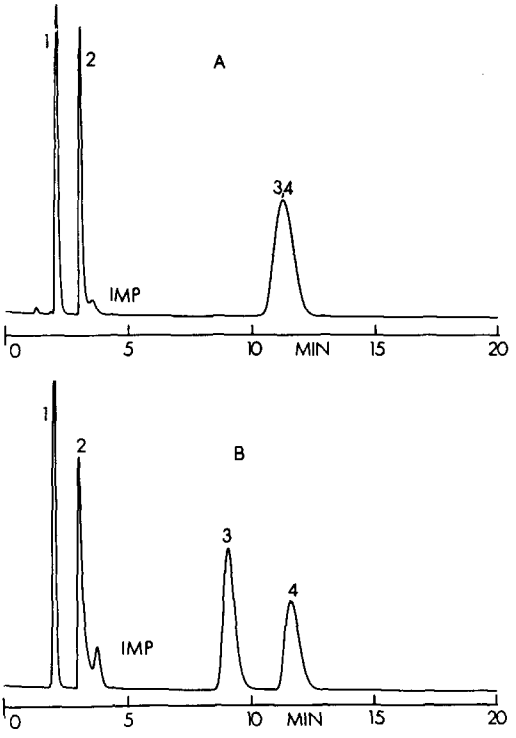

Fig. 6. Chromatography of uracil (1), NAP (2), caffeine (3) and phenol (4) on C_{18} columns after passage of 0.1 *M* NaOH in 80% aqueous methanol with and without dissolved silicate. The solutes were chromatographed using the conditions of Fig. 1. (A) 165 ml each of 0.1 *M* NaOH in 80% aqueous methanol and 25 m*M* HNO_3 in 80% aqueous IPA were passed through the column. Operating pressure was 180 p.s.i. (B) The same volumes of the same solutions as in (A) were cycled through the column except the 0.1 *M* NaOH in 80% aqueous methanol was saturated with silicate, see text. Operating pressure was 260 p.s.i. IMP = Impurity.

silica-saturated basic solution for that containing no silicate. Loss of bonded carbon was reduced by *ca.* 40% in the C_{18} system and by *ca.* 50% in the C_4 system by virtue of silica-saturation of the 0.1 *M* NaOH solution. Increased column lifetime in terms of caffeine–phenol selectivity was observed for both the C_4 and C_{18} columns using this approach. Undoubtedly, with adjustment of the organic modifier content of the wash solutions in the C_4 system as appropriate for cleaning, the column lifetime could be improved further. Fig. 6 presents the chromatograms on the C_{18} columns after passage of *ca.* 165 ml of the unsaturated (Fig. 6A) and saturated (Fig. 6B) silicate solutions of basic pH. It is obvious that the addition of silicate to the mobile phase improved the column stability. Note that some increase in operating pressure is observed in Fig. 6B, but that the result achieved is acceptable. Thus, silicate-saturated basic pH solutions can be used to wash silica-based reversed phases with extended lifetimes of the columns.

CONCLUSION

This paper examined several variables in the stability of silica-based reversed phases to basic pH. Chromatography of small molecule test probes and %C measurements proved to be valuable in qualitatively evaluating bonded phase stability as a function of passage of hydro–organic solutions of 0.1 *M* NaOH. Increased organic modifier content of the 0.1 *M* NaOH wash solution decreases the operating lifetime of the reversed-phase column as measured by resolution and operating pressure. High organic modifier content of an acidic wash following the 0.1 *M* NaOH solution also high in organic modifier further decreases column lifetime, probably because cleaved ligand chains are more effectively washed away. High coverage and endcapped bonded phases containing long chains (*i.e.* C_{18}) based on a acid-washed silica showed an increased lifetime under basic pH conditions relative to those cases where any one of these conditions was not met. The use of a silica precolumn does not serve to enhance system usefulness. Finally, the use of silica-saturated (via soluble sodium silicates) mobile phases of high pH can increase column lifetime for long or short (*i.e.* C_4) chain columns over the case where silica-presaturation is not used.

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