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Aluminum ion mediated stabilization of silica-based anion-exchange packings to caustic regenerants

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SUMMARY

Silica-based chromatography columns can be effectively regenerated with sodium hydroxide solutions containing millimolar levels of aluminum salts. The presence of aluminum ions in the caustic wash significantly reduced and in some cases virtually eliminated silica dissolution from silica-based anion-exchange columns. Wide-pore polymer coated anion-exchange packings for protein separations were washed in excess of 100 cycles with 0.1 *M* and 0.5 *M* sodium hydroxide containing aluminum nitrate with no detectable deterioration of the column bed or chromatographic performance.

The protection results from incorporation of aluminosilicate sites on the silica support surface beneath the polymeric coating. This aluminosilicate is insoluble in the operational pH range (5–9), therefore aluminum (as Al_2O_3) is undetectable at a sensitivity of 800 parts per 10^9 in the chromatographic effluent. The protective aluminosilicate can be removed by an acid wash.

The physical/chemical changes in the column packing which result from caustic washing were studied by solid-state NMR and other physical techniques. The extent of modification, proposed protection mechanism and the influence on chromatographic performance are discussed.

INTRODUCTION

The complexity of biological mixtures poses specific problems for the chromatographic isolation of proteins. It is not unusual for some fraction of the loaded sample mass to remain adsorbed to the column after normal operation. Depending on the sample origin, typical strongly bound materials may consist of proteins, nucleic acids, lipids, phospholipids or lipopolysaccharides. These compounds can deposit on the chromatographic surface and restrict mass transport, block adsorption sites and create non-specific binding sites.

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In many cases column fouling can be minimized with meticulous sample preparation and/or the use of guard columns. Even with such precautions, strongly binding materials still find their way to the chromatographic column eventually leading to decreased performance and finally irreversible column deterioration. Once performance is lost, a mobile phase which both desorbs and solubilizes the deposits must be passed through the column in order for it to be recovered. Reasonably effective regenerants include solutions of high ionic strength, chaotropic agents, acids, and alcohols or other organic modifiers.

Dilute base (0.1 or 0.5 *M* sodium hydroxide) is one of the most popular cleaning agents. Its effectiveness, low cost and low toxicity are especially attractive for process chromatography columns and other equipment used in protein production. At this scale, the need to easily and effectively regenerate columns is paramount, since many of the purified proteins will be used as injectable therapeutics.

The unique properties of silica, such as high surface area, density and mechanical stability, combined with the hydrophilic polymer coating technologies now available, have led to an increased application of these packings in process operations. The use of base for regenerating silica-based chromatography columns would be preferred if silica dissolution at high pH could be minimized or eliminated.

Three general approaches have been taken to extend the useful upper pH limit of silica-based chromatographic sorbents. Polymeric organic coatings were applied to porous silicas to simultaneously confer chromatographic properties and high pH protection[1]. Silicas have been coated with various metal oxides or hydroxides to improve the pH stability of the silica support[2–4]. Silica guard columns have been used to presaturate the solvent stream with dissolved silica to suppress dissolution of the chromatographic column[5]. All of these techniques provided some enhanced pH stability above the normal upper limit of pH 8. None of these procedures, however, are effective at the pH extremes (pH 13–14) shown to be effective for cleaning anion-exchange columns.

We have shown that silica-based anion-exchange columns can be regenerated with sodium hydroxide solutions containing low levels of aluminum salts which effectively suppress silica dissolution at high pH[6]. The protection mechanism appears to result from the formation of an aluminosilicate layer on the support surface maintained by the continuous presence of aluminum ions in the sodium hydroxide solution. This aluminosilicate layer is insoluble under normal chromatographic pH conditions which therefore prevents any detectable leaching of Al_2O_3 during gradient operation.

THEORETICAL

Silica can exist in several forms ranging from crystalline to amorphous, each type having a characteristic solubility in aqueous solution. Amorphous species generally have the highest solubility of all common forms of silica. Silicas used as chromatographic supports consist almost exclusively of the porous amorphous form.

Silica dissolves in aqueous solutions below pH 9 to form free monosilicic acid, $Si(OH)_4$, according to eqn. 1:

$$(SiO_2)_x + 2H_2O \rightarrow Si(OH)_4 + (SiO_2)_{x-1} \quad (1)$$

The equilibrium solubility of pure amorphous silica between pH 2 and 9 ranges between 100 and 150 ppm[7]. Above pH 10.7 monosilicic acid ionizes to form the soluble silicate[8] according to eqn. 2:

$$Si(OH)_4 + OH^- \rightleftarrows Si(OH)_5^- \qquad K_{eq} = 1.8 \cdot 10^4 \tag{2}$$

At high pH the equilibrium strongly favors formation of the soluble silicate, reducing the concentration of monosilicic acid which leads to complete dissolution of the amorphous solid.

Because of this solubility behavior, the use of high-pH solutions for elution or cleaning of silica-based chromatography columns has been avoided. The practical upper limit of eluent pH for use on siliceous columns without significantly shortening column lifetime is approximately pH 8.5.

It is well known that low levels of certain impurities can reduce the rate of dissolution of silica as well as the solubility at equilibrium. Lewin[9] found that silica dissolution at pH 8 was retarded in the presence of salts of Al, Be, Fe, Ga, Gd, and Y. Dmitrevskii *et al.*[10] showed that the presence of Al^{3+}, Ca^{2+} and Mg^{2+} sharply reduced silica solubility in alkaline solution. Iler[2] and Lieflander and Stöber[11] have shown that when 5–50% of a silica surface is occupied by aluminosilicate the solubility falls off drastically.

This phenomenon has been put to practical use to reduce the attack of caustic wash solutions on glass. Aluminum[12], zinc[13] and beryllium[14] have been included in caustic solutions for washing soda-lime glass bottles. Of all polyvalent metals studied, aluminum appears to have the most dramatic impact for suppressing silica solubility.

The nature of "aluminate" solutions has been studied by Raman spectroscopy, ^{27}Al NMR spectroscopy and ion-exchange chromatography. From pH 8 to 12 the principal aluminum species appears to be a polymer with octahedral Al and OH bridges[15]. Above pH 13 the tetrahedral $Al(OH)_4^-$ predominates and is formed from aluminum salts including the nitrate, sulfate, chloride and acetate according to eqn. 3:

$$Al^{3+} + 4OH^- \rightleftarrows Al(OH)_4^- \tag{3}$$

The aluminate ion, $Al(OH)_4^-$ is geometrically similar to the tetrahedrally coordinated silicon in a silica surface. It is postulated that this facilitates insertion or exchange into the SiO_2 surface creating an aluminosilicate site having a fixed negative charge[2]. Iler has proposed that these fixed anionic sites repel the approach of hydroxyl ions in solution, thus reducing the rate of dissolution of silica.

The equilibrium solubility of silica in the presence of aluminate ion is also substantially reduced. This is found to be the case even when the silica surface contains as little as 5% aluminosilicate[2]. The aluminosilicate itself is known to be quite insoluble; however the mechanism by which such low level incorporation can suppress silica solubility is not well understood.

EXPERIMENTAL

Materials

PAE-1000 packing and uncoated 1000-Å pore diameter silica (both 10 μm) were from Amicon (Danvers, MA, U.S.A.). Bovine serum albumin (BSA, fraction V powder) and ovalbumin (OVA, grade V) were from Sigma (St. Louis, MO, U.S.A.). Tris(hydroxymethyl)amino methane, sodium hydroxide, sodium chloride, and aluminum nitrate [$Al(NO_3)_3 \cdot 9H_2O$] were from J. T. Baker (Phillipsburg, NJ, U.S.A.) and were of the highest grade available. HPLC-grade deionized water was produced by using a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.).

Apparatus

Dynamic solubility test. Two systems were operated in parallel, each consisting of an Altex 110B pump (Beckman, Palo Alto, CA, U.S.A.) and an LKB 2112 Redirac fraction collector (Pharmacia, Piscataway, NJ, U.S.A.).

Column longevity model system —cycle test. Two chromatographic systems were configured to carry out automated model studies of column longevity. System 1 was controlled by a Waters Model 840 data station (DEC Pro 350 based) and consisted of two M510 pumps, a refrigerated WISP 712 autosampler and a Model 490 variable-wavelength programmable UV detector operating at 280 nm. The chromatographic gradient was monitored by using an Anspec Model AN-400 conductivity detector (Anspec, Ann Arbor, MI, U.S.A.) in-line after the UV detector. An Autochrome Model 101 3-solvent select valve (Autochrome, Milford, MA, U.S.A.) was installed on the inlet line of the "B pump" to allow computer selection of strong buffer, caustic or deionized water.

System 2 consisted of a Waters 720 system controller, two Waters M6000A pumps, a refrigerated WISP 712 autosampler and a Kratos Spectroflow 757 variable-wavelength UV detector (Kratos Analytical Instruments, Ramsey, NJ, U.S.A.) operating at 280 nm. An Autochrom 3-solvent select valve was installed on the inlet line of the "B pump" as in system one. The chromatographic gradient was monitored by using an in-line Anspec AN-400 conductivity detector after the UV detector, and both detector outputs were monitored simultaneously by using a Waters data module.

BSA frontal uptake. All BSA frontal uptake experiments to determine column loading capacity for proteins were carried out on a Waters liquid chromatograph consisting of a Model 720 system controller, two M6000A pumps, a Kratos Spectroflow 757 variable-wavelength UV detector operating at 288 nm and a Waters data module. The protein solution (BSA, 2.5 mg/ml) was loaded onto the column through an Altex 110B pump plumbed into the system via a Valco 6-port sample valve (Valco, Houston, TX, U.S.A.).

Methods

Column packing. Stainless-steel columns (25 × 0.41 cm I.D.) were packed by using a Haskel pump and an upward stirred slurry technique. Packing material was slurried in 30 ml of 2-propanol and packed at 5000 p.s.i. with methanol as the packing solvent. Columns were flushed with deionized water before use.

Dynamic solubility test. Stainless-steel columns (25 × 0.41 cm I.D.) packed with

PAE-1000 sorbents were flushed at 1 ml/min with 15 ml of 0.5 *M* sodium hydroxide and kept at room temperature for 4 h to precondition the column. Caustic solutions containing various levels of aluminum nitrate (such that the final sodium hydroxide concentration was either 0.1 or 0.5 *M*) were then pumped through the column at 1 ml/min collecting fractions of 5.5 ml. Fractions 2–8 were diluted 1:1 with deionized water and analyzed for silica by atomic absorption spectroscopy. It was empirically determined that the silica level seen in fractions 6–8 represents a plateau which predicts with reasonable accuracy the silica level obtained while washing with caustic during an automated 7-day column longevity study.

Column longevity model system —cycle test. The design of the automated cycle test models extended column lifetime under rigorous use conditions. The key elements of the test include (1) a chromatographic separation of BSA and OVA on a 25 × 0.41 cm I.D. column, (2) a caustic wash of specified duration, and (3) readjustment of column pH in preparation for the next chromatographic cycle. The actual hardware connections and software details are dependent upon the high-performance liquid chromatography (HPLC) system used, therefore only a general description of the test system is given.

The system was initially set up with 0.01 *M* Tris–HCl of pH 8 in pump A. The three solvent lines for pump B contained (A) 0.1 *M* Tris–HCl + 0.5 *M* sodium chloride, pH 8, (B) caustic (as specified), and (C) deionized water. A sample mixture containing 6.2 mg/ml BSA and 4.9 mg/ml OVA was maintained at 4°C in the autosampler.

The system was programmed to carry out a 20-min linear gradient from 100% A to 100% B, followed by a 5-min hold at 100% B. At 25 min, a signal from the data system advanced the solvent select valve, starting the caustic wash. The duration of the caustic wash depended on the caustic concentration: 4 column volumes of 0.5 *M* caustic or 10 column volumes of 0.1 *M* caustic. At the end of the caustic wash the solvent select valve was again advanced, and 2 ml of deionized water was pumped through the column. The solvent select valve was again advanced equilibrating the column for 10 min with buffer B, after which the chromatographic system was equilibrated for 15 min with buffer A in preparation for the next chromatographic run. A schematic of the cycle test is shown in Fig. 1.

Injections (50 μl) of the BSA–OVA mixture were repeated over the course of the test totalling 100 cycles. Resolution (R_s) of the BSA–OVA mixture was calculated according to eqn. 4 and plotted *vs.* cycle number.

$$R_s = \frac{2(t_2 - t_1)}{\Delta t_1 + \Delta t_2} \tag{4}$$

In this equation t_1 and t_2 are the retention times of OVA and BSA respectively, and Δt_1 and Δt_2 are their peak widths at the baseline. Early column failure was indicated by a loss of resolution and a catastrophic column bed collapse.

BSA frontal uptake measurement. Stainless-steel columns (25 × 0.41 cm I.D.) containing PAE-1000 packing were washed at 1 ml/min with 20 ml of 0.01 *M* Tris–HCl + 1 *M* sodium chloride, pH 8, then equilibrated in 0.01 *M* Tris–HCl, pH 8. By rotating the Valco 6-port valve, a BSA solution (2.5 mg/ml in 0.01 *M* Tris–HCl, pH 8) was pumped onto the column at 1 ml/min through an Altex 110B pump. The column

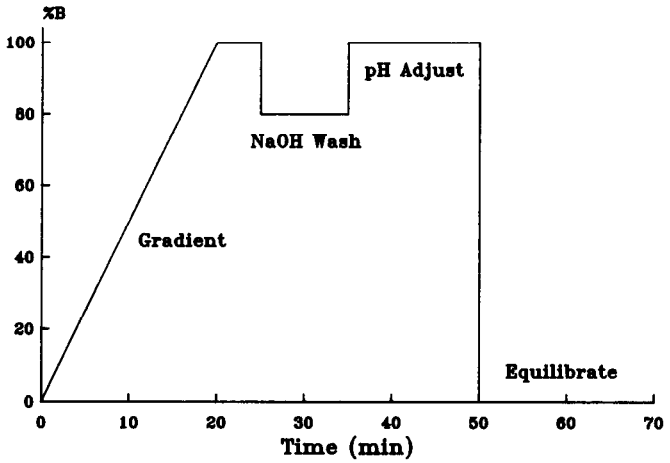

Fig. 1. Schematic representation of the segments comprising one cycle of the automated column longevity system. One longevity test consisted of 100 cycles carried out continuously for 7 days.

effluent was monitored at 288 nm to assure that the absorbance of the BSA solution remained on scale at a detector setting of 1.0 a.u.f.s. Column saturation was judged when the detector output was 20% of the full-scale BSA absorbance. Volumetric and mass loading capacities were determined from the calculated mass of BSA bound and the column volume (3.3 ml) or mass of packing material (1.3 g packing/column).

RESULTS AND DISCUSSION

Aluminum-ion mediated suppression of silica solubility

To accurately assess the effect of increasing aluminum ion concentration on silica solubility in a chromatographic column, a non-equilibrium test system was employed. Shaker-flask type equilibrium solubility tests for silica in the presence of aluminum ion predict erroneously low silica solubility levels compared to silica levels actually observed in a pumped system.

Aluminum nitrate-containing sodium hydroxide wash solutions were prepared to give the final Al^{3+} and OH^- concentrations indicated in Table I allowing for the consumption of OH^- by Al^{3+} as shown in eqn. 3. If this allowance is not made, the final solution pH can fall below 13 and precipitation of Al_2O_3 can occur.

Control columns washed with sodium hydroxide solutions containing no aluminum salts exhibited high concentrations of SiO_2 in the column effluent. Columns washed with caustic solutions containing high aluminum nitrate levels exhibited significantly lower SiO_2 dissolution than controls. Since our purpose was to determine the lowest practical aluminum concentration needed to effectively protect the silica for a minimum of 100 wash cycles, the range of 0.005–0.01 *M* Al^{3+} was determined to be optimal for these adsorbents. In addition to aluminum nitrate, we have studied other aluminum salts including the chloride, acetate and sulfate and found no difference in their ability to suppress silica solubility[6]. Gallium chloride also suppressed silica solubility, although less effectively (data not shown). An alumina-containing pre-column was also used effectively as an aluminum ion source to suppress silica

TABLE I

DYNAMIC SOLUBILITY TEST RESULTS —PAE 1000

Data were collected as described in Experimental. Silica values represent the average of two separate determinations. Increasing aluminum nitrate concentration reduces the dissolved silica levels observed in the column effluent.

$[Al^{3+}]$ (mol/l)	0.1 *M NaOH* + 1.0 *M NaCl*, ppm SiO_2	0.5 *M NaOH*, ppm SiO_2
0	250	480
0.002	110	320
0.005	52	130
0.01	43	120
0.02	33	80
0.04	33	80
0.08	19	51

dissolution. No attempt was made to optimize precolumn or alumina particle dimensions (data not shown).

To maintain efficient suppression of silica solubility at high pH the continuous presence of aluminum ions in the caustic solution is required. A column was flushed with 1 l of caustic containing aluminum nitrate until silica dissolution was significantly reduced (Fig. 2A). The wash solution was then changed to 0.1 *M* sodium hydroxide. Removal of the aluminum salt from the caustic wash resulted in a rapid stripping of adsorbed aluminum from the silica surface and a concomittant increase in SiO_2 levels (Fig. 2B).

Column longevity studies

Column longevity was assessed as a function of repeated caustic wash cycles. For this purpose, an automated test system which would model column conditions during preparative chromatography was designed. Column performance was judged during each chromatographic cycle by monitoring the separation of a protein test mixture containing BSA and OVA. Fractions (15 ml) were collected during the chromatographic gradient and caustic wash portions of the cycle for determination of SiO_2 and Al_2O_3 levels.

In this test system, each chromatographic separation was followed by a caustic wash cycle. In practice, column washing may be done much less frequently depending on the sample composition, the tendency toward column fouling, and the stringency of the validation procedures. Two different wash protocols using 0.1 and 0.5 *M* sodium hydroxide were chosen to best represent the range of conditions typically used to clean preparative chromatography columns.

0.1 M sodium hydroxide wash

As a control, PAE-1000 columns were washed with 10 column volumes of a solution containing 0.1 *M* sodium hydroxide + 1.0 *M* sodium chloride. The silica level during the caustic wash increased to approximately 400 ppm before bed collapse between cycle 30 and 40 (Fig. 3A). This represents dissolution of approximately 30% of the silica support during the wash cycles. Total wash volume before bed collapse was

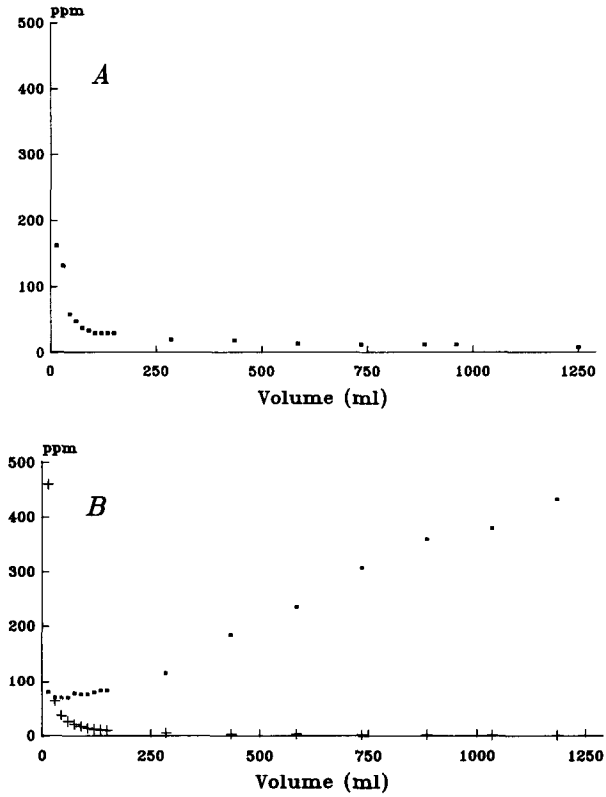

Fig. 2. (A) Dynamic solubility test results for PAE-1000 column (250 × 4.1 mm I.D.) washed at 1 ml/min with 0.18 *M* sodium hydroxide + 0.02 *M* aluminum nitrate. For details of test conditions, see Experimental. Silica solubility (in ppm) decreased sharply as the protective aluminosilicate layer formed. (B) Dynamic solubility test results obtained when aluminum-containing sodium hydroxide wash solution was replaced by 0.1 *M* sodium hydroxide. Accumulated aluminosilicate was rapidly lost and silica solubility increased steadily to levels comparable to untreated silica. ■ = SiO_2; + = Al_2O_3.

approximately 1 l. Silica levels during the chromatographic gradient were approximately 12 ppm. A large void (*ca.* 10 cm) was formed at the head of the 25-cm column after bed collapse. It is noteworthy that column performance as measured by BSA–OVA resolution remained reasonably constant until shortly before bed collapse (Fig. 3B).

Silica levels during the caustic wash were reduced to approximately 25 ppm throughout the entire 100-cycle test for PAE-1000 columns washed with caustic containing optimized aluminum nitrate levels (Fig. 4A). Furthermore, silica levels during the chromatographic gradient were reduced to approximately 2 ppm and aluminum (as Al_2O_3) was undetectable in the gradient fractions at 800 ppb[a] sensitivity. Column performance as measured by resolution of the BSA–OVA mix was unchanged through 100 cycles (Fig. 4B). No evidence for void formation at the inlet was observed in either column.

[a] Throughout this article, the American billion (10^9) is meant.

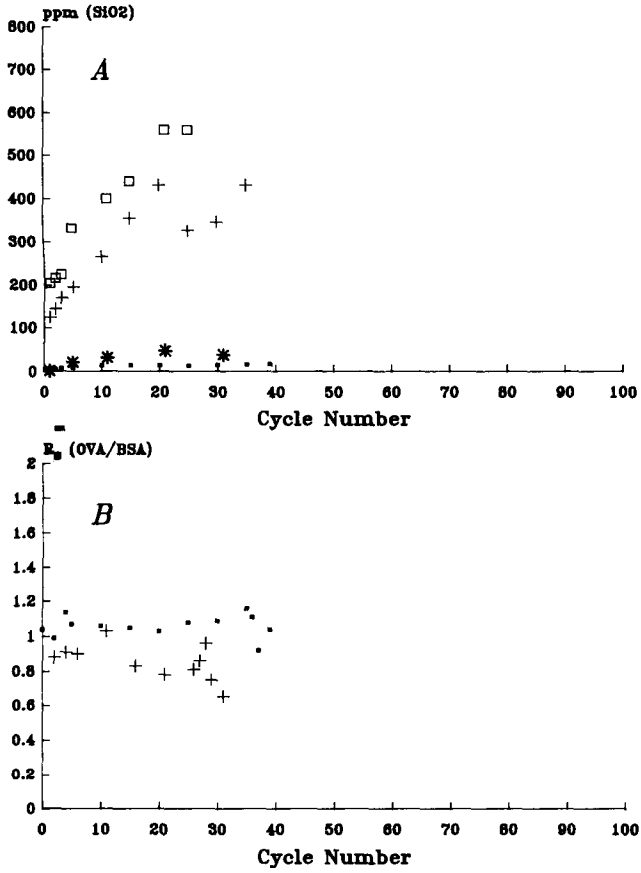

Fig. 3. Cycle test results for control PAE-1000 columns (250 × 4.1 mm I.D.) washed with 10 column volumes of 0.1 *M* sodium hydroxide + 1.0 *M* sodium chloride after each chromatographic run. Different symbols indicate results of duplicate experiments. For details of cycle test conditions, see Experimental. (A) Silica level during caustic wash (□, +) and chromatographic gradient (■, ∗). (B) Resolution (R_s) of BSA–OVA mixture for duplicate experiments (■, +).

Cycle testing of PAE-1000 under these optimized conditions was repeated for extended periods until eventual column failure (data not shown). Chromatographic performance was maintained unchanged through 300 cycles. Gradual deterioration in performance, probably due to channeling in the column bed, was seen from that point until complete bed collapse occurred at cycle 425. Put in perspective, this represents washing a 25 × 0.41 cm I.D. analytical column with approximately 14 l of 0.1 *M* sodium hydroxide. At the end of the test, approximately 40% of the silica support had been dissolved.

0.5 M sodium hydroxide wash

PAE-1000 columns were washed with 4 column volumes of 0.5 *M* sodium hydroxide or 0.5 *M* sodium hydroxide containing aluminum nitrate (Figs. 5 and 6). Despite the higher pH wash, control columns exposed to a 4-column-volume wash

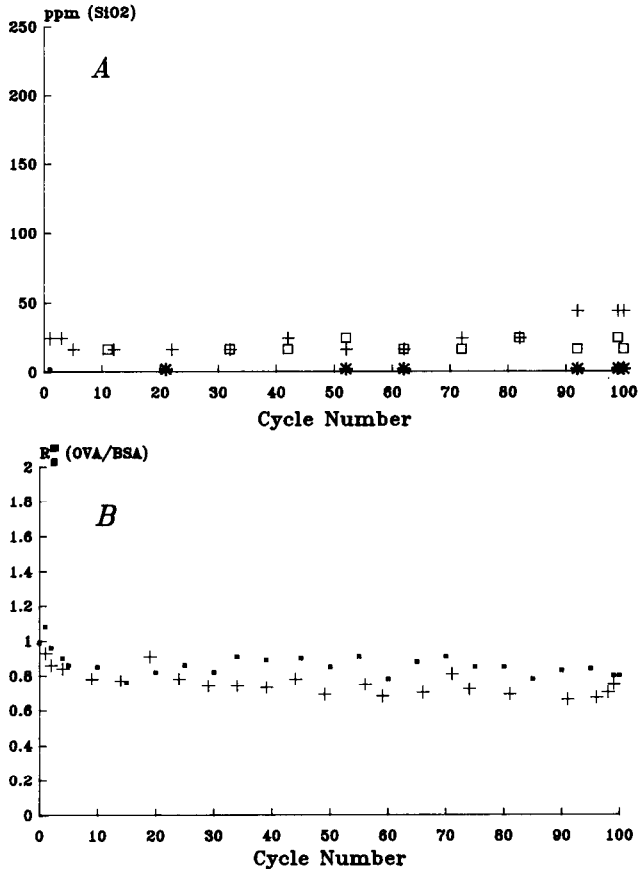

Fig. 4. Cycle test results for duplicate PAE-1000 columns (250 × 4.1 mm I.D.) washed with 10 column volumes of 0.12 *M* sodium hydroxide + 0.005 *M* aluminum nitrate + 1.0 *M* sodium chloride. (A) Silica level during caustic wash (□, +) and chromatographic gradient (■, ∗). (B) Resolution (R_s) of BSA–OVA mixture for duplicate experiments (■, +).

survived over 50 cycles as compared to 36 cycles for controls washed with 10 column volumes of 0.1 *M* sodium hydroxide + 1.0 *M* sodium chloride (Fig. 5A). This again represents dissolution of approximately 30% of the silica support. Column performance was maintained until shortly before bed collapse (Fig 5B). The presence of aluminum nitrate significantly reduced silica levels during both the caustic wash and chromatographic gradient (Fig. 6A). Column performance remained unchanged throughout the 100-cycle test (Fig. 6B), and there was no evidence for void formation at the column inlet. Chromatographic peak shape was maintained throughout the cycle test (Fig. 7).

Extended cycle testing of PAE-1000 washed with 0.5 *M* sodium hydroxide containing aluminum nitrate was repeated until eventual column failure (data not shown). Chromatographic performance remained unchanged through over 200 wash cycles. Noticeable column deterioration then progressed until complete bed collapse occurred at cycle 275. Based on dissolved silica in the wash solutions, approximately 40% of the silica support had been dissolved before bed collapse.

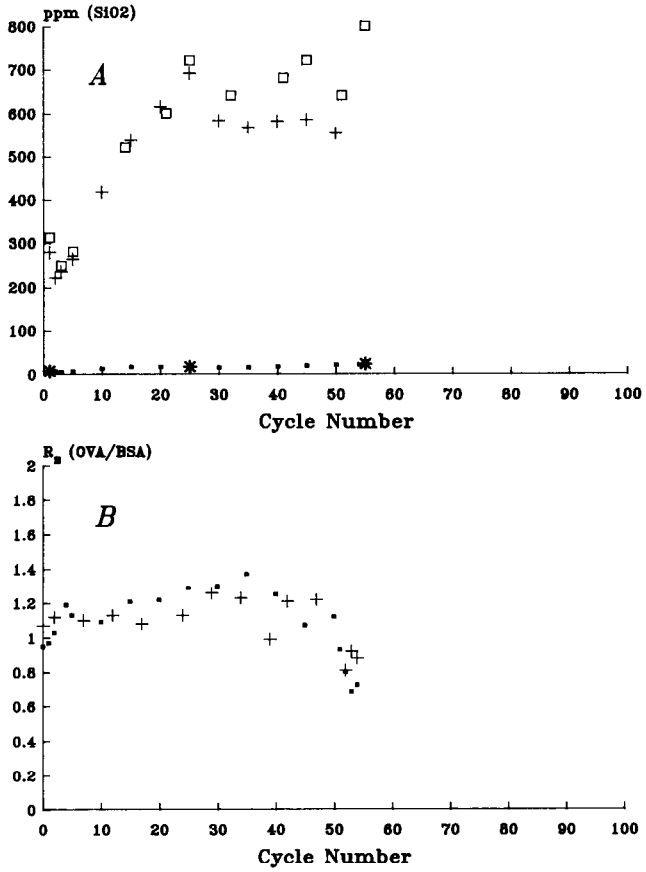

Fig. 5. Cycle test results for duplicate control PAE-1000 columns (250 × 4.1 mm I.D.) washed with 4 column volumes of 0.5 *M* sodium hydroxide after each chromatographic run. (A) Silica level during caustic wash (□, +) and chromatographic gradient (■, ∗). (B) Resolution (R_s) of BSA–OVA mixture for duplicate experiments (■, +).

For comparison, cycle testing was carried out by using less than optimal wash conditions, *i.e.* 0.002 *M* aluminum nitrate in 0.5 *M* sodium hydroxide. The column showed no evidence of deterioration during the 100-cycle test. The silica solubility data in Table I suggests that an extended test would show a reduced total lifetime compared with the above test using optimal aluminum nitrate levels.

The fact that aluminate ion exists in solution above pH 13 as the tetrahedral $Al(OH)_4^-$ is crucial to the use of aluminum salts as additives in caustic wash solutions for silica-based anion-exchange packings. Below pH 13, polymeric alumina predominates and can precipitate, rapidly poisoning the anion-exchange column (data not shown). Above pH 13 the $Al(OH)_4^-$ monomer predominates and has no deleterious effect on either the adsorbed, cross-linked organic coating or the chromatographic performance. The $Al(OH)_4^-$ apparently can diffuse through the organic coating and exchange into the underlying silica surface to form a protective aluminosilicate layer. This significantly reduces silica dissolution, allowing repeated cleaning with caustic containing aluminum salts with no significant deterioration in column performance.

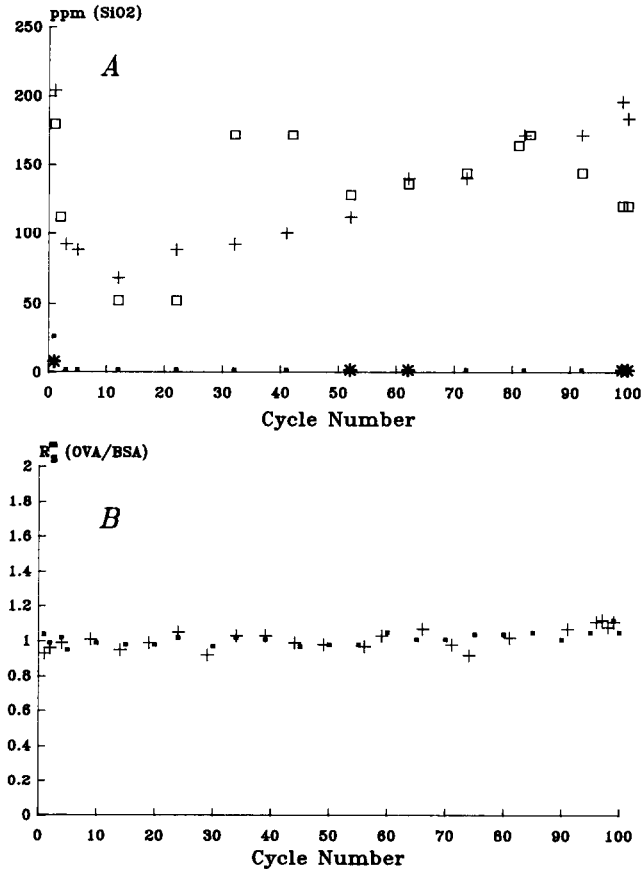

Fig. 6. Cycle test results for duplicate PAE-1000 columns (250 × 4.1 mm I.D.) washed with 4 column volumes of 0.52 *M* sodium hydroxide + 0.005 *M* aluminum nitrate. (A) Silica level during caustic wash (□, +) and chromatographic gradient (■, ∗). (B) Resolution (R_s) of BSA–OVA mixture for duplicate experiments (■, +).

Characterization of the aluminosilicate layer

To investigate the physical/chemical changes occurring on the silica support after exposure to caustic solutions containing aluminum nitrate, sorbent was recovered from the column and analysed by magic angle spinning solid state ^{27}Al NMR. The presence of Al–O–Si bonds from an aluminosilicate was confirmed, therefore the aluminum appears to be chemically incorporated into the silica surface as opposed to being simply chemisorbed upon the surface[16].

Studies were carried out to determine the extent of Al_2O_3 incorporation into the support during the course of the cycle test. For this purpose, 25 × 0.41 cm I.D. columns of PAE-1000 were washed at 1 ml/min with volumes of 0.12 *M* sodium hydroxide + 1 *M* sodium chloride + 0.005 *M* aluminum nitrate corresponding to 1, 5, 10 or 100 wash cycles. After an exhaustive water wash to remove unbound aluminum, the silica was recovered from the column, dried and weighed. Weight percent aluminum was determined by atomic absorption spectroscopy after digestion with hydrofluoric acid–perchloric acid.

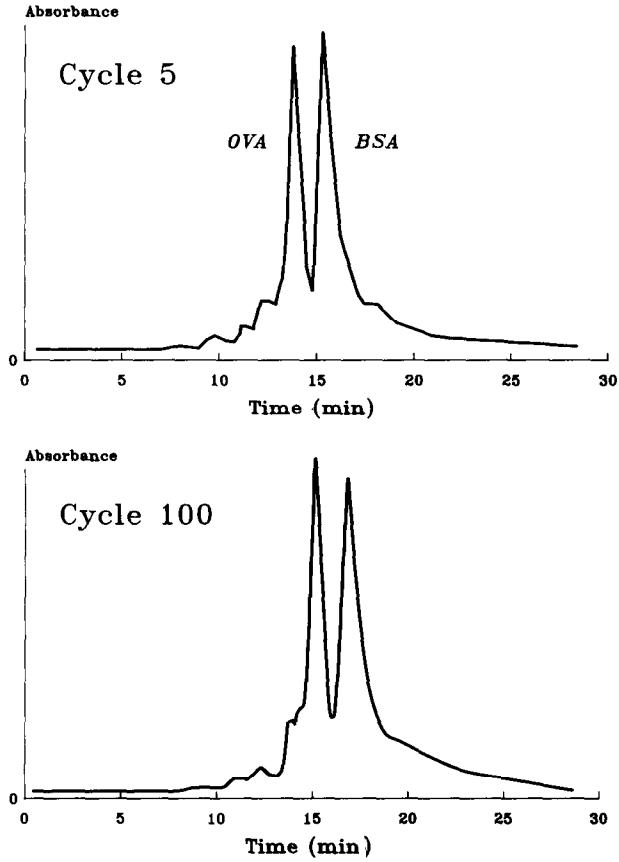

Fig. 7. Representative chromatograms from the beginning and end of a cycle test with aluminum nitrate-containing caustic. Column, 25 × 0.41 cm I.D. PAE-1000; wash, 4 column volumes of 0.52 *M* sodium hydroxide + 0.005 *M* aluminum nitrate; gradient, as described in Experimental section.

TABLE II

BSA FRONTAL LOADING CAPACITY ON PAE-1000 COLUMNS

Data were collected as described in Experimental. Column loading capacity values were the result of at least two determinations. No significant change in loading capacity was seen for columns exposed to 100 wash cycles relative to controls.

Treatment	*BSA loading capacity*		
	mg/column	*mg/ml*	*mg/g packing*
None	145	44	112
110 cycles 0.12 *M* NaOH + 0.005 *M* $Al(NO_3)_3$ + 1 *M* NaCl	155	47	120
100 cycles 0.52 *M* NaOH + 0.005 *M* $Al(NO_3)_3$	139	42	106

The protective aluminosilicate layer was found to form rapidly with greater than 50% deposited in the first cycle, and 67% adsorbed by cycle 10. The final level incorporated (0.33%, w/w) represents 1.6 aluminum atoms/nm^2 which corresponds to 20% of a monolayer assuming 8 metal atom sites per nm^2 (ref. 2). This number agrees well (perhaps coincidentally) with the maximum aluminum incorporation seen by Iler[2] for colloidal silica at pH 8.

Iler[2] suggested that the rate of silica dissolution was suppressed in the presence of aluminum ions by the formation of negatively charged aluminosilicate sites which repel the approach of hydroxyl ions. Adsorbed aluminum also reduced the equilibrium solubility of the silica despite formation of less than a complete monolayer. One might speculate that through the dynamic equilibrium process, the presence of aluminosilicate sites creates a short range order in the silica surface, reducing the equilibrium solubility in high-pH solutions. We found no evidence for significant crystallinity detectable by X-ray diffraction techniques in these and related materials[17].

Several acids were evaluated as stripping agents for those cases in which trace aluminum buildup on-column is to be avoided. Both 0.1 *M* hydrochloric acid and 0.1 *M* orthophosphoric acid were quite effective at removing bound alumina from columns exposed to 100 caustic wash cycles. Phosphoric acid, being less corrosive to stainless steel systems was the method of choice. A 30-ml wash of 0.1 *M* orthophosphoric acid removed approximately 85% of the accumulated aluminum from a 25 × 0.46 cm I.D. column. For all practical purposes, however, since the aluminum leaching level during the chromatographic gradient is less than 800 ppb, acid stripping should not be required after caustic washing.

BSA loading capacity studies

Although extensive cycle testing with aluminum-containing caustic showed no evidence for performance deterioration of PAE-1000 with regard to the separation of BSA and OVA, this did not necessarily demonstrate bonded phase integrity. Gradient elution protein chromatography is known to be sensitive to column bed integrity and channeling but relatively insensitive to column length[18] and hence the mass of functional sorbent. It may therefore be possible to strip a substantial fraction of the bonded phase from the column without significantly affecting analytical scale column performance.

BSA frontal loading capacity studies were carried out on PAE-1000 columns before and after exposure to 100 caustic wash cycles to demonstrate conclusively that washing with caustic containing aluminum nitrate did not promote bonded phase loss. BSA frontal loading capacity was determined before and after cycle testing as described in materials and methods. BSA does not bind to uncoated silica sorbents under the specified anion-exchange chromatography conditions. A loss of the coating would be detected by a reduction in BSA loading capacity. Column loading capacity remained virtually unchanged after exposure to 100 caustic cycles (Table II), therefore no significant bonded phase loss occurred.

PAE-1000 packings were prepared by adsorption and subsequent cross-linking of cationic polymers[1]. This chemistry produces a continuous pellicular coating which is quite resistant to deterioration at high pH. Silica-based anion-exchange packings for protein separations can also be prepared by covalent attachment of organosilanes[19] or preformed cationic polymers[20,21]. Caustic attack rapidly strips covalent bonded

phases (data not shown), whereas slow dissolution of the silica from beneath an adsorbed, cross-linked coating can proceed up until bed collapse with virtually no effect on the chromatographic performance.

Physical changes due to caustic washing

Cycle test results showed no detectable deterioration in chromatographic performance after 100 washes with aluminum-containing caustic. However, silica levels during the caustic wash indicated that some minor erosion of the inorganic support did occur. Long-term cycle tests also showed that columns eventually did collapse due to removal of approximately 40% of the silica support. Pore volume, pore diameter and surface area measurements were made on sorbents recovered after cycle testing to determine whether physical changes in the support could be detected after 100 wash cycles.

Washing with caustic (either 0.1 *M* or 0.5 *M* sodium hydroxide) caused rapid bed collapse in control experiments due to removal of silica which in turn weakened the bed structure (Table III). A 45–60% increase in pore volume and up to 2-fold increase in surface area was observed relative to untreated packing material. An overall decrease in average pore diameter was also seen which is due to the creation of a larger population of small pores, rather than loss of large pores.

TABLE III

PHYSICAL CHANGES IN PAE-1000 AFTER CAUSTIC WASHING

Packing materials were recovered from the columns, washed to neutrality by repeated suspension and settling in deionized water, then dried under vacuum. The organic coating was pyrolized in air by a stepwise process to prevent particle fracture. Surface area and pore diameter were then determined by standard nitrogen BET and mercury porosimetry techniques.

Treatment	*Pore volume* (cm^3/g)	*Nominal pore*	
		diameter (Å)	*surface area* (m^2/g)
None (control)	0.86	590	47
31 cycles 0.1 *M* NaOH + 1 *M* NaCl (collapsed)	1.25	520	73
100 cycles 0.12 *M* NaOH + 1 *M* NaCl + 0.005 *M* $Al(NO_3)_3$	0.86	590	47
425 cycles 0.12 *M* NaOH + 1 *M* NaCl + 0.005 *M* $Al(NO_3)_3$ (collapsed)	1.22	520	64
54 cycles 0.5 *M* NaOH (collapsed)	1.36	470	95
100 cycles 0.52 *M* NaOH + 0.005 *M* $Al(NO_3)_3$	1.18	560	57

Washing with aluminum nitrate-containing 0.1 *M* sodium hydroxide for 100 cycles resulted in virtually no change in the physical characteristics of the support. This would suggest very effective suppression of silica dissolution under the chosen wash conditions, which was confirmed by the 425-cycle lifetime seen in extended tests. Sorbent recovered after bed collapse following 425 wash cycles showed pore volume, surface area and pore diameter changes very similar to those for control columns washed with caustic alone until bed collapse.

Washing with aluminum nitrate-containing 0.5 *M* sodium hydroxide for 100 cycles caused alterations in pore volume, surface area and pore diameter intermediate between those seen for untreated and collapsed sorbents. This is reasonable based upon the dissolved silica level predicted by dynamic solubility tests and seen during caustic washing in cycle tests, as well as the lower lifetime (225 cycles) relative to 0.1 *M* sodium hydroxide washing. If additional stability is desired using 0.5 *M* sodium hydroxide wash solutions, the aluminum nitrate concentration can be increased based upon the silica solubility data shown in Table I.

CONCLUSIONS

The addition of millimolar amounts of aluminum salts to sodium hydroxide wash solutions above pH 13 significantly suppressed silica dissolution from silica-based anion-exchange packings. A constant source of aluminum must be present during the high pH wash to provide continuous protection of the silica support. Chromatographic performance, the major determinant of column lifetime, is virtually unaffected by slow dissolution of the silica support beneath the polymeric anion-exchange coating until shortly before column bed collapse.

BSA loading capacity analysis before and after cycle testing showed that the adsorbed polymeric anion-exchange coating used to prepare PAE-1000 packings was stabile through at least 100 aluminum nitrate-containing caustic wash cycles. Column performance as determined by the chromatographic resolution of BSA and OVA remained unchanged for 200–300 caustic wash cycles depending upon the sodium hydroxide concentration. This represents up to a 10-fold improved column lifetime relative to control columns washed with aluminum-free sodium hydroxide. Covalent chromatographic coatings showed significant deterioration when washed with caustic solutions containing aluminum nitrate, most likely due to the cleavage of the organosilane linkage.

Solid-state ^{27}Al NMR identified the presence of an aluminosilicate layer on the silica support after exposure to caustic solutions containing aluminum nitrate. The amount of alumina accumulated after 100 wash cycles represented less than a monolayer, yet provided sigificant suppression of silica solubility. Adsorbed aluminum did not appear to leach from the column during gradient elution at the 800 ppb detection level. The trace level of insoluble aluminosilicate on the chromatographic support can be significantly reduced by a brief wash with 0.1 *M* phosphoric acid.

The unique properties of silica-based anion-exchange sorbents can be used to their full advantage in process chromatographic applications if aluminum-containing caustic washes are employed. Processes will benefit from improved throughput and resolution as well as the ability to regenerate the column with dilute caustic solutions.

This technique may have additional applications. High-pH analytical separations may be possible by including low levels of aluminum salts in the chromatographic eluent. This is of particular interest for reversed-phase separations of basic compounds.

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