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Stability of silane modifiers on alumina nanoporous membranes

Victor Szczepanski, Ivan Vlassiouk, Sergei Smirnov *

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, NM 88003, United States

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Abstract

Stability of silanes covalently bound to the surface of nanoporous alumina membranes was investigated and the reasons for variation have been identified as due to activation of Al–O–Si bond caused by local increase of pH from primary and secondary amines of the linker molecules. Transformation of amines into amides or passivation of alumina surface by as little as five atomic layers of deposited silica before silanization dramatically improve stability of immobilized molecules.

Keywords: Nanoporous alumina; Silanization; Stability; Membrane modification; Local pH effect

1. Introduction

Anodized aluminum oxide membranes (AAO) can be easily synthesized via anodization of metal aluminum and high control of pore diameter and length can be achieved. The length can be extended over tens of microns. Choosing appropriate anodization voltage and the electrolyte allow to controllably vary the diameter from 10 to 300 nm. Due to these unique properties AAO membranes found various applications in such areas as biological and chemical separations [1–3], biosensors [2–6], and solution flow regulation [7,8]. Most of these applications require surface modification of AAO by molecules with desired properties. The typical approach employs surface hydroxyl groups by reacting them with trialkoxyaminosilanes and consequent functionalization using different routes for primary amine modification [9]. Silanization of alumina is relatively new [2,3,6,7,10] and not as widely used as silanization of silica that has been employed for decades. In our previous work [3,6], we applied such a procedure for AAO amination and subsequent DNA immobilization using gluteraldehyde linker.

A variety of sensors for biological analytes (including DNA) rely on stability of covalently immobilized molecules. We aim at developing sensors based on nanoporous alumina membranes and have shown that optical, IR, and electrochemical detections can be realized on these substrates [2,5]. An important aspect of applicability of such sensors is their lifetime and reusability. In this letter we report on our study of stability of covalently bound silanes on alumina nanoporous (20 and 200 nm diameter) filter membranes. By comparing different silanes and their modifiers we conclude that a limited stability of covalently bound molecules on alumina is likely due to facile activation of Al–O–Si bond breaking by local increase of pH. The latter is promoted by primary and secondary amines from the silane linkers. Two approaches for resolving this issue were demonstrated: (a) use of silanes with no secondary amines and (b) passivation of alumina with silica deposited by atomic layer deposition technique.

2. Experimental

Two aminosilanes were used in this study: 3-(2-aminoethyl)aminopropyl trimethoxysilane (AEPS, 98%) and 3-aminopropyl trimethoxysilane (APS, 97%) (Fig. 1), both from Aldrich, which differ not only by the length but also by an extra amino group in AEPS. Tetraethoxysilane (98%) was also purchased from Aldrich. Fresh AAO membranes (Anodisc 13 from Fisher) were first silanized by either aminosilane for approximately 1 h in 5% dry acetone solution:

$$\sim \text{Al–OH} + \text{CH}_3\text{O–Si(CH}_3\text{O)}_2\text{–RNH}_2 \rightarrow \sim \text{Al–O–Si(CH}_3\text{O)}_2\text{–RNH}_2 + \text{CH}_3\text{OH} \quad (1)$$

washed thoroughly in acetone and then baked at 120 °C in an oven for either 20 min or overnight before reacting with DNP,

* Corresponding author. Tel.: +1 505 646 1547; fax: +1 505 464 2649.
E-mail address: snsm@nmsu.edu (S. Smirnov).

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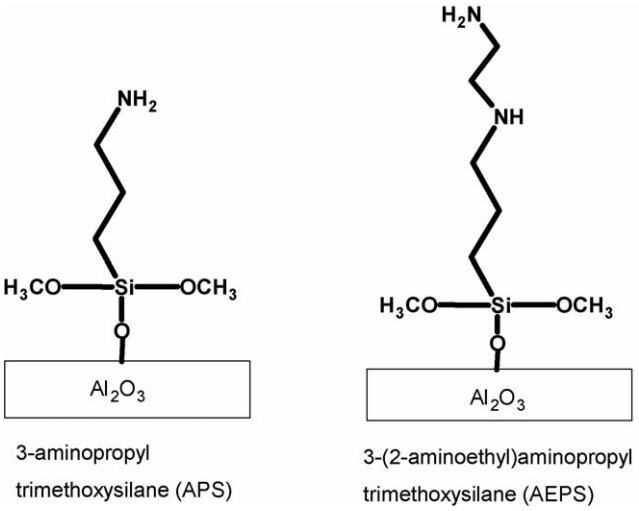

Fig. 1. Structures of immobilized APS and AEPS.

6-(2,4-dinitrophenyl)amino hexanoic acid succinimidyl ester (from Molecular Probes), by placing membrane in 1 mM acetone solution of DNP overnight. After thorough washing in acetone, modified membranes were tested using UV absorption of DNP (see Fig. 2). Absorption at either peak of DNP, 350 or 420 nm, with the latter being more convenient due to lesser interference with membrane absorption.

As was shown before [2], due to the high surface area of AAO membrane, the surface density of immobilized molecules could be easily measured using either UV/Vis or IR spectra. The surface density of immobilized molecules on AAO can be calculated from the optical density, A, and known molar extinction coefficient, $\varepsilon = 7250\ \mathrm{M^{-1}\ cm^{-1}}$ at 420 nm, of DNP [10]:

$$n_s\,(\mathrm{cm^{-2}}) = \frac{N_A}{1000}\frac{A}{\varepsilon}\frac{4L}{d} \quad (2)$$

where factor $4L/d$ represents the surface area enhancement due to a large membrane thickness, L, as compared to pore diameter, d, and the coefficient 1000 in denominator accounts for the

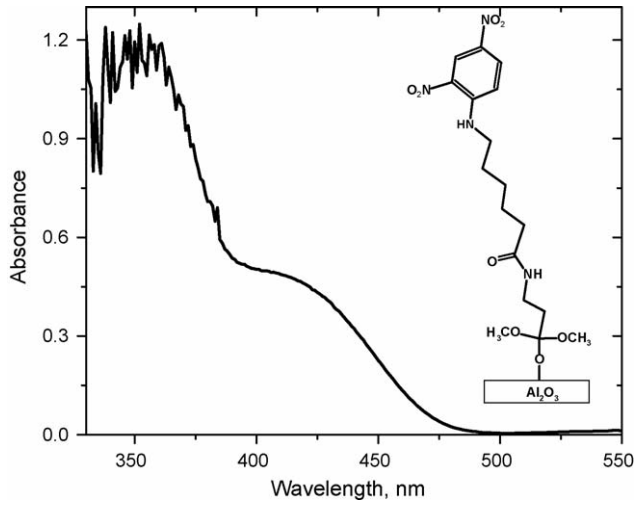

Fig. 2. UV/Vis spectrum of modified AAO membrane with DNP and structure of immobilized DNP; case with APS linker is shown.

appropriate unit conversion from ε in $\mathrm{M^{-1}\ cm^{-1}}$ to n_s in $\mathrm{cm^{-2}}$. For the membranes used in this study: with 200 nm pore diameter (very short portion of 20 nm on one side) and 60 μm thickness the surface area enhancement factor is close to 10^3. Surface densities of immobilized DNP were approximately equal for the two silanes but slightly depended on pretreatment of AAO: boiled in distilled water for 20 min membranes had higher density, $n_s \sim 5 \times 10^{13}\ \mathrm{cm^{-2}}$, which was still approximately four times less than the density achieved on a flat silica surface for similar molecules [10]. Not boiled membranes demonstrated lower surface density, $n_s \sim 2 \times 10^{13}\ \mathrm{cm^{-2}}$, probably because of fewer surface hydroxyls available that are either created or liberated from weakly soluble impurities as a result of boiling.

Atomic layer deposition (ALD) of silica layers from a gas phase was performed in a glass vacuum chamber with Teflon valves using procedure of Ferguson et al. [12] Ten membranes were simultaneously modified by exposing first to ∼130 Pa (∼1 Torr) of tetraethoxysilane for 3 h, followed by pumping out the residual gas and subsequent hydrolysis in 1300 Pa (10 Torr) of water vapor for 3 h. In both cases 1300 Pa (10 Torr) of NH_3 gas was always present as a catalyst and the temperature of 30 °C was maintained. Five of such cycles resulted in five monolayers of silica adlayer. The procedure took 1.5 days, but could be performed faster with commercial automatic systems.

Streaming potential was measured in a homemade cell constructed from two stainless steel flanges between which a membrane under study was placed via dielectric o-rings. The potential was measured in the two-point scheme using the flanges as the electrodes upon water passing through the membrane. The pressure gradient between the two water filled compartments connected to the flanges was applied using nitrogen gas.

3. Results and discussions

Membranes modified with just a silane or with DNP remain practically unchanged in organic solvents but if left in distilled water or in phosphate buffer saline (PBS) solution (pH = 7.6, 0.1 M NaCl) for a long period of time, surface concentration of DNP declined noticeably within a week (see Fig. 3). The effect is most dramatic for AEPS linker as compared to APS (compare circles and squares). The rate of decline becomes similarly fast with APS linker that was exposed to PBS prior to reacting with DNP (compare triangles and squares). The difference between short (20 min) and long (overnight) heating in the oven after silanization correlates (empty versus filled circles or squares) with similarly higher initial density for longer heating. It is expected for reaction (1) which starts as physisorption and completes only upon heating.

We speculate that the surface density decline in aqueous solutions is to a great extent due to the basicity of amines present in the silanes that increase local pH near the surface. The speculation is corroborated by observation that when the primary amine of APS was changed into amide, the rate of silane loss from the surface slowed down (squares versus triangles in Fig. 3) in accordance with high pK_a of primary and secondary amines

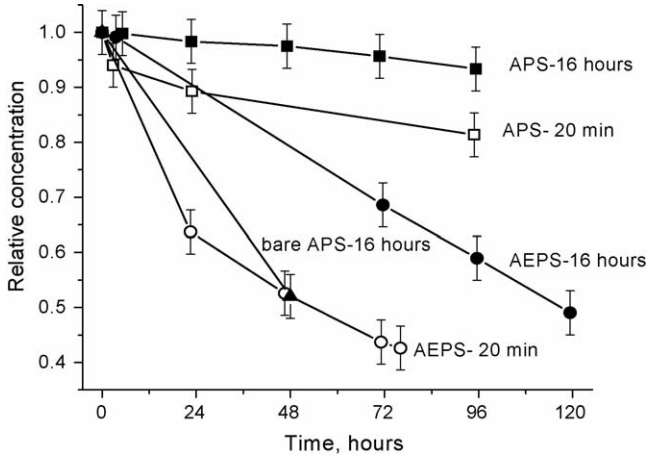

Fig. 3. Kinetics of survival for immobilized molecules on AAO as a function of exposure to PBS solution. Filled squares (■) and empty squares (□) correspond to DNP linked to AAO surface via APS, while filled circles (●) and empty circles (○) are those for AEPS linker, respectively. Empty symbols (□ and ○) are for a short 20 min baking after silanization, while filled symbols (■, ● and ▲) are for overnight baking. Triangles (▲) show the relative concentration of DNP obtained after AAO modified with APS (no DNP was attached) was first exposed to PBS solution for 2 days and then DNP was immobilized using the standard procedure (with overnight baking). Similar results were observed in water.

($pK_a > 9$) and its decreasing in zwitterions of amides ($pI < 6$). AEPS has two amines, one primary and one secondary. The primary terminal amine changes into amide by reacting with succinimidyl ester of DNP, but the remaining secondary amine should still cause an increase in local pH. Thus APS offers a greater stability between the two linkers. It should be noted that stability of the AAO surface modified by alkoxysilane without aminogroup was almost infinite on this timescale because such highly hydrophobic membranes do not wet at all.

Alumina dissolves in both strong acids and strong bases but has the minimum solubility in slightly acidic solutions. Thus maintaining local pH in the vicinity of minimum solubility is critical to prolonged stability of the covalently attached layer. Alternatively, passivation of the surface with a thin layer of less reactive oxide should improve the stability as well. The benefits of a decreased chemical activity of the passivated layer can be negated if the layer becomes inactive to silane chemistry modification. We speculate that because Si–O bond is less polar than Al–O bond, the overall Si–O–Al linker is highly polarized and thus is more vulnerable to activation by a base or an acid.

To overcome this vulnerability, we chose to passivate alumina surface with a thin silica layer even though its solubility may not be better than that of alumina itself. Silicon oxide has acidic pH of zero charge, $pH_{pzc} \sim 2.9$, as opposed to basic, $pH_{pzc} \sim 9.1$, of alumina [11], and its solubility is also pH dependent. Nevertheless, after silane modification, the Si–O–Si linker should be less likely chemically activated than Al–O–Si bond. Having the latter covered by a few atomic layers of silica should provide a sufficient cushioning and shield it from reacting.

Following literature procedure [12], five silica layers were built on membranes by atomic layer deposition (ALD) by alternating exposure to ~130 Pa (~1 Torr) of tetraethoxysilane (reaction (3)) and water vapor (1300 Pa) (10 Torr) (reaction (4)):

$$\sim \text{surface–OH} + \text{Si}(\text{OC}_2\text{H}_5)_4 \rightarrow \sim \text{surface–O–Si}(\text{OC}_2\text{H}_5)_3 + \text{C}_2\text{H}_5\text{OH} \quad (3)$$

$$\sim \text{surface–O–Si}(\text{OC}_2\text{H}_5)_3 + 3\text{H}_2\text{O} \rightarrow \sim \text{surface–O–Si}(\text{OH})_3 + 3\text{C}_2\text{H}_5\text{OH} \quad (4)$$

In both cases 1300 Pa (10 Torr) of NH_3 was present as a catalyst [12].

After five of such cycles, the membranes can be modified in a similar manner as described above. To confirm that the modified membranes did have the outer layer of silica, streaming potential measurements were performed on treated and untreated membranes at pH = 7.0 in a homemade cell. As Fig. 4 illustrates, the streaming potential of unmodified bare AAO membrane was negative and increased with applied pressure in accordance with Helmholtz–Smoluchowski Eq. (5) [13–15]:

$$\Delta V = \frac{\varepsilon_0 \varepsilon \zeta}{\eta \lambda} \Delta P \quad (5)$$

where ζ is the surface zeta potential, ε and η are the solvent dielectric constant and viscosity, respectively, λ the conductivity of the electrolyte, and ΔP is the pressure gradient. As it is apparent from Eq. (5), the streaming potential decreases with electrolyte concentration. In nanopores the concentration dependence of $\Delta V/\Delta P$ becomes nonlinear and saturates at low concentrations, where the Debye length exceeds the pore radius [15,16]. The relation of $\Delta V/\Delta P$ amplitude to the absolute value of zeta potential, ζ, becomes more complicated than in Eq. (5) but the sign is given correctly, e.g. $\Delta V/\Delta P$ is positive for a negatively charged surface and vice versa [15,16].

We used deionized water for the streaming potential measurements to maximize the signal. The negative sign of $\Delta V/\Delta P$

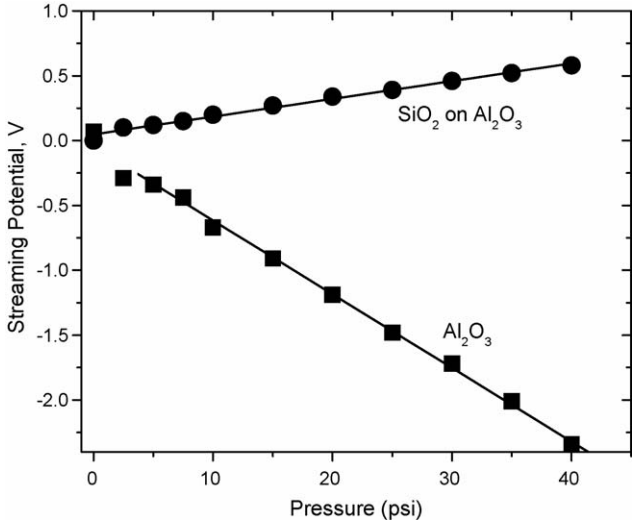

Fig. 4. Streaming potential across unmodified membrane (■) as a function of applied pressure in distilled water and the same for membrane modified with five atomic layers of silica (●). Boiling of the latter for 15 min in distilled water completely recovers the streaming potential to that of naked membrane.

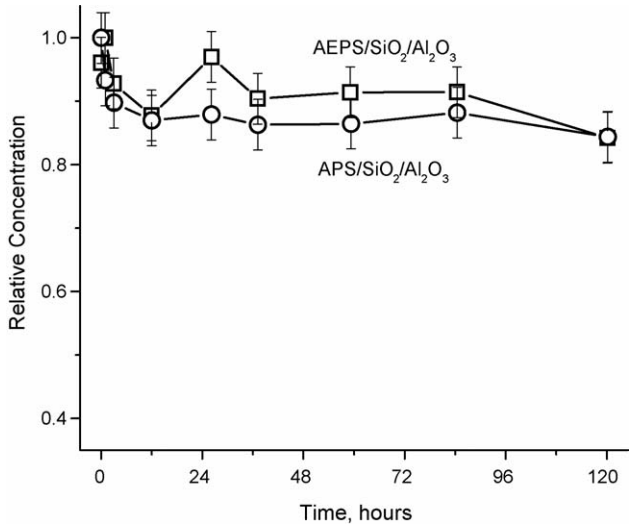

Fig. 5. Kinetics of survival for immobilized DNP molecules on AAO that was initially modified with five atomic layers of silica. Both linkers, AEPS (□) and APS (◯), are shown. Neither membrane was boiled prior to silanization. Silanes were baked for 20 min before reacting with DNP. The kinetics are given for PBS solution; similar results were observed in water.

for bare alumina membranes (squares in Fig. 4) indicates a positive surface charge, as would be expected at neutral pH for a surface with $pH_{pzc} = 9.1$. Slightly larger values of $\Delta V/\Delta P$ were previously reported by Hernandez et al. [16] for similar alumina membranes with larger pore diameters (100–200 nm). Silica treated membranes, on the other hand, showed a positive slope (circles in Fig. 4), in agreement with a negative surface charge of silica at pH = 7.0 ($pH_{pzc} = 2.9$). A smaller positive slope of $\Delta V/\Delta P$ for silica treated membranes was previously reported for alumina membranes modified with silica by sol–gel method [17]. When membranes were boiled in distilled water for 15 min, both types of membranes, unmodified and silica modified, showed identically negative slope close in value to that of untreated membranes.

Fig. 5 shows kinetics of survival for silica treated membranes with DNP immobilized on them by AEPS and APS linker. Both membranes were baked for 20 min after silanization prior to reacting with DNP. The boiling of membrane step was eliminated in their preparation since it stripped off the silica layer. The latter could be due to a difference in expansion coefficients between alumina and silica. It is unambiguous that silica treated membranes showed dramatic improvement in the survival of immobilized molecules: after initial fast drop, there was practically no decline in concentration over the period of 5 days independently of what linker, APS or AEPS, was used (compare Figs. 3 and 5). The effect is most pronounced for the AEPS linker, where the pH effect of amino group was the greatest. The initial drop was insignificant and similar to that observed without silica adlayers for short baking. It was likely due residual physisorbed molecules that did not wash away.

Of course, the stability of silanes covalently linked to the surface of alumina (and silica) depends on other contributions such as surface density of modifiers, overall surface hydrophobicity, temperature, and solution pH. After all, even bare alumina and silica are soluble themselves. Nevertheless, there appears a significant difference in the survival rate between the otherwise very similar silane linkers that is most directly correlated with presence an amino group(s). The stability improves with either transforming the amines into amides or by passivating the surface with a silica adlayer that creates a less pH sensitive bond with silane linkers.

While this article was in preparation for publication, a paper by Wang et al. [18] appeared with similar studies on the silane linkers' stability on silicon oxide. The authors also observed poor long-term survival of aminosilane linkers and blamed 'hydrogen bonding between the organosilane monomer and silicon surface and among the organosilane monomers' as responsible for the instability. We go a step further and link the instability with local increase of pH and offer two means of alleviating the problem: by transforming amines into amides and by passivating alumina with an adlayer of silica. Even though Wang et al. claim no benefit from baking after silanization, we are sure of its importance. Fig. 3 illustrates the improvement in stability after increased duration of the baking time from 20 min to overnight (empty versus filled circles and boxes). Lack of baking drastically deteriorates both the yield of immobilized DNP and its stability.

4. Conclusions

Thus we demonstrated that silanes covalently bound to the surface of nanoporous alumina membranes had different stability depending on the type of linker used. In agreement with our speculation, local increase of pH from amines on the silanes was a likely cause for activating Al–O–Si bond and making APS a more stable linker due to the lack of secondary amines. Passivation of alumina surface by as little as five atomic layers of deposited silica dramatically improved stability of immobilized molecules for the same conditions.

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References

[1] D.T. Mitchell, S.B. Lee, L. Trofin, N. Li, T.K. Nevanen, H. Soderlund, C.R. Martin, JACS 124 (2002) 11864–11865.
[2] E.D. Steinle, D.T. Mitchell, M. Wirtz, S.B. Lee, V.Y. Young, C.R. Martin, Anal. Chem. 74 (2002) 2416–2422.
[3] I. Vlassiouk, A. Krasnoslobodtsev, M. German, S. Smirnov, Langmuir 20 (2004) 9913–9915.
[4] P. Kohli, M. Wirtz, C.R. Martin, Electroanalysis 16 (2004) 9–18.
[5] M. Wirtz, C.R. Martin, Sens. Update 11 (2003) 35–64.
[6] I. Vlassiouk, P. Takmakov, S. Smirnov, Langmuir 21 (2005) 4776–4778.
[7] D.J. Odom, L.A. Baker, C.R. Martin, J. Phys. Chem. B 109 (2005) 20887–20894.
[8] W. Chen, J.-H. Yuan, X.-H. Xia, Anal. Chem. 77 (2005) 8102–8108.
[9] G.T. Hermanson, Bioconjugate Techniques, Academic Press, New York, 1996.
[10] A. Krasnoslobodtsev, S. Smirnov, Langmuir 18 (2002), 3181–3184 and references therein.

[11] G.E. Brown, V.E. Henrich, W.H. Casey, D.L. Clark, C. Eggleston, A. Felmy, D.W. Goodman, M. Gratzel, G. Maciel, M.I. McCarthy, K.H. Nealson, D.A. Sverjensky, M.F. Toney, J.M. Zachara, Chem. Rev. 99 (1999) 77–174.
[12] J.D. Ferguson, E.R. Smith, A.W. Weimer, S.M. George, J. Electrochem. Soc. 151 (2004) G528–G535.
[13] A. Szymczyk, A. Pierre, J.C. Reggiani, J. Pagetti, J. Membr. Sci. 134 (1997) 59–66.
[14] B. Winkler, R.E. Baltus, J. Membr. Sci. 226 (2003) 75–84.
[15] C.L. Rice, R.J. Whitehead, J. Phys. Chem. 69 (1965) 4017–4024.
[16] A. Hernandez, F. Martinez, A. Martin, P. Pradanoz, J. Coll. Interf. Sci. 173 (1995) 284–296.
[17] M. Nystrom, M. Lindstrom, E. Matthiasson, Coll. Surf. 36 (1989) 297–312.
[18] A. Wang, H. Tang, T. Cao, S.O. Salley, K.Y.S. Ng, J. Coll. Interf. Sci. 291 (2005) 438–447.