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Amino-Modified Diamond as a Durable Stationary Phase for Solid-Phase Extraction

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We report the formation of a highly stable amino stationary phase on diamond and demonstrate its use in solid-phase extraction (SPE). This process consists of spontaneous and self-limiting adsorption of polyallylamine (PAAm) from aqueous solution onto oxidized diamond. Thermal curing under reduced pressure or chemical cross-linking with a diepoxide was shown to fix the polymer to the particles. The resulting adsorbents are stable under even extreme pH conditions (from at least pH 0−14) and significantly more stable than a commercially available amino SPE adsorbent. Coated diamond particles were characterized by X-ray photoelectron spectroscopy (XPS) and diffuse reflectance Fourier transform-infrared spectroscopy (DRIFT). Model silicon surfaces were characterized by spectroscopic ellipsometry and wetting. Solid-phase extraction was demonstrated using cholesterol, hexadecanedioic acid, and palmitoyloleoylphosphatidylcholine as analytes, and these results were compared to those obtained with commercially available materials. Breakthrough curves indicate that, as expected, porous diamond particles have higher analyte capacity than nonporous solid particles.

Solid-phase extraction (SPE) is a much-used technique for sample preparation, purification, concentration, and cleanup.[1,2] It has, in many cases, replaced liquid−liquid extraction and it often precedes high-performance liquid chromatography (HPLC) in analytical analyses. SPE has been referred to as on/off chromatography because ideally it is designed to either perfectly retain or completely release (not retain) analytes of interest.

Diamond is an extraordinary material because of its chemical inertness, hardness, high thermal conductivity, and optical transparency. Diamond's chemical inertness and hardness make it an ideal material in a number of applications. Indeed, there is increasing recognition (vide infra) that synthetic diamond, which has become relatively inexpensive, might function well as a stationary phase for HPLC and SPE.

In this paper, we demonstrate the spontaneous and self-limiting adsorption of a water-soluble polymer to diamond and its application as an SPE stationary phase. This polyelectrolyte (polyallylamine, PAAm,[3,4] see Scheme 1) contains pendant primary amines along its backbone, and is commercially available in a variety of molecular weights. Primary amines are one of the most useful reactive functional groups in organic and bioconjugate chemistry.[5,6] These amine groups should allow PAAm coated diamond particles to function in a manner analogous to commercially available amino phases for SPE and HPLC. PAAm adsorption and its subsequent reactivity are confirmed on diamond particles by X-ray photoelectron spectroscopy (XPS) and diffuse reflectance Fourier transform-infrared spectroscopy (DRIFT) and on model silicon surfaces by spectroscopic ellipsometry and wetting. Stability tests show that under strongly basic and acidic conditions, the adsorbed PAAm coating on diamond is moderately stable. These results are compared to the stability of a commercially available SPE amino phase. Through chemical cross-linking or by thermal curing, we show that the PAAm coated diamond can be given complete stability at both very high and very low pH. We then successfully demonstrate the use of PAAm coated diamond as an adsorbent for SPE and compare our results to those obtained with materials from two different vendors.

There are now a few recent examples of the use of diamond in SPE and HPLC. For example, Chang and co-workers have used uncoated diamond nanoparticles terminated with carboxyl groups for the extraction of proteins.[7] They also reported protein capture using uncoated diamond nanoparticles,[8] and in another study, they demonstrated the use of polylysine coated diamond nanocrystals for capturing oligonucleotides from solution.[9] No SPE was performed in this study, and the polylysine was not cross-linked

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(3) In this paper we will use PAAm as the abbreviation for poly(allylamine) and not PAA, which is the common abbreviation for polyacrylic acid. The use of "PAAm" is also to distinguish this (nearly) neutral polymer from polyallylamine hydrochloride, which has been widely used for building polyelectrolyte multilayers and has the abbreviation "PAH".
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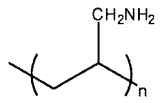
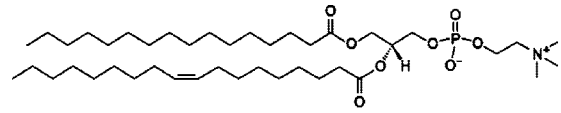
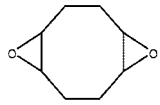
Scheme 1. Compounds Employed in This Study



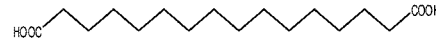
Polyallylamine (PAAm)



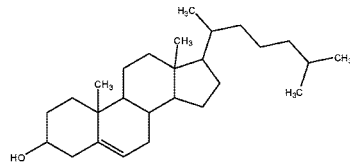
Palmitoyloleoylphosphatidylcholine (POPC)



1,2,5,6- Diepoxycyclooctane



1,16-Hexadecanedioic acid



Cholesterol

or cured. Nesterenko and co-workers have also demonstrated HPLC with uncoated diamond as a stationary phase.[10,11]

EXPERIMENTAL SECTION

Reagents. (See Scheme 1) Poly(allylamine) (M_w approximately 65 000, 20 wt % solution in water, Aldrich, Milwaukee, WI), 1,2,5,6-diepoxycyclooctane (96%, Aldrich), 1,16-hexadecanedioic acid (≥98%, Aldrich), cholesterol (95%, Aldrich), 2-propanol (99.5%, Mallinckrodt Chemicals, Phillipsburg, NJ), and palmitoyloleoylphosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) were used as received. Diamond powder (70 μm and sintered) was provided by U.S. Synthetic (Orem, UT).

Substrates. Silicon wafers (test grade, n-type, ⟨1-0-0⟩ orientation, 2−6 Ω cm) were purchased from UniSil (Santa Clara, CA) and cleaved into approximately 1.5 cm × 1.5 cm pieces. Porous diamond particles were prepared by pressing 2 μm diamond grit and then by crushing and sizing it. The 38−65 μm fraction was employed as an adsorbent for SPE in this study.

Surface Cleaning. Prior to any surface treatment, diamond powder was cleaned in 100 mL of piranha solution (70% H_2SO_4/30% concentrated H_2O_2) at 100 °C for 1 h and then thoroughly washed with deionized water. Silicon wafers were lightly brushed with a camel hair brush using 2 wt % sodium dodecylsulfate solution in water, then rinsed thoroughly with ultrapure water obtained from a Milli-Q (OM-154) water system from Millipore, and finally plasma cleaned at high power (16 W applied to the rf coil) for 1 min using a plasma cleaner (model PDC-32G) from Harrick Plasma (Ithaca, NY).

Hydrogen Termination of Diamond Powder. Diamond powder was hydrogen terminated by heating it in a furnace in an atmosphere of 5% H_2 in Ar at 900 °C for 28 h (this is a noncombustible gas mixture). Hydrogen termination of diamond can also be performed using pure H_2 gas.[12] The powder was shaken twice during this process to facilitate hydrogen termination over all surfaces of the particles.

Poly(allylamine) (PAAm) Deposition. A 0.375 wt % solution of poly(allylamine) was made by dissolving 0.75 g of PAAm (20 wt % solution in water) in 40 mL of ultrapure water. Piranha cleaned diamond powder (5 g) was poured into this solution, and polymer adsorption was allowed to take place for 1 h at room temperature. The solution was shaken every 5 min for approximately 10 s to completely expose the diamond particles to the PAAm solution. After adsorption, PAAm functionalized diamond powder was sonicated in ultrapure water for approximately 10 min. The water was exchanged 4−5 times during this sonication procedure. Finally, the PAAm functionalized diamond powder was captured on a filter funnel and washed with copious amounts of ultrapure water for approximately 30 min. This coating and cleaning procedure was employed for both the 70 μm and porous 38−65 μm diamond material employed in this study, with the exception that the 38−65 μm material was not filtered. The porous particles were repeatedly sonicated in approximately 200 mL of ultrapure water, where the water was decanted and replaced with fresh water 5−6 times during the cleaning procedure. The particles were then dried in a vacuum oven. For deposition of PAAm onto native silicon oxide on silicon, silicon wafers were immersed in 10 mL of a 0.1 wt % solution of PAAm for 35 min. In practice, we have found that this is more than enough time to saturate the surface with this polymer. The wafers were then washed with ultrapure water and dried with a jet of nitrogen.

Curing of PAAm Functionalized Diamond Powder. Curing of PAAm functionalized diamond powder took place in a vacuum oven at reduced pressure and at elevated temperature (approximately 115 °C, for 2.5 h). The vacuum for this oven was provided by a rotary vane pump. The system contained a dry ice/

(10) Nesterenko, P. N.; Fedyanina, O. N.; Volgin, Y. V. *Analyst* **2007**, *132*, 403–405.

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acetone cooled trap that prevented back streaming of oil from the pump.

Chemical Cross-Linking of PAAm Functionalized Diamond Powder. Adsorbed PAAm on diamond powder was cross-linked with 1,2,5,6-diepoxycyclooctane. This molecule has two strained epoxide rings that make it reactive with the primary amine groups of PAAm. Since cross-linking of adsorbed PAAm with 1,2,5,6-diepoxycyclooctane results in the immobilization of hydrophobic cyclooctyl rings and hydrophilic -OH groups on the surface, this reaction will lead to the formation of a stationary phase with some mixed mode character. A 3.65 wt % solution of 1,2,5,6-diepoxycyclooctane was made in isopropanol, and 5 g of PAAm functionalized diamond powder was immersed in 40 mL of this solution. Alcohols are effective solvents for catalyzing amine−epoxide reactions. The reaction was performed in a sealed, thick-walled glass reaction vessel at 80 °C for 12 h. After this reaction, the powder was sonicated first in approximately 70 mL of isopropanol for 5 min and then in approximately 70 mL of dichloromethane for 5 min. The solvents were exchanged with clean solvent three times during sonication with each solvent. Finally, the powder was captured on the glass frit of a filter funnel (ChemGlass, 15 mL, fine pore size frit), and washed with copious amounts of dichloromethane for 15 min. To cross-link PAAm on planar silicon, the PAAm functionalized surface was immersed in 10 mL of 0.6 wt % 1,2,5,6-diepoxycyclooctane in isopropanol. This reaction was also performed in a sealed, thick-walled glass tube at 80 °C for 12 h. After the reaction, the surfaces were rinsed first with isopropanol and then with dichloromethane.

Stability Studies. Approximately 2.5 M NaOH and 2.5 M HCl solutions were prepared for pH stability studies. An amount of 0.2 g of each adsorbent was immersed separately in either the NaOH or HCl solution for 38 h. Finally, the particles were captured on a filter funnel as before (vide supra) and rinsed with copious quantities of ultrapure water.

Surface Analysis. X-ray photoelectron spectroscopy (XPS) was performed with an SSX-100 instrument from Surface Sciences (Bend, OR) using an Al Kα source and a hemispherical analyzer. An electron flood gun was employed for charge compensation, and this charge compensation was further enhanced with a fine Ni mesh approximately 0.5−1.0 mm above the surface. FT-IR was performed with a Magna-IR 560 spectrometer from Nicolet (Madison, WI). Spectroscopic ellipsometry was performed with an M-2000 instrument from the J.A. Woollam Company (Lincoln, NE). The optical constants of silicon dioxide were used to model adsorbed PAAm films, which is appropriate because of the similarity between the optical constants of SiO_2 and most organic materials, especially over much of the visible region of the spectrum and because of the thinness of the PAAm films. Advancing water contact angles were measured with a contact angle goniometer, model 100-00 from Ramé-Hart (Netcong, NJ).

Solid-Phase Extraction. SPE of different analytes was performed with commercially available amino columns (Phenomenex Strata NH_2, 55 μM, 70 Å, 1.2 cm^3 of packing material, Torrance, CA, and Varian Bond Elut NH_2, 47−60 μM, 58−87 Å, 0.95 cm^3, Lake Forest, CA) and with packings prepared in our laboratory. For our experiments, the material in a commercially available cartridge (from Phenomenex) was replaced by our amino stationary phase. A control experiment was performed that showed that neither the plastic cartridge nor the frits retained analytes. The same volume (approximately 1.40 cm^3) of packing material was used in all of our experiments. To improve packing, the cartridges were washed with water under negative pressure from the house vacuum during loading. Finally, the columns were dried using house vacuum.

Prior to SPE, cartridges containing our amino-diamond phase were first conditioned with six column volumes of 0.5 M NH_4OH to deprotonate any protonated amine groups and then with three column volumes each of isopropanol, 50% isopropanol/50% hexane, and hexane. Analyses of individual analytes were then performed by loading 0.1 mL of cholesterol in chloroform (0.05 mg/mL), 0.1 mL of palmitoyloleoylphosphatidylcholine in chloroform (0.05 mg/mL), or 0.1 mL of 1,16-hexadecanedioic acid dissolved in a 4:1 mixture (v/v) of chloroform and isopropanol (0.5 mg/mL).

A lipid separation protocol was used to test PAAm coated diamond. In this procedure, neutral lipids are eluted with chloroform, fatty acids are eluted with 2% acetic acid in diethyl ether, and phospholipids are eluted with methanol. This method was originally developed by Kaluzny and co-workers for performing solid-phase extraction of lipid mixtures on amino SPE phases.[13] This procedure, or a modified version of it, has been used a number of times to this end[14–16] (we used a slightly modified version of the Kaluzny protocol, employing chloroform instead of 2:1 chloroform/2-propanol in the first elution).

In practice, amino modified diamond SPE adsorbents could be repeatedly used without noticeable degradation. Before each reuse, the column was washed with methanol several times to remove any traces of palmitoyloleoylphosphatidylcholine from the previous run. ESI-MS was performed on the final methanol wash to verify the absence of palmitoyloleoylphosphatidylcholine in the column.

Solid-Phase Extraction of the Three Component Mixture. Solid-phase extraction was also performed on a mixture of cholesterol, hexadecanedioic acid, and palmitoyloleoylphosphatidylcholine. A solution of these three components was made by dissolving 0.4 mg of palmitoyloleoylphosphatidylcholine, 4.0 mg of hexadecanedioic acid, and 5.0 mg of cholesterol in a mixture of chloroform (9 mL) and isopropanol (1 mL). The diamond amino column was conditioned using the same procedure as mentioned before. The Phenomenex and Varian materials were similarly conditioned but not with the NH_4OH solution. A volume of 0.1 mL of the three component solution was loaded onto the column after conditioning, and cholesterol, hexadecanedioic acid, and palmitoyloleoylphosphatidylcholine were eluted with three column volumes of chloroform, 2% acetic acid in diethyl ether, and methanol, respectively.

Breakthrough Curves. The analyte used for determination of breakthrough volumes was 1,16-hexadecanedioic acid. The column was first conditioned using the procedures mentioned above. After conditioning, the analyte solution was loaded onto the cartridge. The column was kept wet, and the flow rate was kept constant during the process. Equal volumes of the fractions

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(14) Bateman, H. G.; Jenkins, T. C. *J. Agric. Food Chem.* **1997**, *45*, 132–134.
(15) Bodennec, J.; Koul, O.; Aguado, I.; Brichon, G.; Zwingelstein, G.; Portoukalian, J. *J. Lipid Res.* **2000**, *41*, 1524–1531.
(16) Åkesson-Nilsson, G. *J. Chromatogr., A* **2003**, *996*, 173–180.

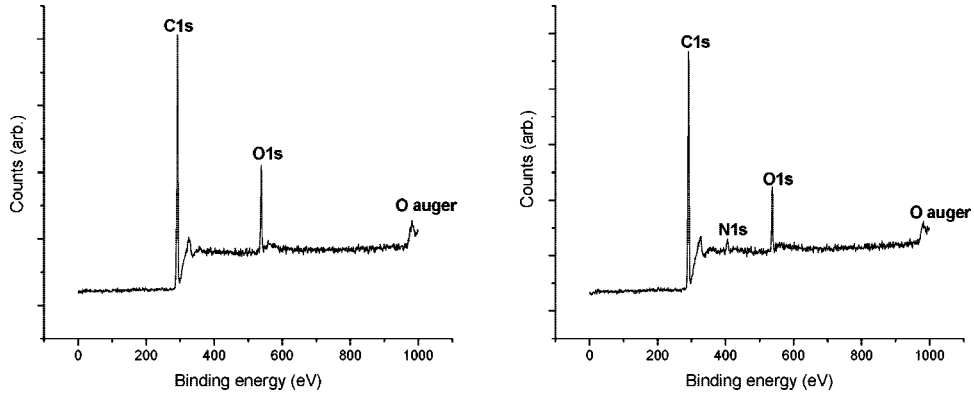

Figure 1. XPS survey scans of piranha cleaned diamond powder (left) and PAAm functionalized diamond powder (right).

eluting from the column were collected in separate vials. Finally, these fractions were analyzed by ESI-MS.

Breakthrough curves had sigmoidal shapes. The breakthrough volume was calculated from the point on the curve corresponding to 5% of the average value at the maximum (plateau region).

Electrospray-Mass Spectrometry (ESI-MS). ESI-MS was performed on an Agilent Technologies LC/MSD TOF system by direct infusion of several microliters of sample along with the mobile phase: 75% MeOH and 25% water with 5 mM ammonium formate. In positive ion mode, the charging voltage and the capillary voltage were set at 900 and 3500 V, respectively, and the skimmer was operated at 60 V. The nebulizer was at 35 psi, and the gas temperature was 350 °C. The flow rate of the nitrogen drying gas was set at 12 L/min. All of the instrument parameters in negative ion mode were identical to those in positive ion mode except the capillary voltage and drying gas flow rate, which were set at 4000 V and 8 L/min, respectively.

The ESI-MS is a communal instrument that gets heavy use in the BYU chemistry department; it is not uncommon for us to see spurious peaks in our spectra from the instrument. The peak at m/z 199.01 (vide infra) showed up only in the mass spectra of three fractions in the SPE of 1,16-hexadecanedioic acid, i.e., in panels b−d of Figure 2, which were taken on the same day. This peak did not show up in the breakthrough volume determination study, which used 1,16-hexadecanedioic acid as the analyte, or in the 2% acetic acid/diethyl ether fraction during SPE of the three component mixture, i.e., cholesterol, 1,16-hexadecanedioic acid, and POPC, which were taken on other days. Thus it was inferred that this peak was due to contamination and was therefore removed.

RESULTS AND DISCUSSION

PAAm Adsorption, Thermal Curing, and Chemical Cross-Linking. Prior to polyelectrolyte adsorption, diamond particles were cleaned in piranha solution, which is an extremely reactive solution that is widely used for removing organic contamination from silicon and glass surfaces. Caution: Piranha solution reacts explosively with a number of organic solvents and materials, e.g., acetone, and should be handled with great care. The fact that this 1 h cleaning does not appear to affect the diamond particles is consistent with diamond's tremendous chemical stability. This work is to be compared to an earlier study that showed polylysine deposition onto diamond. In our work, effective deposition of an amine-containing polymer (PAAm) was demonstrated on cleaned but otherwise unfunctionalized diamond. In these earlier studies, diamond surfaces were oxidized by heating in 9:1 sulfuric acid/nitric acid for 1 day to form surface carboxyl groups that facilitated polylysine adsorption.[9,17] We believe that fewer surface attachment points, as would be expected with our method, is preferable because a large number of surface carboxyl groups should decrease the capacity for SPE of an adsorbed amine-containing polymer.

After adsorption of polyallylamine (PAAm), coated diamond particles were characterized by XPS, Fourier transform-infrared spectroscopy (FT-IR), and chemical stability tests. The nitrogen-to-carbon ratio on this material, measured by the ratio of the N1s to the C1s XPS signals, was 0.036 ± 0.002, where all such ratios quoted in the text were obtained from XP narrow scans. No nitrogen signal was discernible by XPS prior to PAAm adsorption (see Figure 1). Diffuse reflectance FT-IR showed no C−H stretches after piranha cleaning and before PAAm adsorption but showed clearly defined signals in the C−H stretching region after PAAm adsorption.

Chemical stability tests were performed by immersing PAAm coated diamond particles into 2.5 M HCl or 2.5 M NaOH for 38 h. Following this treatment, approximately one-third of the nitrogen was removed from the surface; after exposures to acid and base, the N1s/C1s ratios decreased to 0.024 ± 0.003 and 0.026 ± 0.003, respectively. These results were compared to the stability of a commercially available SPE stationary phase (Phenomenex Strata NH_2, 55 μM, 70 Å), which is primarily composed of silica, where the Si2p peak (a substrate peak) is a better reference peak for XPS than the C1s signal. Prior to stability tests, the N1s to Si2p ratio by XPS was 0.135 ± 0.002. After immersion of these particles in 2.5 M NaOH for 6 h, the particles dissolved completely. To further verify the dissolution of these particles, the resulting clear solution was filtered. It easily passed through the filter, leaving no material behind. The Phenomenex particles were also immersed in 2.5 M HCl for 36 h. A small decrease in the N1s/Si2p ratio was observed (down to 0.117 ± 0.012), which suggests that a little less than 15% of the nitrogen-containing coating on the particles had been lost. Thus, the deposited PAAm coatings on diamond are somewhat less stable in acid than the comparable amino coating on a commercially available SPE packing material, while being much more stable to base.

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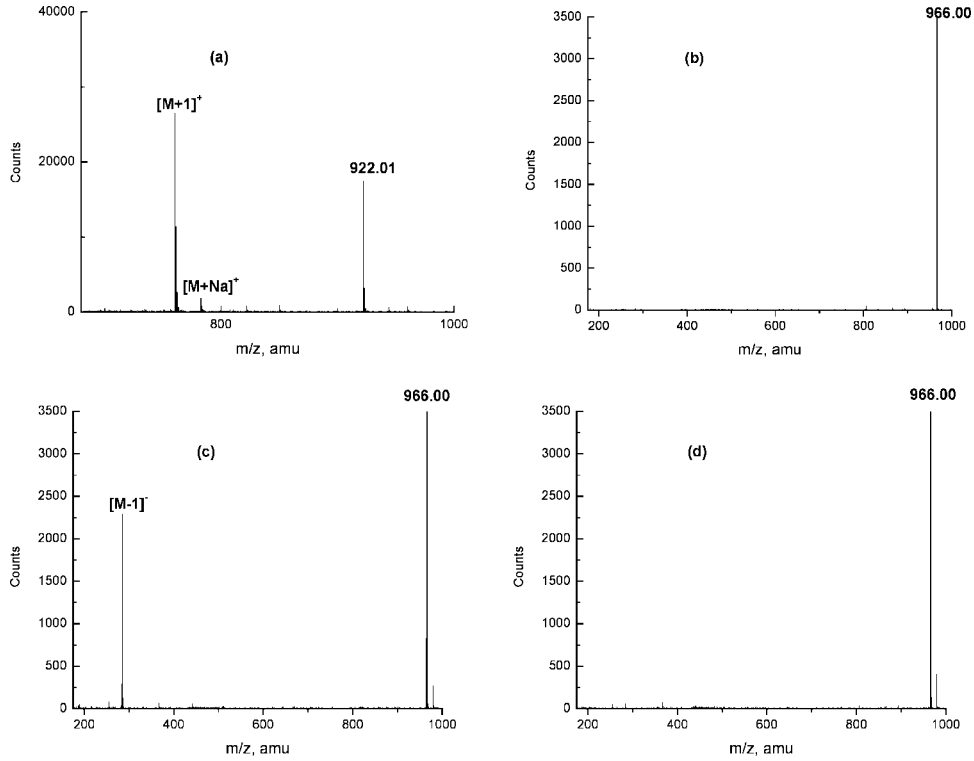

Figure 2. ESI-MS spectra of SPE fractions of individual analytes (not from a mixture of these analytes) from sequential elution of amino-coated diamond with chloroform, 2% acetic acid in diethyl ether, and methanol. (a) Positive ion spectrum of POPC eluted in methanol showing the $[M + H]^+$ and $[M + Na]^+$ peaks of POPC and negative ion spectra of 1,16-hexadecanedioic acid eluted in (b) chloroform (no analyte present), (c) 2% acetic acid in diethyl ether, and (d) methanol (no analyte present). An unidentified peak at *m*/*z* 199.01 is not shown in panels b−d. The reference peak at *m*/*z* 966.00 is much higher in intensity than shown in panels b−d.

While these results might suggest that direct adsorption of PAAm onto clean diamond may create a stationary phase that is useful in a variety of situations, we wondered if it would be possible to create an even more stable stationary phase. Two approaches were taken to accomplish this: thermal curing and chemical cross-linking. For thermal curing, the PAAm coated diamond particles were heated under vacuum to 115 °C for 2.5 h. Such conditions have been shown to create amide linkages in polyelectrolyte multilayers that contain carboxyl and amine groups.[18] While the number of carboxyl groups on our diamond surfaces appeared to be small by XPS, after curing, a series of small peaks appeared in the FT-IR spectrum at the positions expected for the amide stretches,[19] i.e., (1690−1620 cm^{-1}) due to C=O stretching (the amide I band), and (1570−1520 cm^{-1}) due to coupled C−H stretching and N−H bending (the amide II band). Stability tests were then performed on this cured material. Prior to curing, the N1s/C1s ratio of the PAAm coated diamond powder was 0.036 ± 0.002 (vide supra). After curing, this ratio remained essentially constant (i.e., 0.036 ± 0.001). After immersion of this cured material into 2.5 M HCl and 2.5 M NaOH for 38 h, the N1s/C1s ratios were essentially unchanged (i.e., 0.034 ± 0.002 and 0.036 ± 0.003, respectively). These results indicate that a thermal cure can significantly improve the high and low pH stability of PAAm coated diamond particles.

Chemical cross-linking was also investigated as a method for increasing the stability of PAAm on diamond. The effect of this change would be to increase the molecular weight of the adsorbed PAAm, which would increase its stability. Accordingly, PAAm coated diamond was treated with 1,2,5,6-diepoxycyclooctane. The O1s/C1s ratio after cross-linking measured by XPS increased to 0.15 ± 0.01 from a value of 0.12 ± 0.01 prior to cross-linking. This increase in the oxygen content of the surface is consistent with the chemisorption of an oxygen-containing species (the diepoxide). The N1s/C1s ratio, by XPS, after this cross-linking reaction was 0.025 ± 0.001. The decrease in the N1s/C1s ratio, compared to the ratio found before cross-linking (vide supra), is presumably due to the increased amounts of carbon and oxygen added to the surface through chemisorption of the diepoxide. After the cross-linking reaction, the pH stability of the material was measured. After immersion of the cross-linked material into 2.5 M HCl and 2.5 M NaOH for 38 h, the N1s/C1s ratios were essentially unchanged at 0.023 and 0.026, respectively.[20] These results indicate that chemical cross-linking can also significantly improve the high- and low-pH stability of PAAm coated diamond particles.

Further information about this cross-linking reaction was obtained using a planar silicon surface. Because of the expected self-limiting nature of PAAm adsorption onto materials, adsorption of PAAm onto silicon oxide is expected to be similar to adsorption of this polymer onto diamond. An additional advantage of a planar substrate is that optical ellipsometry can be performed on it.

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(20) In both of these experiments, two spots were analyzed on each surface and identical results were obtained.

Accordingly, a clean silicon surface with its thin native oxide layer was immersed in a dilute solution of PAAm, where the PAAm was found to adsorb spontaneously onto the surface. The resulting PAAm film was 8.7 ± 1.5 Å thick. After reaction with 1,2,5,6-diepoxycyclooctane, the film thickness increased by 9.6 ± 0.6 Å. These changes in surface chemistry were also followed by wetting (contact angle goniometry). Initially, the clean silicon oxide surface is easily wetted by water. After adsorption of PAAm, the advancing water contact angle of the surface was 32 ± 6°, and after reaction with the diepoxide, the advancing water contact angle was 65 ± 8°. These increases in film thickness and the corresponding advancing water contact angles are again consistent with the expected surface chemistry.

Solid-Phase Extraction with PAAm Coated Diamond Particles. PAAm coated diamond particles were loaded into columns and used for solid-phase extraction. A demonstration of the retention and release of analytes was performed with cholesterol, 1,16-hexadecanedioic acid, and palmitoyloleoylphosphatidylcholine (POPC) (see Scheme 1).

A modified literature SPE procedure for amino-coated packing material that fractionates biological tissue extracts into neutral lipids, fatty acids, and phosphatidylcholines was used to demonstrate the effectiveness of our new stationary phases.[13] The columns were first conditioned with 0.50 M NH_4OH to deprotonate them, a high pH solution that would only be appropriate for a very stable stationary phase, followed by isopropanol, 50% isopropanol/50% hexane, and hexane. Individual analytes, and later a three component mixture, were then loaded onto the columns, after which they were eluted sequentially with chloroform (to elute neutral lipids), 2% acetic acid in diethyl ether (to elute fatty acids), and methanol (to elute phosphatidylcholines). In some cases, comparison SPE columns made of piranha cleaned, untreated diamond powder or hydrogen-terminated diamond powder were also employed. ESI-MS confirmed the presence or absence of the analytes in the fractions that were taken. During SPE, these analytes eluted only in their respective eluents and not in other eluents, i.e., cholesterol eluted only in chloroform, 1,16-hexadecandioic acid eluted only in 2% acetic acid in diethyl ether, and POPC eluted only in methanol. Solid-phase extraction was done in this manner many times using the same diamond material; the same column could be reused multiple times without showing any significant signs of deterioration.

On hydrogen-terminated diamond packing material, POPC eluted in the first fraction (chloroform). In contrast, POPC eluted in the last fraction (methanol) but not in the first two fractions (chloroform and 2% acetic acid in diethyl ether) for uncoated (oxidized) diamond and for PAAm coated diamond (the as-deposited, not cured or cross-linked coating). Figure 2 shows an ESI-MS spectrum of the corresponding methanol fraction from PAAm coated diamond that is dominated by three peaks: the $[M + H]^+$ and $[M + Na]^+$ peaks for POPC and a reference peak at 922 amu. SPE was then performed with 1,16-hexadecanedioic acid using the as-deposited PAAm coated diamond. This analyte elutes only in the second fraction (2% acetic acid in ether) as the $[M - H]^-$ ion, as it should, where Figure 2 shows ESI-MS negative ion spectra for the chloroform, 2% acetic acid, and methanol fractions. Finally, with the use of this procedure, cholesterol was shown to elute only in the first fraction (chloroform).

With the employment of the above procedure, cholesterol, 1,16-hexadecanedioic acid, and POPC were separated on uncured PAAm modified diamond as a three-component mixture. As expected, upon elution with chloroform, cholesterol appears exclusively in the first fraction, as shown by m/z 369 and 409 peaks, due to the $[M - OH]^+$ and $[M + Na]^+$ peaks of cholesterol, respectively. In the second fraction, 1,16-hexadecanedioic acid appears exclusively upon elution with 2% acetic acid in diethyl ether. Finally, POPC appears exclusively in the final fraction with methanol elution.

A comparison of the extraction performance of the diamond amino column was also made by doing SPE of the three-component mixture on Phenomenex and Varian amino columns. The diamond amino column was conditioned as before. However the 0.5 M ammonia solution was not used during the conditioning of the Phenomenex and Varian amino columns. Otherwise the rest of the procedure for the commercial materials was identical to that used for the diamond amino column. It was observed that the three-component mixture could be separated using the diamond amino column, but it could *not* be separated effectively using our method (essentially the Kaluzny[13] procedure) with the Varian and Phenomenex amino columns. On these columns, 1,16-hexadecanedioic acid did not elute in the 2% acetic acid in diethyl ether fraction, as we would have expected. Rather, it eluted with POPC in the final methanol fraction.

To further characterize the as-deposited, thermally-cured, and chemically cross-linked diamond SPE materials, breakthrough curves were obtained for 1,16-hexadecanedioic acid. From these breakthrough curves, the column capacities were calculated to be 0.16 mg, 0.11 mg, and 0.11 mg for the as-deposited, thermally-cured, and chemically cross-linked columns, respectively. It is clear from these results that the column capacity decreases somewhat for this analyte upon thermal curing and chemical cross-linking. According to our proposed mechanism, thermal curing should convert some ammonium ions that are adjacent to carboxylates into amides, which will reduce the possible number of active amine groups in the coating. Tighter attachment of the polymer coating to the diamond substrate may also reduce the accessibility of some of its amines. Chemical cross-linking of adsorbed PAAm should decrease diffusion of analytes into the coating, decreasing the column capacity.

The pressure-flow behavior of amino-coated diamond (70 μm particles) was compared to that of a commercially available amino-coated silica-based SPE material (Phenomenex Strata NH_2). Different solvents have different viscosities; therefore, pressure-flow behavior of the column varies with the solvent type. The pressure-flow behavior of the column was studied using water (a fairly viscous solvent for chromatography) as the fluid. Equal volumes of the diamond and commercially available sorbent (1.02 cm^3) were placed in an SPE column and connected to an HPLC. The flow, F (in mL/min), was determined as a function of back pressure, P (in psi), between 165 and 550 psi. The resulting curves were fit to straight lines. The two materials had nearly equal pressure−flow properties. The linear fit to the pressure−flow curves were $F = 0.0128P + 0.996$ with an R^2 value of 0.997 for the diamond particles and $F = 0.0128P + 1.05$ with an R^2 value of 0.996 for the commercially available material. In a more practical (but less quantitative) sense, liquid could be easily forced through

all the home-built and commercially available SPE columns studied in this work with a plastic syringe that was compressed manually.

Increase in Particle Surface Area to Increase Column Capacity. The breakthrough curve of 1,16-hexadecanedioic acid on amino-coated ~70 μm diamond powder was compared to the same breakthrough curve on commercially available amine-coated silica. This comparison indicates that the capacity of our columns is approximately 1/300th of the capacity of commercially available material. The logical explanation for this difference is the lack of porosity (surface area) of our particles, which are solid and quite large.

To increase the surface area of our packing materials, we sintered 2 μm diamond particles and crushed and sized the resulting porous solid. These porous particles were then coated with PAAm and packed into columns for SPE. The N1s/C1s ratio by XPS for these coated, porous diamond particles was 0.046. This ratio is larger than the value obtained with nonporous particles (vide supra) and is attributed to the greater surface area of this porous material. The capacity of the resulting columns (1.40 cm^3 of diamond material), as measured by the breakthrough curve of 1,16-hexadecanedioic acid, was 3.26 mg for the 38−65 μm fraction (2.3 mg/cm^3) (see Figure 3), which is a greater than a 20-fold increase in column capacity compared to the solid ~70 μm diamond particles (The Phenomenex Strata NH_2 sorbent has a capacity of 17.5 mg for 0.45 cm^3 of material or 39 mg/cm^3). Thus, our porous diamond particles have about 1/17th the capacity of the commercially available sorbent. Further work in our group will focus on smaller particles and better amino coatings that should further improve column capacity.

CONCLUSION

We have reported the formation of a highly stable amino stationary phase on diamond and demonstrated its use in solid-phase extraction. The pH stability of this amino coating on diamond was compared to the pH stability of a commercially available SPE amino adsorbent. After chemical cross-linking or thermal curing, this amino coating on diamond was stable under extremely acidic and basic conditions, while a commercially available amino SPE adsorbent disintegrated and dissolved in a high pH medium, and approximately 15% of its amino coating was lost in a highly acidic medium. Solid-phase extraction was demonstrated on amino coated diamond using cholesterol, palmitoyloleoylphosphatidylcholine, and hexadecanedioic acid, and these results were compared to SPE on commercially available sorbents.

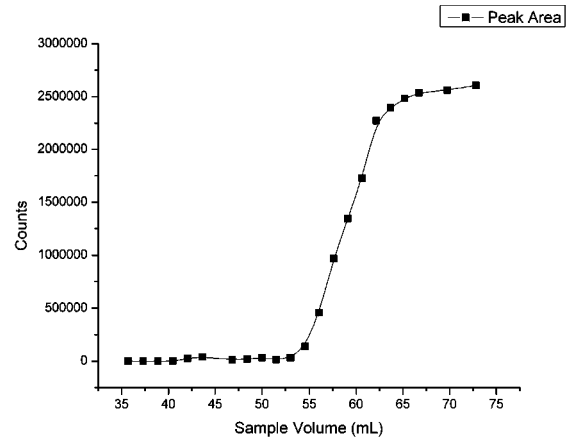

Figure 3. Breakthrough curve for 1,16-hexadecanedioic acid on PAAm coated, sintered, and crushed 2 μm diamond particles (38−65 μm fraction of porous diamond powder). Each point represents the area of the analyte peak from a negative ion ESI-MS analysis of the fractions that were collected.

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SUPPORTING INFORMATION AVAILABLE

Diffuse reflectance FT-IR spectra of the C−H and amide stretching regions and representative N1s, C1s, and Si2p narrow XP scans. This material is available free of charge via the Internet at http://pubs.acs.org.

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