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Amine-phenyl multi-component gradient stationary phases

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ABSTRACT

Continuous multi-component gradients in amine and phenyl groups were fabricated using controlled rate infusion (CRI). Solutions prepared from either 3-aminopropyltriethoxysilane (APTEOS) or phenyltrimethoxysilane (PTMOS) were infused, in a sequential fashion, at a controlled rate into an empty graduated cylinder housing a vertically aligned thin layer chromatography (TLC) plate. The hydrolyzed precursors reacted with an abundance of silanol (Si—OH) groups on the TLC plates, covalently attaching the functionalized silane to its surface. The extent of modification by phenyl and amine was determined by the kinetics of each reaction and the exposure time at each point along the TLC plate. The local concentrations of phenyl and amine were measured using diffuse reflectance spectroscopy and X-ray photoelectron spectroscopy, respectively. The profile of the multi-component gradients strongly depended on the order of infusion, the direction of the gradient and the presence of available surface silanol groups. A slightly higher amount of phenyl can be deposited on the TLC plate by first modifying its surface with amine groups as they serve as a catalyst, enhancing condensation. Separation of water- and fat-soluble vitamins and the control of retention factors were demonstrated on the multi-component gradient TLC plates. Uniformly modified and single-component TLC plates gave different separations compared to the multi-component gradient plates. The retention factors of the individual vitamins depended on the order of surface modification, the spotting end, and whether the multi-component gradients align or oppose each other.

1. Introduction

A single-component gradient has one chemical component whose surface concentration changes in a predictable manner from one end of a substrate to the other. Such materials have been used to study cell adhesion and migration, directed transport, and protein adsorption among other phenomena as described in a number of reviews [1–6]. An one-component gradient, however, may not be sufficient for a number of applications, particularly those that rely on the interaction of an analyte species with more than one functional group located in close proximity to each other on a surface. Separation science and bifunctional heterogeneous catalysis are two areas where such interactions are vitally important. The presence of two or more components, each having different characteristics (e.g., charge, hydrophobicity), can provide an avenue for improved retention and selectivity, particularly if the two or more components act together in a synergistic fashion.

Multi-component gradients can be inherently more complex to prepare and characterize, and can also be more molecularly complex on the microscopic length scale. One popular method to fabricate multi-component gradients involves the selective removal of one component on a substrate followed by backfilling with another. An early example of this approach involved using scanning tunneling microscopy-based replacement lithography to remove and replace alkanethiols self-assembled on gold with a second functionalized thiolate present in solution [7]. Bohn et al. have also described an electrochemical gradient technique to form a multi-component surface whereby functionalized thiols adsorb at certain locations along a potential gradient on gold and then the remaining uncoated areas are backfilled with a second thiol. This approach can be followed by immobilizing a specific reagent such as an antibody to the surface via reaction with a terminal functional group on the gradient surface, thus extending applications [8–12]. A variation of this method has also recently been described

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that involves the electrochemical desorption/readsorption of surface modifiers while withdrawing the substrate from solution [13]. Other approaches to the formation of multi-component gradients include gradually exposing a suitable substrate to a modifying solution followed by full immersion [14–16], creation of a gradient monolayer via microfluidic lithography followed by backfilling [17], the use of a photo-mask followed by electrochemical oxidation and selective immobilization [18], modulating the exposure time of a self-assembled monolayer on gold to a reducing agent [19] and cross diffusion of different organothiols through a sephadex gel on a gold slide [20–22].

We have also fabricated two-component gradients using controlled rate infusion (CRI) [23–25] and infusion-withdrawal dip coating (IWDC) [26–28]. These two methods involve a variation of the sol–gel process [29–31]. In CRI, a reactive organoalkoxysilane is gradually infused at a controlled rate into a reaction vessel where it reacts with Si—OH groups on the surface of a siloxane-coated substrate in a time-dependent fashion [23–25]. Gradient formation is governed by the rate of infusion, the rate of reaction, and the concentration of the silane. In IWDC, the siloxane-coated substrate is suspended in a sol prepared from tetramethoxysilane and synchronized syringe pumps are used to infuse an organoalkoxysilane into the deposition reservoir, while the mixed sol (and/or the slide) is withdrawn to produce a film [26–28]. In both of these approaches, one component is unreacted Si—OH and/or $Si(OR)_x$ groups due to incomplete condensation and the other is the pre-determined organic modifier (e.g., CH_3, NH_2, C_6H_5, etc.). Thus, a gradient in the organic modifier also contains a counter gradient in surface silanol (Si—OH) groups.

Here, we describe a simple method to generate continuous multi-component silane gradients using CRI with 3-aminopropyltriethoxysilane and phenyltrimethoxysilane as the organoalkoxysilane precursors. These multi-component materials have a gradient in amine and phenyl that either oppose or align with each other in a predictable fashion. Also present are surface silanol groups, whose concentration will vary along the surface, albeit in a more unpredictable manner depending on the extent of modification by the amine and/or phenyl moieties. We also demonstrate a practical application of such materials in the area of separation science with the separation of a mixture of vitamins on modified thin-layer chromatography (TLC) plates. We show that the retention factors (R_f) and the degree of separation are highly dependent on the manner and extent to which the TLC plates are modified.

2. Material and methods

2.1. Reagents

3-Aminopropyltriethoxysilane (98%, APTEOS) and phenyltrimethoxysilane (97%, PTMOS) were purchased from Alfa Aesar. Vitamins B1 and C were purchased from Sigma–Aldrich while vitamins D3 and K1 were purchased from Alfa Aesar. Ethanol (200 proof, USP/ACS grade) was purchased from Pharmaco-Aaper. Merck Silica gel 60 F_{254} TLC plates (20 cm × 20 cm) were used. All plates were from the same batch. According to the manufacture, the TLC plates have a specific surface area of 480–540 m^2/g, a pore volume of 0.74–0.84 mL/g, and a layer thickness of 210–270 μm.

2.2. Fabrication of stationary phases

TLC plates were cut into pieces of 8 cm × ~1.8 cm and soaked in ethanol to first remove impurities that adhered to the surface and then dried in air. Once dried, they were placed in an oven at 110 °C for 10 min to drive off excess adsorbed water and then cooled to room temperature. The plates were used without modification, with uniform modification by amine (A), phenyl (P), or both (A + P, P + A) and with single gradients of A or P and dual gradients (A + P aligned, A + P opposed, P + A aligned and P + A opposed). The uniformly modified substrate was prepared by soaking the activated TLC plates in either APTEOS solution for less than a minute or in PTMOS solution for 12 min. A + P uniform TLC plates were fabricated by first soaking the activated TLC plates in amine solution (for less than a minute) followed by the phenyl solution (for 12 min) or in the reverse order for P + A uniform plates. The composition of the APTEOS solution was ethanol: APTEOS: distilled water (20:0.1:0.1, v/v/v) while the PTMOS solution was ethanol: PTMOS: 0.01 M HCl: 0.02 M NaOH (15:3:1:1, v/v/v/v). The PTMOS solutions were prepared by first hydrolyzing PTMOS in 0.01 M HCl followed by addition of 0.02 M NaOH after 15 min. The solution was further stirred for another 15 min.

The multi-component gradients were prepared using controlled rate infusion (CRI) [23]. In this method, the activated TLC plate was placed vertically in a 25 mL graduated cylinder and either the APTEOS or PTMOS solution was infused at a rate of 20.48 mL/min or 1.38 mL/min, respectively, using a syringe pump (New era NE-1000). Infusion was stopped ~0.5 cm from the top of the plate. All the TLC plates modified with the APTEOS solution were immediately rinsed with ethanol and air dried while those modified with PTMOS solution were directly air dried and placed in an oven for 1 h at 160 °C to form a more stable coating. Both the steps were sequentially applied to produce the multi-component TLC plates. For aligned gradients, the TLC plates were placed in the graduated cylinder in the same direction each time, while for opposed gradients, the slide was inverted between depositions. It is important to note that the concentration of PTMOS in solution was significantly higher and the infusion time significantly longer than for APTEOS, leading to a much higher degree of modification of the TLC plates by phenyl in contrast to amine. Fig. 1 depicts a cartoon of the fabrication of amine-phenyl multi-component gradients.

2.3. Characterization of stationary phases

The extent of amine and phenyl modification across the length of the TLC plates (gradient profile) was assessed via X-ray photoelectron spectroscopy and by diffuse reflectance spectroscopy, respectively. XPS was performed with a ThermoFisher ESCAlab 250 imaging X-ray photoelectron spectrometer (Al KR (1486.68 eV)), using a 500 μm spot size, 50 eV pass energy, 0.1 eV step size. The TLC plate was first cut into five 1.5 cm length pieces, the powder scrapped off each piece, and mixed to form a uniform sample. Approximately 1 mg of powder was then firmly pressed on conducting tape on a 5 cm × 2 cm sample holder and XPS spectra were collected at three different points on each powdered sample. The mean and standard deviation are reported. Charge corrections of the peaks were done using the C1s peak at 284.6 eV. The area under the N1s peaks was calculated using commercially available software (Avantage Version 4.4). For the phenyl modified TLC plate, the plate was cut into 5 pieces of ~1.8 cm × 1.5 cm along its length starting at ~0.5 cm from the top and each piece examined using a diffuse reflectance spectrophotometer (Agilent technologies, Cary Series 6000i UV–Vis–NIR). The reflectance relative to that of an unmodified TLC plate was converted using the Kubelka-Munk (K-M) equation and the K-M function plotted against wavelength. The average value at 260 nm obtained from three separate TLC plates was used to create the profile plots.

2.4. Separations

The separations of vitamins B1, C, D3, and K1 were carried out in triplicate on unmodified, amine and phenyl uniformly modified and gradient TLC plates. The TLC plates were spotted with 3 spots,