Journal of Chromatography A 1730 (2024) 465090

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Continuous stationary phase gradient preparation on planar chromatographic media using vapor phase deposition of silane

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ARTICLE INFO

Keywords:
Thin-layer chromatography
Chlorosilane
Silanization
Diffuse reflectance spectroscopy (DRS)
Aromatic compounds

ABSTRACT

A new, versatile, and straightforward vapor phase deposition (VPD) approach was used to prepare continuous stationary phase gradients (cSPGs) on silica thin-layer chromatography (TLC) plates using phenyldimethylchlorosilane (PDCS) as a precursor. A mixture of paraffin oil and PDCS was placed at the bottom of an open-ended rectangular chamber, allowing the reactive silanes to evaporate and freely diffuse under a controlled atmosphere. As the volatile silane diffused across the length of the TLC plate, it reacted with the surface silanol groups thus functionalizing the surface in a gradient fashion. Characterization of the gradient TLC plates was done through UV visualization and diffuse reflectance spectroscopy (DRS). Visualizing the fluorescent gradient plates under UV radiation shows the clear presence of a gradient with the side closest to the vapor source undergoing the most modification. More quantitative characterization of the shape of the gradient was provided by DRS. The DRS showed that the degree of modification and shape of the gradient was dependent on the concentration of silane, VPD time, and relative humidity. To evaluate the chromatographic performance, a mixture of three aromatic compounds (acetaminophen (A), aspirin (As), and 3-hydroxy-2-naphthoic acid (3H)) was spotted on the high (GHP) and low phenyl (GLP) ends of the gradient TLC plates and the results compared to the separations carried out on unmodified and uniformly modified plates. The GHP TLC plates showed retention factors (Rf) of 0.060 ± 0.006, 0.391 ± 0.006, and 0.544 ± 0.006, whereas the unmodified plate displayed Rf values of 0.059 ± 0.006, 0.092 ± 0.003, and 0.037 ± 0.002 for the analytes A, As, and 3H, respectively. From the Rf values, it was observed that each modified plate exhibited different selectivity for the analytes. The GHP TLC plates exhibited better separation performance, and improved resolution compared to the GLP, unmodified, and uniformly modified plates. Overall, VPD is a new, cost-effective method for creating a gradient on the stationary phase which has the potential to advance chromatographic separation capabilities.

1. Introduction

Stationary phase gradients (SPGs) have considerable promise in the field of chromatography [1–11]. Unlike traditional chromatographic techniques with a stationary phase with constant properties, SPGs offer a continuous variation in chemical properties like polarity and hydrophobicity. As the analytes travel through the separation bed containing a gradient in functionality, they encounter different chemical environments, providing a new avenue for diverse interactions with the stationary phase. This can lead to more selective separations. Moreover, the variation of ligand composition can make the sample molecules interact with the stationary phase in a unique and synergistic way, which can offer better separation of the sample components. This synergistic interaction cannot be achieved using a uniform stationary phase [10]. Additionally, SPGs have been shown to be a useful strategy to alter the selectivity of a separation, reduce the analysis time, and improve the separation of complex mixtures [6,8]. One popular and simple approach involves the serial connection of individual columns or support media to form what has been termed discontinuous stationary phases [6,8]. However, this approach can lead to band broadening and limits the possibility of simultaneous interactions of multiple functional groups on a solute with the individual functional groups on the stationary phase [6]. An alternate approach involves creating a continuous stationary phase gradient (cSPG) in a single housing material [1–5]. This approach is more challenging and often involves the time-dependent coupling of a functionalized organosilane precursor to a bare silica support [4].

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https://doi.org/10.1016/j.chroma.2024.465090
Received 16 April 2024; Received in revised form 11 June 2024; Accepted 12 June 2024
Available online 13 June 2024

In previous work, we used both bond-breaking and bond-forming strategies to create cSPGs on particle-packed columns [4,5]. We have also used a technique that we have termed controlled rate infusion (CRI) to create cSPGs on silica thin-layer chromatography plates (TLC) [9–12]. CRI is a simple time-dependent technique, where silane precursors are infused at a constant rate into a container that contains a vertically oriented planar substrate or support material, in this case, a TLC plate [9–12]. The bottom of the substrate is exposed to the reactive solution for a longer period than the top, and a cSPG is formed [9–12]. CRI offers advantages such as the ability to change the gradient shape by adjusting the infusion rate, but it also has drawbacks, including self-polymerization of the precursors and the uneven wicking of the solution in the silica gel layer. Additionally, to effectively create the gradient, it is necessary to match the rate of infusion with the kinetics of the reaction, which can be challenging when using this method for preparing cSPGs [12].

In this work, we report on a new vapor phase deposition (VPD) approach to create cSPGs for chromatography using silica-coated TLC plates as a proof-of-concept example. VPD has been a valuable approach for fabricating polarity gradients on glass slides and silicon wafers [13–28]. VPD relies on the principle of diffusion where molecules spontaneously move from higher to lower concentration due to random movement of the particles. In this approach, a volatile silane is dissolved in a viscous oil and placed a fixed distance from a hydroxylated surface [22]. The silane vapor diffuses over the substrate where it reacts with the surface silanol (Si-OH) groups. The side of the substrate closest to the vapor source is more heavily modified than the edge furthest away [22]. The chemistry involved in this process is straightforward and doesn't require expensive equipment, making it easy to implement in a laboratory setting. The vapor phase approach is scalable, allowing for the preparation of gradients with different lengths. Additionally, modification using a VPD method offers a homogenous and gradual distribution of coating layers compared to solvent-based methods, which tend to produce coatings with irregular surface topologies [28–30].

VPD has been used to prepare gradients on glass slides and silicon wafers for high throughput studies of adsorption, desorption, and adhesion [18,19], to study the migration of cells and movement of liquid droplets on surfaces [22–26,31], to investigate the elongation and binding of DNA [13,27] and to uniformly modify TLC plates [32], but, to our knowledge, has not been applied to make stationary phase gradients on TLC plates. The size of a typical TLC plate is significantly larger than the average size of the substrate used in prior experiments, which was typically 2–3 cm [18–22,25–28,33–35]. Also, the porous, high surface area silica surface on a TLC plate is much different from that of a planar glass substrate that contains only a few layers of surface silanol groups. Both these differences can bring challenges to gradient fabrication.

In this work, using phenyldimethylchlorosilane (PDCS) as a precursor, we have been able to create phenyl-functionalized cSPGs on silica-coated TLC plates. To characterize the gradient, we employed UV light and diffuse reflectance spectroscopy (DRS). Our findings demonstrate that phenyl gradient stationary phases can easily be formed on TLC plates and the degree of functionalization can be easily modified by adjusting the concentration of the silane precursor and controlling humidity. Additionally, we observed that the cSPGs exhibited improved/enhanced separation performance compared to uniform (unmodified and fully modified) TLC plates, as shown by the successful separation of a mixture containing three aromatic compounds.

2. Experimental

2.1. Chemicals and reagents

Bare silica fluorescent TLC plates (Supelco TLC silica gel 60 F_{254}), paraffin oil (light), hexane, acetaminophen, and aspirin were purchased from Sigma-Aldrich. Bare silica non-fluorescent TLC plates (Baker Si250) were purchased from J.T. Baker. PDCS was acquired from Gelest and used as received, while ethanol was sourced from Decon Lab Inc. Ethyl acetate was purchased from Fisher Scientific and 3-hydroxy-2-naphthoic acid was obtained from Acros Organics.

2.2. Fabrication of continuous stationary phase gradients on TLC plates

Stationary phase gradients on TLC plates were prepared by using the VPD of PDCS. Initially, bare silica TLC plates were cut into pieces of 7.5 $\times$ 2.5 cm^2 and soaked in ethanol to remove any impurities from the surface and then dried in air. Once dried, they were placed in an oven at 40 °C for 5 min, then 120 °C for 60 min to remove excess adsorbed water, and then quickly transferred into an open-ended rectangular chamber. The rectangular chambers are positioned on top of a base frame at an approximate angle of 15 degrees, and the entire assembly is enclosed within a large circular glass chamber. The 15-degree angle was identified through a trial-and-error process and chosen because it provided the best gradient shape. A mixture of paraffin oil and PDCS was carefully placed at the bottom of the rectangular chamber, which allowed the reactive silanes to evaporate and freely diffuse under a controlled atmosphere for 30 min. The TLC plates are positioned in a groove within the rectangular chamber, maintaining a fixed distance from the solution to the edge of the plate. In this process, the bottom of the TLC plate was modified to a greater extent than the top, resulting in the creation of a cSPG. The grooves secure the TLC plates in place inside the rectangular chamber. Photographs of the grooves holding the TLC plate are shown in Figure S1. Depending on the lab humidity, nitrogen or water vapor was introduced into the glass chamber to achieve a relative humidity (RH) of 40 % or 60 %. On the other hand, the lowest humidity condition of RH 10 % was achieved through the use of desiccant (Drierite) placed inside the chamber. Once the desired humidity level was attained, both the inlet and outlet were sealed to maintain a consistent humidity inside the chamber. The concentration of PDCS in paraffin oil was varied as stated below and ranged from 15% to 50 % V/V. A cartoon illustrating the VDP setup for the fabrication of a single stationary phase gradient is shown in Fig. 1 while a photograph of the entire deposition system that allows for three gradients to be prepared at the same time is shown in Figure S1.

2.3. Fabrication of the uniformly modified TLC plate

The preparation of uniformly modified TLC plates involved a two-step process using the VPD method. In the first step, a regular gradient was established by following the procedure described in Section 2.2. In the second step, the TLC plate was reversed, and the procedure was

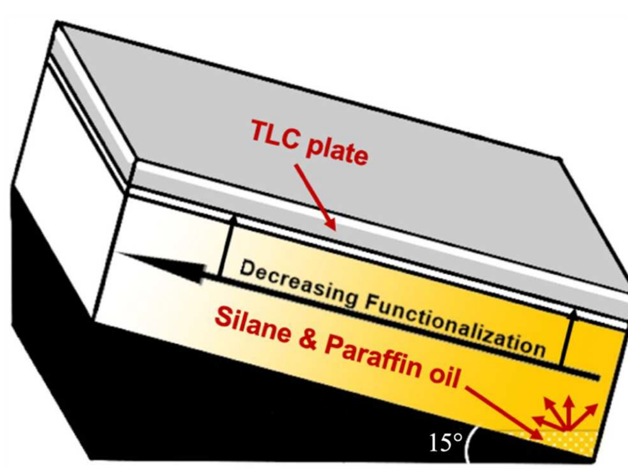

Fig. 1. Cartoon illustrating the VPD setup for the fabrication of a single stationary phase gradient on a TLC plate. A photograph of the entire deposition system is shown in Figure S1.

repeated. This repetition ensured that the modification occurred from a different side of the plate, effectively creating a uniform gradient across the entire plate. Each step of the process had a deposition time of 1 hour for the preparation of the uniformly modified plate. The concentration of PDCS in paraffin oil was 70 % V/V.

2.4. Characterization of continuous stationary phase gradient (cSPG)

2.4.1. UV visualization

Initial evaluation of the distribution of phenyl groups on the gradient and uniform TLC plate was conducted by observing the plates under UV light (254 nm). Following the VPD experiment, the modified TLC plates were positioned under UV light (CAMAG Reprostar II, Camag, Muttenz, CH) for visualization. Subsequently, the resulting image of the gradient plates was captured using a smartphone (iPhone 13 Pro Max).

2.4.2. Diffuse reflectance spectroscopy (DRS)

DRS was used to evaluate the presence and extent of the phenyl group modification on a non-fluorescent TLC plate, where the maximum absorbance (λ_{max}) occurred in the UV region of the spectrum at 263 nm [9] . The Kubelka-Munk (K-M) transformation was used to convert reflectance, R, to K-M units [36].

$$\mathrm{K-M} = (1-\mathrm{R})^2 \Big/ 2\mathrm{R} \tag{1}$$

The DRS spectra were obtained by first collecting a background spectrum from an unmodified non-fluorescent bare silica plate. Each gradient non-fluorescent TLC plate was divided into eight different segments measured at 0.8 cm increments from bottom (closest to the vapor source) to top. Then the DRS spectrum of each segment was taken starting from the bottom and gradually moving to the top. Each segment was examined using a diffuse reflectance spectrophotometer (Agilent Technologies, Cary Series 6000i UV–Vis–NIR).

2.6. Chromatography

A mixture of three different analytes (acetaminophen (A), aspirin (As), and 3-hydroxy-2-naphthoic acid (3H), Fig. 2), was used to evaluate the separation performance of the cSPG. The analytes were dissolved in acetonitrile, each at concentrations of 8 mg/mL, 50 mg/mL, and 10 mg/mL, respectively. The gradient plates were prepared using fluorescent TLC plates with 30 % V/V silane in paraffin oil at 40 % RH and allowed to react for 30 min. The mobile phase was a mixture of 50:50 ethyl acetate and hexane. The development chamber was surrounded by a saturation pad and allowed enough time to equilibrate prior to elution. The chromatographic separation was performed under room humidity. The mixture was spotted ~ 0.5 cm from the edge of the plate. The solvent front for each plate was allowed to reach up to ~6.5 cm. After development, the TLC plates were dried under atmospheric conditions for 5–10 min and placed into an iodine chamber for the visualization of the analytes. The bare silica (unmodified) plate was viewed under UV light. The separation process was performed in triplicate for each trial to facilitate subsequent statistical analysis. The retention factor (Rf) of each compound and the resolution (Rs) between the neighboring peaks were calculated using the following Eqs. (2) and (3) [11,37].

$$Rf\ value = \frac{Distance\ traveled\ by\ compound}{Distance\ traveled\ by\ solvent\ front} \tag{2}$$

$$Rs = \frac{d}{(W_1 + W_2)/2} \tag{3}$$

where d represents the center-to-center distance between two spots and W indicates the base width of the spot.

3. Result and discussion

3.1. Fabrication and characterization of phenyl gradient TLC plates

In this work, we describe a new approach for gradient fabrication on TLC plates that utilizes a VPD method [22,32]. In this method, a mixture of paraffin oil and PDCS is placed at the bottom of an open-ended rectangular chamber. This chamber allows the reactive silanes to evaporate and freely diffuse under a controlled atmosphere in a controlled space, Fig. 1. The volatile silane naturally diffuses from areas of high concentration to low concentration due to random molecular collisions and the presence of the concentration gradient. As a result, the silane vapor diffuses over the TLC plates and reacts with the surface hydroxyl (silanol) groups present on the silica support. The surface of the TLC plate closest to the vapor source experiences the greatest modification compared to the other end, thereby creating a gradient. To ensure the success of this process, the humidity within the glass chamber was maintained at a constant level. This controlled humidity is important as a thin water film is required to facilitate the attachment of the silane to the silica support of the TLC plate [38–40]. The chemistry associated with gradient fabrication is well known and involves the hydrolysis and condensation of vapor phase PDCS with the release of hydrochloric acid and the formation of stable Si-O-Si bonds [41,42].

A simple approach to evaluate the presence and shape of the phenyl gradient on the fluorescent TLC plate was to view the modified plate under UV radiation. The unmodified TLC plate glows green under UV radiation while a uniformly modified TLC plate appears dark. Representative photographs of three different TLC plates under UV radiation are shown in Fig. 3. In Fig. 3A, the green background under UV radiation represents the unmodified bare silica. The bare silica TLC plates contained fluorescent manganese-doped zinc silicate causing the background of the plate to appear green under 254 nm UV radiation. However, when ligands attached to the silica absorb UV light at that wavelength, it prevents the excitation of the fluorescence, resulting in a dark appearance of the plate, Fig. 3C. A gradient plate shows a clear gradient in intensity from green to dark. In Fig. 3B, the darker region at the bottom (closest to the vapor source) signifies the heavily modified side, while the lighter area denotes the less modified end. The gradual

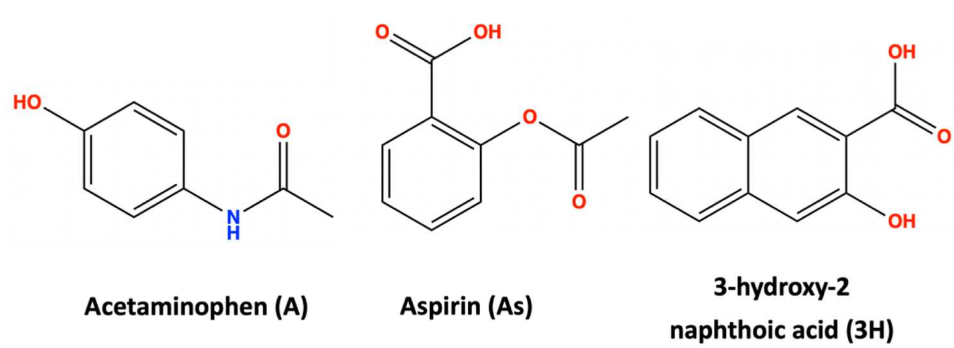

Fig. 2. Structure of the aromatic compounds used to evaluate the chromatographic performance of cSPG TLC plates.

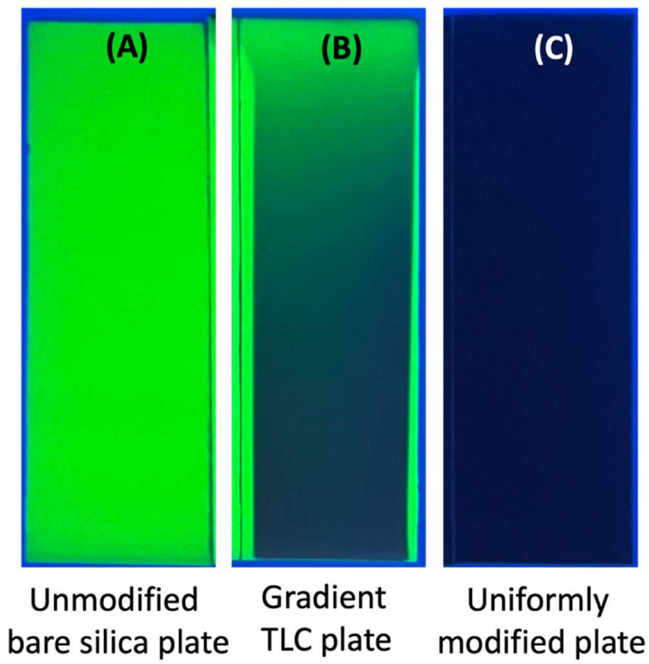

Fig. 3. Photographs acquired under UV radiation of (A) an unmodified bare silica plate, (B) a gradient TLC plate, and (C) a uniformly modified TLC plate; the dark color indicates heavy modification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

color change from dark to light over the length of the plate confirms the formation of the gradient. Additionally, the consistent dark color throughout the length of the plate in Fig. 3C indicates a uniformly modified plate.

To assess the gradient formation more quantitatively and obtain the gradient profile, DRS was used [9]. Fig. 4 shows the gradient TLC plate with the corresponding DRS spectra. Each DRS spectrum was generated by plotting Kubelka-Munk (K-M) intensity against wavelength, as displayed in Fig. 4B. The K-M value, which measures the reflectance and transmittance properties of the sample, was utilized to evaluate the presence and extent of the ligand on the plates. DRS data in Fig. 4B was collected from various distances along the TLC plate, as indicated in Fig. 4A. The DRS spectrum is consistent with prior work [9]. The DRS data reveals that the end of the TLC plate closest to the vapor source has the highest K-M intensity (blue line) and the intensity gradually decreases along the length of the plate (rust orange line), reflecting a decrease in the degree of modification. This gradual variation in K-M intensity with distance indicates the formation of a gradient along the length of the plate. DRS spectra for three gradient plates prepared in the same batch are shown in Figure S2.

To obtain the gradient profile, the K-M intensity at 263 nm was plotted against the distance in centimeters starting from the bottom of the plate. The graphs were constructed using K-M intensity data from three gradient plates produced in the same batch. In Fig. 5A, three lines, represented by blue, green, and red, respectively, depict the K-M intensities at different distances from three plates produced at the same time. The graph exhibits descending lines from left to right, indicating a gradual decrease in K-M intensity across all three plates. At a distance of 0.8 cm from the bottom, the K-M intensity is highest for all plates, and it gradually decreases as it approaches the top at 6.8 cm. This observation suggests a gradual distribution of the phenyl ligand along the length of the plate, with the highest concentration at the bottom (closest to the vapor sources) and the lowest concentration at the top. From the plot, it is observed that the three lines representing the K-M intensities for each plate almost overlap with each other, indicating good within-a-batch reproducibility among the three different plates produced in the same batch.

In Fig. 5B, the average K-M intensities as a function of distance from TLC plates produced in two different batches are shown by the green and red lines. Each line represents the average K-M intensity from three different plates made in the same batch, with the error bar indicating the standard deviation. As seen in Fig. 5B, the batch-to-batch reproducibility is also good, with the two batches exhibiting similar trends and patterns.

3.2. Variation in the gradient profile

Variables that can influence the gradient profile and degree of modification of the TLC plate include both the concentration of the silane and the humidity. In this work, the first variable examined was the concentration of silane in the paraffin oil. Several different gradient TLC plates were prepared by changing the concentration of the silane from 15 % to 50 % V/V. Upon initial examination of these gradient materials

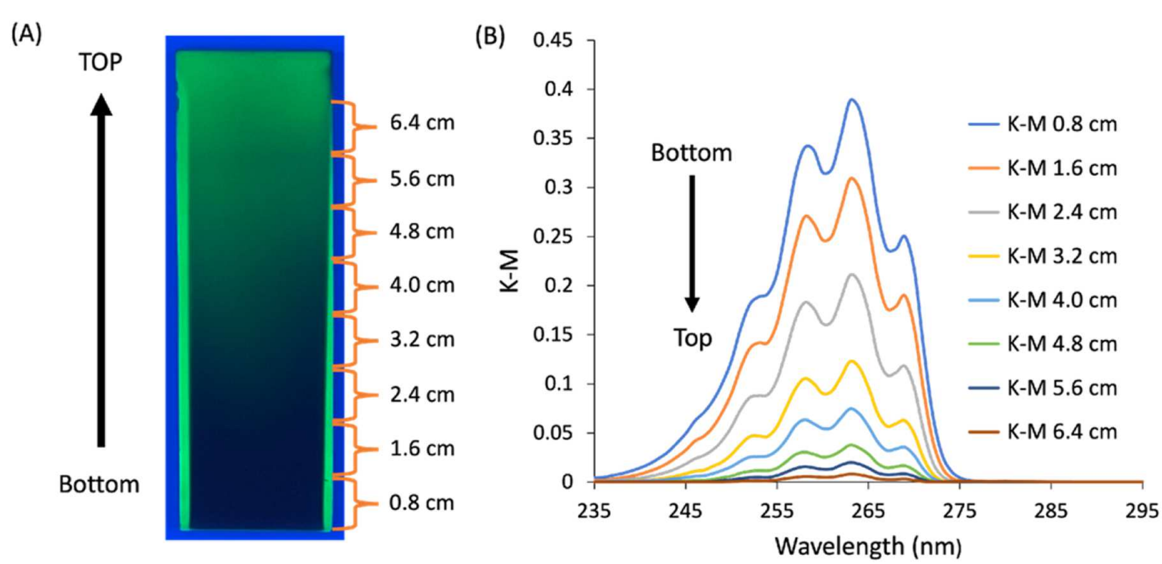

Fig. 4. (A) Photograph of a gradient TLC plate under UV radiation. The plate was divided into eight sections as shown and (B) diffuse reflectance spectra were acquired on each section. The bottom of the plate is closest to the vapor source. Numbers on the legend indicate the position of the segment along the gradient plate. The segment size was 0.8 cm.

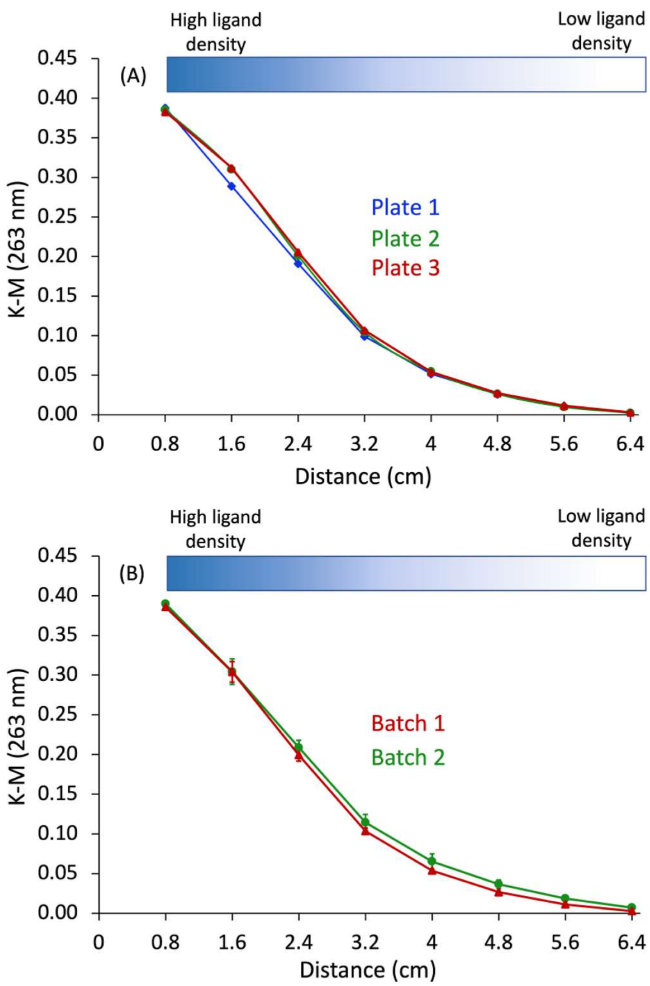

Fig. 5. Gradient profiles collected from diffuse reflectance spectra illustrating the (A) within-a-batch reproducibility for three identical plates modified at the same time (Plate 1, 2, and 3) and (B) batch-to-batch reproducibility for two different batches (Batch 1 and 2). The error bars in panel B indicate the standard deviation of N=3 replicates. All gradient plates were prepared using a 30 % V/V silane concentration at 40 % RH for 30 min. The gradient bar on top of each plot is depicted to guide the eye.

under UV radiation at 254 nm, variable fluorescent intensity (quenching) was noted. The gradient TLC plate prepared from the highest concentration of silane exhibited the lowest fluorescent intensity (darker color) while the TLC plate prepared from the lowest concentration showed the highest fluorescent intensity (lighter color). Photographs of the plates prepared under UV radiation using different concentrations of silane are shown in Figure S4.

To more quantitatively evaluate the gradient profile, DRS was used and the average K-M intensity at 263 nm was used to create the profile plots shown in Fig. 6A. The DRS spectra are shown in Figure S3. A uniformly modified TLC plate prepared from a concentration of 70 % V/V silane exhibits a nearly constant K-M intensity (orange curve) across the length of the plate. For an unmodified bare silica plate, no signal was observed due to the absence of the phenyl functional group on the plate (data not shown). The gradient plates show the K-M intensity decreased from the bottom (closest to the vapor source) to the top for all concentrations of PDCS. As the concentration was changed, a change in K-M intensity was noted. For the 50 % V/V experiment (maroon), the highest K-M intensity was 0.51 ± 0.02 and it gradually decreased to 0.010 ± 0.001. A similar trend was observed at the lowest concentrations (30 and 15 % V/V) with the lowest K-M intensity (purple and indigo).

As can be seen in Fig. 6, the gradient profiles appear to exhibit

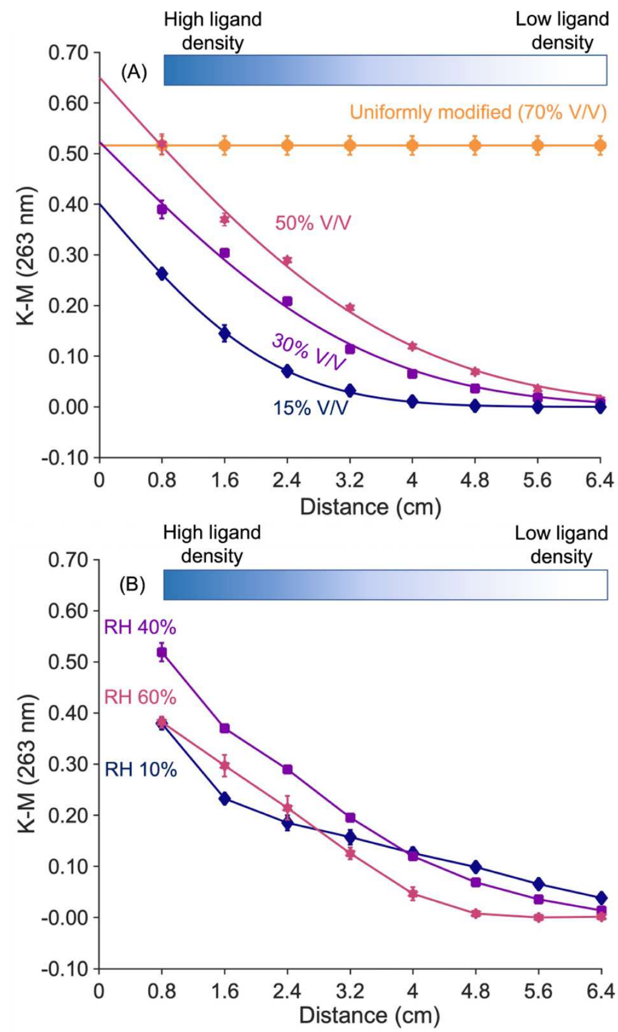

Fig. 6. Effect of different parameters on the shape of the stationary phase gradient. (A) The silane concentration was varied as follows: 50 % V/V (maroon), 30 % V/V (purple), and 15 % V/V (indigo). The concentration for the uniformly modified plate was 70 % V/V (orange). Fitted curves are for a one-dimensional diffusion model as described in the text except for the uniform plate, where the average K-M value was used. (B) The relative humidity was varied as follows: RH 10 % (indigo), RH 40 % (purple), and RH 60 % (maroon). The error bars in the graphs indicate the standard deviation, with N=3 replicates. Line colors in both figures were chosen based on the plasma color map. A gradient bar on top of each plot is presented to guide the eye. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fickian-type diffusion profiles driven by a concentration gradient in the vapor phase. To further verify this observation, the concentration profile data points for 50 %, 30 %, and 15 % concentrations were fitted using a one-dimensional diffusion model based on Eq. 4 as described in reference [43] where a is the relative concentration of the vapor, D is the apparent diffusion coefficient, and t is the time of the experiment (30 min). The fits are shown by the solid lines in Fig. 6 with the exception of that shown for the uniform plate.

$$C(x) = \frac{a}{2} erfc\left(\frac{x}{2\sqrt{Dt}}\right) \quad (4)$$

As can be seen, the fits are good and at these concentrations, the process is controlled by the diffusion of the silane in the vapor phase. The average diffusion coefficient obtained from this fitting is approximately $1.8 \pm 0.8 \times 10^{-3}$ cm^2/s, which is consistent with previously reported values for silanes in the gas phase [14,20].

Humidity is also known to be an important variable for the silanization reaction between the silica surface and the silane precursors [38–40,42]. Previous research has shown that the hydration state of a surface plays a significant role in the surface coverage and the rate of the silanization reaction [38,39]. In addition, it has been demonstrated that in the absence of moisture, the silanization reaction between chlorosilane and silica surfaces does not occur unless elevated temperatures of 300 °C or higher are used [40]. The effect of the humidity on the shape of the cSPG is shown in Fig. 6B. The highest K-M intensities were observed at 40 % RH (indigo), with a slightly lower intensity recorded at 60 % RH (maroon). The general shape of the gradients is similar at these two humidity levels. Gradients formed at 10 % humidity had K-M intensities somewhat between those prepared at 40 and 60 % RH. The shape was a little different as well. It is not clear what the reason is for this behavior other than it's hard to maintain the humidity level during deposition when it approaches 10–20 %.

3.3. Separation of aromatic compounds

A useful application of a phenyl gradient TLC plate would be the separation of compounds with similar functional groups and properties. One such example is a mixture of three aromatic compounds: acetaminophen, aspirin, and 3-hydroxy-2-naphthoic acid, shown in Fig. 2. These compounds possess different functional groups (-OH, -COOH, -NH) in their structures, which have the potential to form hydrogen bonds with the surface silanol groups present on the TLC plate. Also, the aromatic ring in their structures can engage in π-π interactions with the modified gradient phenyl stationary phase [9]. Additionally, the gradient TLC plate offers a unique advantage with its gradual variation of the phenyl group on the surface. This allows for the possibility of neighboring group effects, which is not achievable with uniform plates [8]. As analytes travel through the separation bed, they have the opportunity to encounter diverse chemical environments and thus help to improve selectivity during the separation process.

In this work, the separation performance for these three analytes was investigated using four types of TLC plates: bare silica (unmodified), gradient plates spotted on the high phenyl (GHP) and low phenyl (GLP) end, and uniformly modified plates. On the GHP and GLP plates, the analyte mixture encounters a chemical gradient with either decreasing or increasing surface phenyl concentrations, respectively. On each plate, the mixture was spotted in one spot (M = mixture), while the pure compounds (A, As, 3H) were separately spotted in three spots alongside the mixture for identification purposes. Photographs of the chromatographic separations after exposure to iodine vapor are shown in Fig. 7.

The chromatographic findings, including the retention factors (Rf) and resolution (Rs) between neighboring peaks, are summarized in Tables 1 and 2. On a bare silica (unmodified) plate, compounds A and 3H showed limited mobility from the spotting point (Rf 0.059 ± 0.006 and 0.037 ± 0.002) primarily due to their strong interactions with the silica gel stationary phase. The compound As exhibited slightly higher mobility (Rf 0.092 ± 0.003), suggesting a greater affinity for the mobile phase compared to the stationary phase. Significant tailing was observed on the bare silica plates for the analytes, and the analytes in the mixture were not well resolved. The addition of phenyl groups and the subsequent loss of silanol groups makes the stationary phase more hydrophobic. This surface modification had a profound effect on the retention of As and 3H but a slight effect on the retention of compound A. On the uniformly modified TLC plate, the retention factor for As and 3H significantly increased to 0.52 ± 0.01 and 0.65 ± 0.010, respectively, while compound A only moved slightly, 0.121 ± 0.002.

For the gradient plates, the separation was strongly dependent on whether the solutes were spotted on the low or the highly modified end. Previous research has shown that the spotting end and thus the direction of the gradient on the plate is important, as it influences the separation

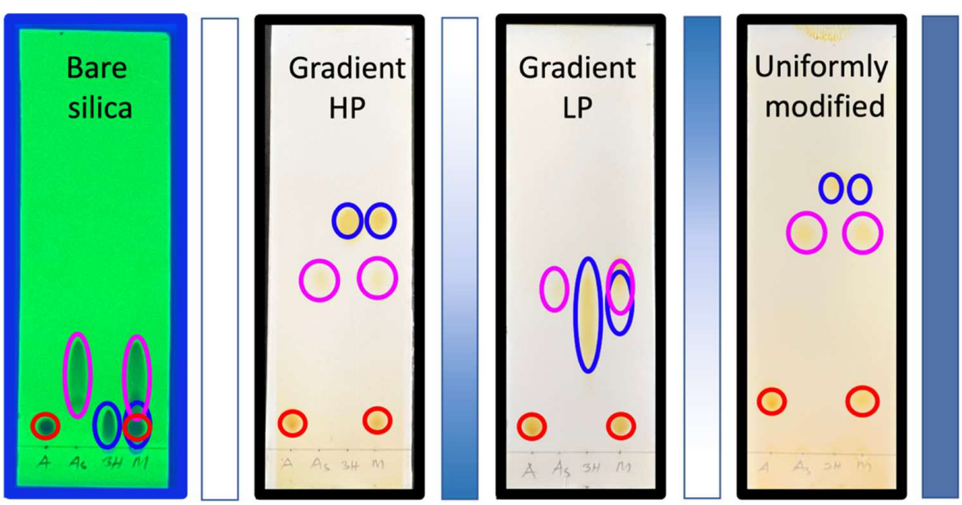

Fig. 7. Photographs of TLC plates used for the separation mixture containing acetaminophen (A), aspirin (As), and 3-hydroxy-2-naphthoic acid (3H) after iodine treatment. The plates are bare silica (unmodified), high phenyl end (GHP) sample application, low phenyl end (GLP) sample application, and uniformly modified. On each plate, individual spots A, As, and 3H represent the single compound, while the spot labeled M represents the mixture of all three compounds. A rectangular bar on the right side of each plate represents the plate (bare, gradient GHP, gradient GLP, and uniform). Mobile phase: 50:50 ethyl acetate:hexane. For the bare silica plate, the analytes are detected/visualized under UV light, while for the gradient and uniformly modified plates, iodine (I_2) chamber is used for detection/visualization. The spots are circled for clarity.

Table 1
Average Rf values along with their corresponding standard deviations (N = 3).

Analytes	Rf bare silica	Rf high end phenyl (GHP)	Rf low end phenyl (GLP)	Rf uniformly modified
Acetaminophen (A)	0.059 ± 0.006	0.060 ± 0.006	0.091 ± 0.003	0.121 ± 0.002
Aspirin (As)	0.092 ± 0.003	0.391 ± 0.006	0.52 ± 0.02	0.52 ± 0.01
3-Hydroxy-2-napthoic acid (3H)	0.037 ± 0.002	0.544 ± 0.006	0.54± 0.02	0.65 ± 0.01

Table 2
Resolution (Rs) value acquired from various TLC plates for three aromatic compounds.

Resolution Rs	Bare silica	High end phenyl (GHP)	Low end phenyl (GLP)	Uniformly modified
$Rs_{A,As}$	1.09 ± 0.05	7.3 ± 0.5	8.9± 0.2	8.6 ± 0.7
$Rs_{A,3H}$	0.19 ± 0.04	10.08 ± 0.4	4.0 ± 0.2	11.3 ± 0.4
$Rs_{As,3H}$	1.7 ± 0.1	2.6 ± 0.1	0.19 ± 09	2.50 ± 0.02

due to different interactions arising from varying degrees of functionalization [9–11]. When the mixture was spotted on the GHP end, a good separation was observed, and less tailing was observed for compounds As and 3H as compared to that observed on GLP and the bare silica plate. All three analytes are well separated and appear as nice round spots. The presence of a high amount of phenyl at the spotting end helps reduce the tailing, which is an added benefit. The incorporation of a significant amount of phenyl groups leads to a decrease in the number of silanol groups in the GHP end. These silanol groups are known for generating strong electrostatic interactions with analytes, similar to what is observed with unmodified bare silica plates, where analytes exhibit minimal movement from the spotting line. Instead of these strong electrostatic interactions, the inclusion of phenyl groups offers comparatively weaker hydrophobic interactions (such as π-π interactions and dipole-dipole interactions) which facilitate less tailing and enhanced analyte migration along the plate. The analytes don't migrate as much as they do on the uniformly modified plate, which is expected. As the analytes start to experience more and more silica as they move up the plate, they start to slow down, and thus the retention factors are not as large as they are on the uniform plate. There is not a very dramatic difference between the separation on the uniform phenyl plate and the GHP gradient plate. This is not surprising, because the separation mainly occurs on the high phenyl region of the plate, as modulated by the ethyl acetate component of the mobile phase. It is likely that mobile phase optimization could show enhanced performance of the GHP separation that could not be achieved on the uniform plate. However, more studies are needed.

In contrast, when the mixture was applied to the GLP plate, a very different separation outcome was observed. Specifically, in contrast to that observed on bare silica, compound As and 3H managed to separate from A. However, compounds As and 3H overlapped with each other during their movement, resulting in a poor separation. Also, tailing was significant for compounds As and 3H on the GLP plate, which is attributed to the high amount of silica functionalities near the spotting point. The separation of these analytes on GLP was better than that observed on bare silica but not as good as that observed on the uniformly modified plate or the GHP plate.

Overall, the separation was excellent on the gradient plate spotted on the high phenyl end. The spots are round with little to no tailing and they move about halfway up the plate and provide complete separation with better resolution compared to the other plates. This result highlights the versatility of gradient TLC plates in customizing separations to meet specific objectives.

4. Conclusion

A VPD approach for the preparation of cSPGs on TLC plates was developed. It involves a simple experimental procedure where a TLC plate was exposed to silane vapor, generated within a rectangular chamber under a controlled atmosphere. The resulting gradient surfaces were analyzed using UV visualization and diffuse reflectance spectroscopy (DRS). The shape of the gradient surfaces was dependent on factors such as silane concentration, experimental duration, and humidity. Comparative chromatographic analyses between unmodified, fully modified, and cSPG plates showed that cSPG plates displayed improved separation performance, highlighting their potential as a new tool for advancing chromatographic separations. While this work has focused on fabricating continuous stationary phase gradients using vapor phase deposition of PDCS, this procedure can be extended to other organosilanes and diverse analyte mixtures. In this study, we employed small molecules with an isocratic mobile phase to assess the performance of cSPG plates. Our future investigations will involve coupling cSPG plates with a gradient mobile phase to evaluate their effectiveness in separating larger molecules, such as proteins.

CRediT authorship contribution statement

Md Abdullah Al Macktuf: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Sarah C. Rutan:** Writing – review & editing, Supervision. **Judith Bautista:** Investigation, Conceptualization. **Maryanne M. Collinson:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We express our sincere gratitude for the support provided by the National Science Foundation (NSF) under grants CHE-1609449 and CHE-2305102, which significantly contributed to the success of this research endeavor. Special thanks to Dr. Indika U. Arachchige and his lab for providing access to the diffuse reflectance spectroscopy (DRS) instruments, essential for the characterization aspects of our study. We would like to acknowledge Dr. Rajib Sarkar for his valuable assistance with DRS instrument and Hannah Klose and Al-Noor Ikram for initial studies. We would also like to express our appreciation to Thomas Cecil, Alan Branigan, and John Arnold for their valuable suggestions and assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2024.465090.

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