Separation of Transition and Heavy Metals Using Stationary Phase Gradients and Thin Layer Chromatography

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Abstract

Stationary phase gradients for chelation thin layer chromatography (TLC) have been investigated as a tool to separate a mixture of metal ions. The gradient stationary phases were prepared using controlled rate infusion (CRI) from precursors containing mono-, bi-, and tri-dentate ligands, specifically 3-aminopropyltriethoxysilane, N-[3-(trimethoxysilyl)propyl] ethylenediamine, and N-[3-(trimethoxysilyl)propyl] diethylenetriamine. The presence and the extent of gradient formation were confirmed using N1s X-ray photoelectron spectroscopy (XPS). XPS results showed that the degree of modification was dependent on the aminosilane precursor, its concentration, and the rate of infusion. The separation of four transition and heavy metals (Co^{2+} , Pb^{2+} , Cu^{2+} , and Fe^{3+}) on gradient and uniformly modified plates was compared using a mobile phase containing a stronger chelating agent, ethylenediaminetetraacetic acid (EDTA). The retention of the metal ions was manipulated by varying the surface concentration of the chelating ligands. The order of retention on unmodified plates and on plates modified with a monodentate ligand was $\text{Fe}^{3+} > \text{Cu}^{2+} \sim \text{Pb}^{2+} \sim \text{Co}^{2+}$, while the order of retention on plates modified with bi- and tri-dentate ligands was $\text{Fe}^{3+} > \text{Cu}^{2+} > \text{Pb}^{2+} \sim \text{Co}^{2+}$. Fe^{3+} and Cu^{2+} were much more sensitive to the concentration of chelating ligand on the surface (displaying lower R_f values with increasing ligand concentration) than Pb^{2+} and Co^{2+} . Complete separation was achieved using a high concentration of the tridentate ligand coupled with a longer time for modification, yielding a retention order of $\text{Fe}^{3+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Pb}^{2+}$.

Keywords: Chelation ion chromatography, planar chromatography, gradients, aminosilanes, organoalkoxysilanes, sol-gel

Introduction

Gradient surfaces[1-3] are potentially useful in separation science because they provide a means to vary the concentration of functional groups along the length of the surface and influence retention and the separation of complex mixtures.[4-9] In general, two approaches have been used to make gradient surfaces for chromatography. The first involves serially connecting columns each containing a different stationary phase[6-9] and the second involves gradually changing the functional group(s) on a single column.[4, 5] The former are examples of discontinuous gradients, while the latter are examples of continuous gradients. Retention factors and thus separation can be manipulated by changing the steepness of the gradient, the functional group(s), and in the case of planar chromatography, the direction of the gradient(s).[4, 5] An advantage that a continuous stationary phase gradient, as opposed to discontinuous one, has is that it provides an avenue for synergistic (cooperative) interactions between neighboring functional groups in the stationary phase to take place.

Recently, we developed a relatively simple and inexpensive method to create a continuous gradient on surfaces suitable for chromatography known as controlled-rate infusion (CRI).[10] In this method, a silica-coated substrate, e.g., a thin layer chromatography (TLC) plate, is exposed to functionalized organoalkoxysilane precursors in a time-dependent fashion; condensation takes place between the hydrolyzed precursors and surface silanol groups forming a modified silica surface whose degree of functionality changes in a predefined fashion from one end to the other.[10] Aminoalkoxysilanes are particularly useful in CRI because they react quickly with surface silanol groups due to the self-catalyzing ability of the amine group.[11] In recent work, we prepared single-component gradients from 3-aminopropyltriethoxysilane to separate a mixture of four weak acids and bases, as well as three commonly used over-the-

counter drugs.[4] Building on this work, aligned and opposed multi-component gradients were prepared from 3-aminopropyltriethoxysilane and phenyltrimethoxysilane and used to separate a mixture of water- and fat-soluble vitamins.[5]

The focus of the present study is on the development of gradient stationary phases suitable for metal ion separation. The separation and quantification of transition and heavy metals, particularly those that are toxic, is of utmost importance. Many separations utilize silica gel supports and focus on manipulating the composition of the mobile phase to improve the separation, which is not always practical.[12-14] Others have modified the inorganic support with chelating ligands[15-23] and/or utilized chelation ion chromatography (CIC) to separate and quantify mixtures containing transition and heavy metals.[24-29] CIC relies on the competition between an immobilized chelating ligand, such as iminodiacetic acid, a β -diketone, or an amine, in the stationary phase and another chelating agent, such as picolinic or dipicolinic acid or trifluoroacetylacetone, in the mobile phase.[24, 25] The principles of CIC have also been applied to TLC.[15-18] Using silica gel TLC plates modified with amines or β -diketones and a mobile phase such as salicylaldehyde or trifluoroacetylacetone in acetone, mixtures of four to six metal ions have been separated.[16, 18]

In this work, we couple gradient stationary phase technology with CIC as a means to control the retention of metal ions and thus influence their separation. Specifically, gradient stationary phases were prepared with either 3-aminopropyltriethoxysilane, N-[3-(trimethoxysilyl)propyl] ethylenediamine, or N-[3-(trimethoxysilyl)propyl] diethylenetriamine using CRI on a TLC plate. The surface density of the ligands on the stationary phase and hence the strength of the chelation, changes along the length of the support. By using ethylenediaminetetraacetic acid (EDTA) in the mobile phase, a competition is created between

the chelating ligands on the stationary phase and a stronger chelating agent in the mobile phase. We demonstrate how changing the aminoalkoxysilane concentration, infusion time, or the number of amine groups can be used to affect the retention factor (R_f) of the selected metal ions and influence separation. We also show how the retention can be changed depending on whether or not a stationary phased gradient is used.

Experimental Section

Reagents: N-[3-(trimethoxysilyl)propyl] ethylenediamine (97%, referred to as diamine) and N-[3-(trimethoxysilyl)propyl] diethylenetriamine (97%, referred to as triamine) were purchased from Acros Organics. 3-Aminopropyltriethoxysilane (98%, referred to as amine) and isobutyltrimethoxysilane (97%, referred to as isobutyl) were obtained from Alfa Aesar. Cobalt (II) acetate, lead (II) acetate, and copper (II) acetate were obtained from Fisher Scientific; iron (III) perchlorate was obtained from GFS Chemicals. Ethylenediaminetetraacetic acid (EDTA), disodium salt, was purchased from Fisher Scientific. Ethanol (200 proof, USP/ACS grade) was obtained from Pharmaco-Aaper. The TLC plates used in this work were silica gel 60 F₂₅₄ from Sigma Aldrich; the plates were 20 x 20 cm with a 250 μ m layer thickness.

Preparation of stationary phase: The 20 x 20 cm TLC plates were cut into pieces of the approximate size of 8 cm x ~1.7 cm using a glass cutter, cleaned by soaking in ethanol for ~5 min, and dried by heating in an oven (~160° C) for 30 min. The plates were then cooled to room temperature before use. Plates used were (1) as-received (no modification), (2) uniformly modified, or as (3) gradient plates prepared using CRI. Before modification, the plates were soaked in an ethanol solution for ~10 min. Both uniformly modified and gradient plates were exposed to a triamine, diamine, or amine solution prepared from 20 : x : 0.004 v/v/v of ethanol: aminosilane: DI H₂O, where x is 0.08, 0.12, or 0.50. The uniformly modified plates were simply

soaked in an aminosilane solution for a set period of time. For the gradient plates, the plates were placed upright in a graduated cylinder, and the aminosilane solution was infused into the graduated cylinder using a syringe pump (Harvard Apparatus PHD 2000 Infusion) at a set rate over a given period of time. The exact parameters required for gradient deposition are determined by the kinetics of precursor reaction with the surface, as has been previously explored in detail for aminosilane gradients.[10, 30] The volumes of aminosilane, infusion rate, and infusion time employed in the present studies are given in **Table 1**. All of the plates were rinsed thoroughly with ethanol after being removed from the aminosilane solution.

Table 1. Aminosilanes, Volumes, Infusion Rates, and Infusion Times for Gradient Preparation.

Aminosilane	Volume of Aminosilane (mL)	Infusion Rate (mL/min)	Infusion Time (min)
Triamine	0.08	1.0	15
	0.12	1.0	
	0.50	3.0	5
		1.0	15
		0.5	30
Diamine	0.08	1.0	15
	0.12		
	0.50		
Amine	0.08	1.0	15

Reduction in residual silanol groups: To reduce the number of residual silanol groups on the TLC plates and thus the streaking of metal ions, the plates were exposed to an isobutyl solution (20 : 0.25 : 0.50 : 0.50 v/v/v/v ethanol : isobutyltrimethoxysilane: 0.02 M HCl : 0.01 M KOH) for 12 min after modification with triamine, diamine, or amine. To prepare the isobutyl solution, the ethanol, isobutyltrimethoxysilane, and 0.02 M HCl were stirred for 30 min to hydrolyze the isobutyl. Then, 0.01 M KOH was added, and the solution was stirred for an additional 30 min.

This two-step process[31, 32] afforded efficient hydrolysis (acid) and condensation (base) of the precursors. After soaking, the plates were air dried and placed in the oven at 160° C for 1 hr.

Separation of metal ions: The metal salts ($\text{Co}(\text{C}_2\text{H}_3\text{O}_2)_2$, $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$, $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$, and $\text{Fe}(\text{ClO}_4)_2$) were dissolved in 0.1 M HCl at a concentration of 5 mg/ml. A mixture of the four metal ions was prepared by dissolving 5 mg of each of the metal salts in 1 ml of 0.1 M HCl. The metal ion solutions and the mixture were spotted on unmodified, uniformly modified, and gradient plates 1 cm above the bottom of the plate. The gradient plates were spotted on either the low amine end (so that they faced an increasing gradient, termed GPLA) or the high amine end (so they faced a decreasing gradient, termed GPHA). After spotting, the plates were allowed to dry for ~5 min before being placed in a pre-saturated mobile phase chamber. The mobile phase was an EDTA solution prepared using 0.1 M EDTA/0.1 M K_2HPO_4 (pH $\approx$ 6.5) : acetone (50 : 50 v/v). The solvent front was allowed to reach 1 cm from the top of the plate. Once the plates were completely dry they were visualized under UV light at 254 nm, and the distance traveled by each metal ion was measured. Photographs of the plates were obtained using a digital camera, examples of which are shown in **Figure 1** for diamine and triamine. As discussed in more detail in the discussion section, streaking is more pronounced for the diamine modified TLC plates compared to triamine.

The retention factor (R_f) for each metal ion was calculated using Eq. 1,

$$R_f = \frac{\text{distance traveled by analyte}}{\text{distance traveled by the solvent front}} \quad (1)$$

Since metal ions tend to streak, the R_f values were calculated from the center of the streak. Standard deviations were calculated using R_f values from three plates. Two of the plates were prepared on the same day, and a third plate was prepared the next day to evaluate reproducibility.

Characterization of the TLC plates using XPS: The gradient profiles of the modified plates were characterized using X-ray photoelectron spectroscopy (XPS). The plate was cut into ten pieces, 0.8 cm in length. The silica powder was scraped from every other piece and analyzed using a ThermoFisher ESCALab 250 imaging X-ray photoelectron spectrometer. For uniformly modified plates, each sample was scanned once. For gradient plates, each sample was scanned at three points and the average value and standard deviation were reported. The binding energies were corrected using the C1s peak as 284.6 eV. Quantification of the N1s peak area was done by commercially available software (Avantage Version 4.4). For ease of comparison, the area under the N1s peak was divided by 2 and 3 for diamine and triamine, respectively.

Results

The separation of four transition and heavy metal ions, Co^{2+} , Pb^{2+} , Cu^{2+} , and Fe^{3+} , was evaluated on unmodified TLC plates, uniformly modified TLC plates, and gradient plates. On an unmodified TLC plate, three of the four metal ions (Co^{2+} , Pb^{2+} , Cu^{2+}) were not significantly retained ($R_f \sim 0.80$) and did not separate. Fe^{3+} was retained ($R_f \sim 0.47$) and separated from the other three metal ions, indicating that it has some affinity for the silica surface, whereas the other metal ions do not. For the gradient plates, the separation was strongly dependent on the spotting end (GPHA vs. GPLA) and the extent at which the plate was modified, which is influenced by the concentration of amine in solution, the infusion time, and the type of chelator. Each of these variables is described in the sections that follow with comparisons made to the uniformly modified plates.

Chelator. The coordination ability of the surface immobilized ligands with the metal ions will be dependent on the number of donor atoms, their orientation on the surface, and their

surface density.[33] To more closely examine how the chelator influences separation, gradients were made from three different aminoalkoxysilanes containing one, two, or three donor atoms. The presence of a gradient and how the surface density of chelating groups changes with distance was evaluated by XPS. **Figure 2** shows representative N1s XPS spectra and corresponding plots of N1s peak area as a function of distance for materials made from amine, diamine, and triamine. Consistent with prior work,[33] the binding energy for the N 1s peak is 399.8 ± 0.1 eV and a small shoulder due to protonated and H-bonded amines can be seen at 401.9 ± 0.1 eV. The uniformly modified substrates show a near constant amount of nitrogen on the surface with distance, while the gradient samples show a gradual increase in N from top to bottom for each chelator. The surface coverage is slightly higher for the materials prepared from amine and is lowest for the triamine, consistent with steric factors as we have noted in prior work.[30, 33]

The effect of the number of N donor atoms on the separation of the metal ions can be seen in **Figure 3**, which shows the R_f values for the four different metal ions on the different TLC plates prepared with $x = 0.08$ chelator. What can be immediately seen is that the retention factors depend on the number of donor atoms in the chelator, with greater retention noted on the triamine modified plates. This result is not unexpected, as multi-dentate ligands such as diamine and triamine will form more stable complexes with a metal ion compared to a monodentate ligand. Such results were also observed in metal uptake studies.[34] Previous detailed XPS undertaken on similar Cu^{2+} -diamine and Cu^{2+} -triamine chelation gradients prepared on modified silicon wafers revealed a N/Cu ratio of 9-11 for the diamine gradient and 4 for coordination to a triamine gradient.[33] Both mono- and bis-complexes were noted with ratios estimated to be 70:20 for diamine and 60:30 for triamine. For 1:1 complexes, the other two sites were filled with

oxygen from neighboring silanol or Si-O-Si groups.[33] The tridentate ligand provides more nitrogen atoms to chelate copper and thus yields a more stable coordination complex.[33]

Of the four metal ions, Cu^{2+} and Fe^{3+} were the most sensitive to the surface bound chelator. Co^{2+} and Pb^{2+} were less affected by the surface bound chelator, as their R_f values only decrease slightly when the number of amine groups increase from two to three. On the amine modified plate, three of the four metal ions were barely retained indicative of weak bonding and coordination of this monodentate ligand to the metal ions. On the other hand, the bi-and/or tri-coordinate ligands were able to retain the metal ions to a varying degree as described in more detail in later sections. A strong chelating ligand is thus required on the stationary phase to retain the metal ions and produce a separation.

Variation in the Triamine Concentration. To evaluate the effect of triamine concentration on the separation of the four metal ions, the volume of triamine was increased in the deposition solution from 0.08 to 0.12 to 0.50 ml. The N1s profiles of the uniformly modified and gradient plates obtained from XPS spectra are shown in **Figure 4**. Predictably, the area under the N 1s peak increases as the concentration of triamine in the deposition solution increases. The gradient profiles increase steadily from the low amine (~1.2 cm) end of the plate to the high amine (~7.2 cm) end, confirming the presence of a gradient.

Figure 5 shows the effect of increasing triamine concentration on the R_f values of Co^{2+} , Pb^{2+} , Cu^{2+} , and Fe^{3+} spotted on uniformly modified and gradient plates. The R_f factors of Co^{2+} and Pb^{2+} on the uniformly modified plate ($R_f = 0.71 \pm 0.07$) are similar to those obtained on an unmodified plate (0.82 ± 0.04), while for Cu^{2+} and Fe^{3+} , the retention increases. For Cu^{2+} , the R_f decreases from 0.82 ± 0.04 on an unmodified TLC plate to 0.39 ± 0.03 on the 0.08 triamine modified plate. For Fe^{3+} , the R_f decreases from 0.47 ± 0.03 to 0.16 ± 0.04 on unmodified and x

= 0.08 uniformly modified TLC plates, respectively. The decrease in R_f values indicates that the Cu^{2+} and Fe^{3+} have a much higher affinity for surface bound triamine than Co^{2+} and Pb^{2+} . With the exception of Pb^{2+} , retention of all the metal ions can be increased by increasing the surface concentration of triamine. As the concentration of triamine increases, a greater density of N groups is available to form a strong chelate with the metal ion, thus increasing retention. Of all the metal ions studied, Fe^{3+} is most sensitive to the surface bound triamine as evident by a decrease in the R_f factor by almost three as the concentration in deposition solution increases from $x = 0.08$ to 0.12 , while Pb^{2+} is the least sensitive.

On GPHA, the separation of the metal ions is almost identical to that seen on the uniformly modified plates, but very different than on GPLA, indicating the importance of the spotting end. The amount of triamine on the high amine end of GPHA and on the uniformly modified plates are very similar (**Figure 4**). The metal ions are thus strongly chelated to the surface near the spotting end. However, on GPLA, the spotting end contains a much lower amount of bound chelator ($\sim 1/3$ less as seen in **Figure 4**). As a result, the metal ions are able to travel up the plate before being retained to a significant degree by the increasing concentration of the bound chelator and their R_f factors are larger on GPLA compared to the uniform substrate or GPHA. This observation is most obvious for Fe^{3+} , which is strongly affected by the amount of triamine on the surface. It is also interesting that when the metal ions are spotted on the low amine end (GPLA), the surface concentration of triamine is not as important. The R_f factors for each ion are similar even though the amount of surface bound triamine differs. For GPHA and the uniformly modified substrate, however, the R_f of Fe^{3+} decreases significantly as the amount of triamine on the surface increases. With the addition of triamine to the stationary phase, Cu^{2+} ,

as well as Fe^{3+} , are now able to be separated from the mixture. Although Co^{2+} has a lower R_f than Pb^{2+} on the $x = 0.50$ triamine plates, some overlap was observed due to the streaking.

Variation in the Infusion Time. The rate of infusion is another variable that can be changed to alter the degree of modification, and hence the retention factors and separation of the metal ions on the TLC plate. A lower degree of modification takes place when the infusion time is fast. In this work, the infusion times were changed from 5 to 15 to 30 min, keeping the triamine concentration constant at 20 : 0.50 : 0.004 v/v/v ethanol : triamine : DI H_2O . **Figure 6** shows the profiles of the 5, 15, and 30 min uniformly modified and gradient plates as evaluated by XPS. As expected, the degree of modification increases with an increase in the exposure time of the TLC plate to the amine. The gradient profiles are very similar with a slightly greater amount of amine on the surface during a 30 min infusion time vs. a 5 min infusion time.

As can be seen in **Figure 7**, the trends in R_f values are very similar to those seen with changing triamine concentration. Co^{2+} , Cu^{2+} , and Fe^{3+} are sensitive to the amount of triamine on the TLC plate. As the time of infusion increases (degree of modification increases), R_f factors decrease. Pb^{2+} is least sensitive to modification, consistent with the data reported in **Figure 5** for increasing triamine concentration, while Fe^{3+} barely moves off of the baseline, even when the infusion time was only 5 min. Fe^{3+} has a high affinity for the stationary phase (triamine + surface silanol groups). Once again, the separation on the uniformly modified plates is similar to GPHA, but very different from GPLA. Clearly, spotting end is very important and influences retention factors; again most notably those of Fe^{3+} . On GPLA, Fe^{3+} moves up the plate where it does not to any significant extent on either the uniform plate or GPHA because of the initial presence of a relatively high amount of triamine near the spotting end.

Co²⁺ and Pb²⁺ were previously inseparable with a 15 min infusion time; however, with a 30 min infusion time, all four metal ions were separated on the uniformly modified plates and on both gradient plates, as can be seen in the photograph of the TLC plate in **Figure 8**. While the metal ions are separated on a uniformly modified plate and on GPHA, Fe³⁺ does not move from the baseline. On GPLA, the four metal ions are still separated, and Fe³⁺ moves up the plate, providing the advantage that these four metal ions could be separated from contaminants or other metal ions that do not move from the baseline.

Varying Diamine Concentration. Similar experiments were performed with diamine where the volume of diamine in the deposition solution (x) was changed from 0.08 to 0.12 to 0.50 ml. **Figure 9** shows the profiles for uniformly modified and gradient plates. As expected, the amount of nitrogen on the surfaces increased with increasing concentration and with increasing exposure time (from the low end to the high end of the gradient). However, compared to the triamine profiles, the N 1s peak areas were slightly higher for diamine. This was noted in previous papers and attributed to steric hindrance in the case of triamine, leading to a lower surface concentration of triamine.[30, 33]

When the metal ions were spotted on the modified plates, streaking was more pronounced with diamine modified plates vs. the triamine modified plates, which can be seen by comparing **Figure 1a** with **Figure 1b**. The streaking is believed to be due to the interaction with surface silanol groups. The addition of an organoalkoxysilane with terminal -CH₃ groups (in this case, isobutyltrimethoxysilane) helps reduce the streaking by removing some of the excess surface silanol groups. It can also diminish the fluorescence of the TLC plate as noted in the darkening of the TLC plates shown in **Figure 8**. Capping all (or most) of the surface silanol groups is not desirable, however, as this would make the plate too hydrophobic and also eliminate remaining

coordination sites being filled by surface silanols. Streaking is one of the disadvantages of modifying the TLC plates with diamine, particularly at the low concentrations. Since diamine only has two N atoms per molecule (as opposed to three in triamine), coordination will be weaker, thus metal ion interactions with surface silanols will likely increase.

R_f values for the different metal ions and different plates are depicted in **Figure 10**. As observed for the triamine modified plates, the separation on GPHA and uniform plates are very similar to each other. For Co^{2+} and Pb^{2+} , there is a slight dependence of R_f with concentration whereas Cu^{2+} and Fe^{3+} are more sensitive to amount of modifier on the surface. Fe^{3+} is significantly less retained on the diamine modified plates compared to the triamine modified plates. Again, triamine is a stronger chelator than diamine. Due to the increased streaking, Co^{2+} , Pb^{2+} , Cu^{2+} are not separable at low concentrations of diamine. While the streaking is reduced at higher concentrations of diamine, Co^{2+} and Pb^{2+} are still inseparable.

Discussion

Many factors can impact the separation of metal ions on functionalized silica, including sorption, electrostatic, and chelation effects.[35] Retention and separation will be influenced by the extent and type of modification of the stationary phase as well as the properties of the mobile phase, most notably pH. At pH ~ 6.5 , the majority of the surface bound amine groups will be protonated, many of the silanol groups will be deprotonated and negatively charged, and EDTA will be negatively charged. Further complications can arise because the TLC plate is not a fully equilibrated system and because differential solvent evaporation from the mixed mobile phase is possible.[36] The difference in the retention and separation of the different metal ions can be explained by their affinities with surface immobilized functionalities and the mobile phase. The metal ions will be displaced in an order consistent with their affinity for the stationary phase

relative to the mobile phase.[23] In this work, the pH of the mobile phase was kept constant at pH 6.5 so that electrostatic interactions will be similar for the different stationary phases while the type (mono-, bi-, or tri-dentate ligands) and concentration of the immobilized chelator was varied to more closely study the chelation effect. Interactions between the metal ions and the silica surface will also be relatively weak compared to chelating effects. [37, 38]

When metal ions are spotted on an amine-modified silica surface with a relatively low density of chelator, they likely form 1:1 and 1:2 metal-ligand complexes with remaining sites filled with oxygen from neighboring silanol and/or Si-O-Si groups.[33] As the surface density of chelating groups changes along the length of the gradient surface, the extent and thus the strength of chelation will inevitably change as we have previously shown in detailed XPS studies.[33] The formation constants are well known for the $M-L_x$ ($L = \text{EDTA, Triamine, Diamine}$) complexes in solution but significantly less known for metals coordinated in various geometries with surface-immobilized ligands.[35]

Fe^{3+} is easily separated from the other three metal ions, even on an unmodified TLC plate. When even a small concentration of a bi- or tri- dentate ligand is added to the surface, the R_f of Fe^{3+} decreases noticeably (going from ~ 0.5 on an unmodified plate to ~ 0.2 or lower on plates modified with a bi- or tri-dentate ligand). These results can be understood by considering Pearson's Hard-Soft Acid-Base (HSAB) theory.[39] Fe^{3+} is considered to be a hard acid while nitrogen and oxygen are hard bases.[40] On an unmodified silica TLC plate, Fe^{3+} strongly interacts with the surface and is retained to a much greater extent than the other metal ions in this study. Furthermore, on the gradient plates that contain both amine and silanol groups, it is very difficult to displace Fe^{3+} since it is tightly bound to the surface.

Co²⁺, Pb²⁺, and Cu²⁺ are considered borderline (not hard or soft) acids in Pearson's HSAB theory,[40] explaining why they are not retained as heavily as Fe³⁺. As a first approximation, differences in their retention can be explained by comparing formation constants (K_f) of solution species (M-EDTA; M-L_x where x = 1),[41-45] **Table 2**. The ratio, K, of their affinity for the stationary phase compared to the mobile phase (EDTA) is given by Eq. 2,

$$K = \frac{K_f (\text{aminosilane})}{K_f' (\text{EDTA})} \quad (2)$$

where K_f (aminosilane) is the formation constant for diamine or triamine and K_f' (EDTA) is the conditional formation constant for the EDTA complex at pH 6.5 ($\alpha = 9.45 \times 10^{-5}$).[45] A higher ratio means the metal ion has a high affinity for the stationary phase compared to the mobile phase; therefore, the R_f for the metal ion will be lower. Even though the values listed in **Table 2** were determined in solution under equilibrium conditions, the order of retention predicted by the values of K is consistent with our experimental results. Clearly, Cu²⁺ has a higher affinity for both diamine and triamine than Co²⁺ and Pb²⁺, which have similar values. It can also be seen that all three metal ions have a higher affinity for triamine than for diamine, which is also consistent with the experimental results (lower R_f values on triamine plates compared to diamine plates.)

Table 2. Formation Constants and Ratio (K) of Affinities for Diamine, Triamine and EDTA with Co²⁺, Pb²⁺, and Cu²⁺.

Metal Ion	Log K _f (Diamine)[43, 44]	Log K _f (Triamine)[43, 44]	Log K _f (EDTA)[45]	Ratio K (Diamine)	Ratio K (Triamine)
Co ²⁺	5.60	8.0	16.45	1.5 x 10 ⁻⁷	3.7 x 10 ⁻⁵
Pb ²⁺	7.00	8.5	18.0	1.1 x 10 ⁻⁷	3.3 x 10 ⁻⁶
Cu ²⁺	10.54	15.9	18.78	6.1 x 10 ⁻⁵	13.9

On an unmodified surface and on a surface modified with a monodentate ligand, the order of retention (affinity for the stationary phase) is $\text{Fe}^{3+} > \text{Cu}^{2+} > \text{Pb}^{2+} \sim \text{Co}^{2+}$. Given that Cu^{2+} and Fe^{3+} have higher affinities for the stationary phase, it is not surprising that their R_f values are influenced by the concentration of aminosilane. Conversely, Co^{2+} and Pb^{2+} have little affinity for the aminosilane stationary phase, and, thus, the concentration of aminosilane has less of an effect on their R_f values. However, the data in **Figure 5, 7, and 10** show that Co^{2+} is more affected by the change in aminosilane concentration on the surface than Pb^{2+} . This difference was used to separate Co^{2+} and Pb^{2+} by increasing the infusion time to 30 min, yielding a retention order of $\text{Fe}^{3+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Pb}^{2+}$. Similar results have been found in studies of metal ion uptake on silica gels supports modified with an amine or hydroxy groups.[34, 46-49] The results of this and other studies indicate that Cu^{2+} and Fe^{3+} are ideal for separation studies since they bind strongly to chelating ligands, while Co^{2+} and Pb^{2+} are more difficult to separate. However, with careful manipulation of the ligand concentration on the surface, separation of all four metal ions can be achieved.

Conclusion

This work utilizes the concepts of chemical gradients and chelation ion chromatography to affect the separation of a mixture of four transition and heavy metal ions (Co^{2+} , Pb^{2+} , Cu^{2+} , and Fe^{3+}). The metal ion separation was studied on unmodified TLC plates and on uniformly modified and gradient plates prepared from three different aminosilanes, three different concentrations of triamine and diamine, and three different infusion times. Separation was achieved by creating a competition between the surface immobilized chelating agents (aminosilanes) and a stronger chelating agent (EDTA) in the mobile phase. Variation in the surface density of amine groups on the stationary phase via changes in the aminosilane

concentration or the infusion time produced small albeit useful changes in the R_f values of the metal ions. The addition of aminosilane with two amine groups was found to be sufficient for separating Cu^{2+} and Fe^{3+} from the mixture, however, a higher concentration of triamine (0.50) and a longer infusion time (30 min) was required for complete separation of all four metals. While separation can be achieved on uniformly modified TLC plates, gradient plates spotted on the low amine end provide the advantage that all of the metal ions move off of the baseline. Future work will focus on separating a greater number of metal ions using two-dimensional gradient TLC.

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505

Figure 1

0.12 Triamine

0.12 Diamine

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10