Functionalization of Silica Gel with a Chiral Moiety

By

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Abstract

The proposed research explores the structural differences of the chiral centers between two enantiomers and how they can be used to accomplish a successful separation. The methods for separating chiral mixtures represent a great importance for drug design or special material synthesis. The non-essential amino acid L-carnitine carries out a remarkable function in the human body, the most understood function being the transport of fatty acids in the mitochondria for oxidation[1]. L-Carnitine has one chiral center and can only be used by mammals in the L form. The method used for the separation of a racemic mixture of D/L carnitine was directly modifying the silica gel (SIG). The modified silica gel can be applied on a thin layer chromatography (TLC) plate or as a packing material for a high-performance liquid chromatography (HPLC) column. A TLC plate is a sheet of glass coated with a thin layer of adsorbent material. It is normally used for separation of molecules of different polarities. Polar molecules travel upwards, while nonpolar molecules stay at the bottom of the plate.[2] We've explored the modifications of SIG to separate an achiral mixture of amino acids on a functionalized silica gel TLC plate as our starting point and have achieved success as a part of the learning of the process necessary to achieve such modification. We were able to make blank glass TLC plate and coat them with functionalized silica gel. We spotted a set of unfunctionalized silica gel TLC plates and a set of functionalized silica gel TLC plates with the same amino acids. During the application of slurry, the layer turned out to be too thick, therefore we could see the solute traveling upwards on the unfunctionalized silica gel TLC plate, but not on the functionalized one.

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Acknowledgments

I want to thank Dr. Stack for giving me professional advice on how to write the manuscript and the methodology of this research. I also want to thank Dr. Styers-Barnett for supervising me during the laboratory and answer any questions regarding the equipment for the experiment.

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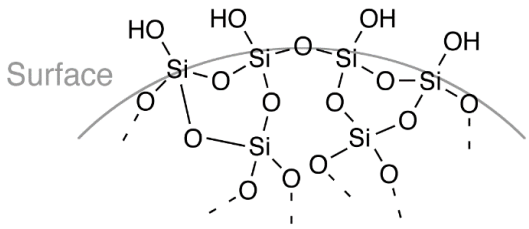
Statement of Purpose

The scope of this research is to study the modification of a SIG stationary phase with a chiral moiety, for the purpose of separating a racemic mixture of D/L-carnitine. Silica gel is a solid material used to perform organic and analytical separation of a mixture[3]. Silica gel consists of an irregular framework of covalently bonded alternating silicon and oxygen atoms with pores and voids at the nanometer scale[3].

Figure 1. Structure of the silica gel[4].



A stationary phase is the solid support in a chromatography system, which interacts with the molecules in the mixture that are being separated[5]. Here, we've used the silica gel as the stationary phase, which we wanted to further modify to observe how that would affect the separation of small chiral molecules. The word chiral is derived from the Greek word *cheir*, which translates to "hand". A chiral object has a nonsuperimposable mirror image[6]. The most universally common example is the difference between your left and right hand. You can't put your left glove on your right hand for the same reason you can't put your right shoe on your left foot[6]. A chiral object will have a mirror image that is similar, but not the same due to the different 3-dimensional arrangement in space. Molecules may also share the property of chirality[6].

The presence of a chiral center is needed to induce chirality in a separation or a chemical reaction[7]. A chiral center can be any atom within a molecule that can make four bonds with four other different atom[6]. In organic chemistry, a chiral center is usually represented by a carbon atom. A pair of molecules that are mirror images of each other are called enantiomers[6], and a mixture of enantiomers is called a racemic mixture. The handedness in chirality relates to the rotation of light by a chiral particle. A particle can exist as left-handed, which means it rotates plane polarized light counterclockwise or it can be right-handed, which means it rotates plane polarized light clockwise[8].

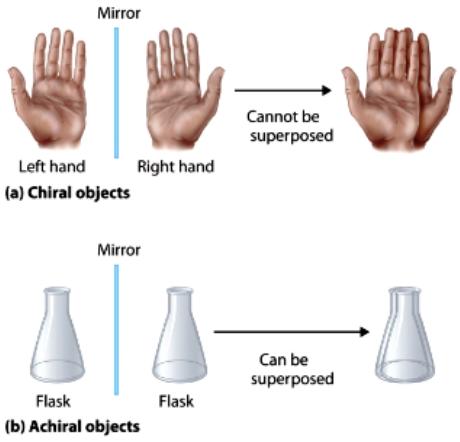


Figure 2. Example of chiral and achiral objects[9].

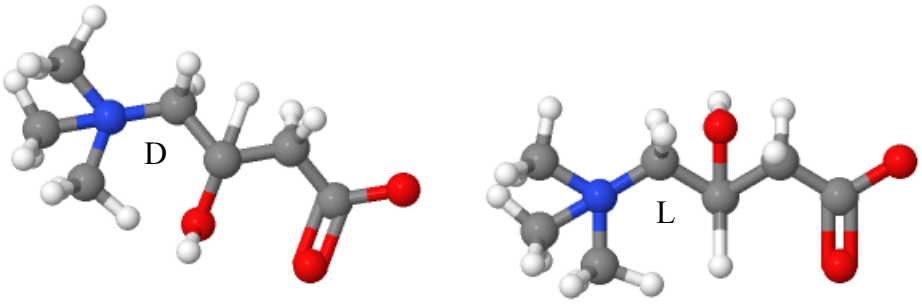


Figure 3. Ball and stick model for the carnitine enantiomers.

We expected the chiral stationary phase to interact with one of the enantiomers more than the other as the racemic mixture passes over the modified silica gel[2]. The enantiomer that interacts more favorably with the stationary phase should be retained longer on the silica gel, which promotes the separation[2]. The anticipated final product of this research project was a functionalized silica gel stationary phase that can separate a racemic mixture of D/L-carnitine enantiomers.

Introduction

Context and Background information: The property of chirality is extremely important in the pharmaceutical industry because all naturally occurring amino acids are a single enantiomer, which is left-handed. The prefix "L" means levorotatory because the enantiomer with this absolute configuration will rotate light to the left. L-Carnitine is naturally biosynthesized in humans and rotates plane polarized light to the left. All the "D" enantiomers are dextrorotatory, and they rotate plane polarized light to the right. Scientists first started to realize the need for chiral separation somewhere between 1957 and 1962, when they developed Thalidomide. This drug was meant to prevent morning sickness in pregnant women. However, it was prescribed as a racemic mixture, which is a mixture that contains both enantiomers of the chiral drug. One of the enantiomers interfered with the proper growth of the fetus, which resulted in thousands of children being born with abnormalities[10].

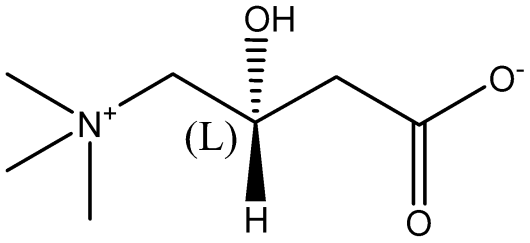


Figure 4. Skeletal structure of L-carnitine

L-Carnitine is a nonessential amino acid synthesized from lysine side chains[1]. Its function is to transport long-fatty acid chains from the cytoplasm to the mitochondria, where they are oxidized to produce $NADH^+$, Co-A, and $FADH_2$ [1]. For this reason, many athletes use it as a supplement to boost their athletic performance[11]. Currently, there are

more than 20 human genetic defects associated with the β fatty acid oxidation. These individuals have carnitine deficiency; thus, they can only operate on ω fatty acid oxidation, which occurs in the endoplasmic reticulum of the liver and kidney cells[12]. It is currently unknown what pathway is more efficient at generating high energy molecules. However, individuals with this disorder are subject to cardiomyopathy, skeletal muscle weakness, and hypoglycemia[13].

L-Carnitine is also a component of the seminal fluid responsible for count and motility, therefore it might treat male infertility. A study on mice that were kept under stress conditions concluded that carnitine supplementation promotes fatty acid oxidation, which gives more energy to the sperm cell, increasing its overall quality[14]. More research asserts the effectiveness of carnitine on reducing the effects of cardiac ischemia[15], diabetic neuropathy[16], and death of lymphocytes in the HIV infected cells[17]. We can conclude that L-carnitine is useful as a boost of energy for athletes, but even more important as a medical supplement for individuals with a deficiency of this amino acid. One study concluded that D-carnitine is toxic because it inhibits the activity of L-carnitine[1]. Therefore, the investigation of separation methods for small molecules of this type is important to scientific research.

There are currently three main ways L-carnitine is produced via either enantioselective synthesis or separation. One method involves an optically pure starting material such as (S)-3-hydroxybutyrolactone. After a series of reactions, the product is L-carnitine[18]. A second way to produce L-carnitine is through separating a racemic mixture of both D/L carnitine over a modified silica gel[19]. There are a couple methods known for

synthesis of carnitine as a racemic mixture at the level of industry production, which are the U.S. Pat. No. 3,135,788 and U.S. Pat. No. 4,070,394[18]. And the last method involves genetically modifying bacteria to instruct its catalytic machinery to generate L-carnitine[20].

Our proposed research focused on the second method for several good reasons. A modified silica gel can be packed inside an HPLC column or applied to a TLC plate. The methods applied to modify the silica gel for a chiral separation of D/L-carnitine can also be applied to separate other small molecules. Moreover, the techniques we intend to use for chirally modifying silica gel are based on Siddiq's methods for separating amino acids. This modified silica gel can be retained for further use compared to other chiral separation methods or selective synthesis methods that are only specific for one chiral compound. Those other methods require stoichiometric equivalencies of a chiral reagent. Our proposed method is less expensive, and it can be applied more broadly and repeatedly.

Final Literature review. Our proposed methods for generating the chiral stationary phase were derived from a previous article using aniline to modify silica gel for separation of amino acids[19]. For our proposed separation to work, we will attempt to modify aniline with a chiral moiety.

Table 1: Adapted from Satinder[7]. Common classification of chiral stationary phases and their respective composition.

Type	Description	Example
1	Brush-type	DNB-glycine, DNB leucine
2	Polysaccharide	Chiralcel-OA, -OB, -OF, -OJ, etc.
3	Inclusion CSPs	Cyclobond 1-3, Chiralpak OP, OT
5	Ligand exchangers	Proline, OH-proline

We were attempting to make the brush type chiral stationary phase and achieved modest success. It has a chiral center that can interact with one enantiomer of carnitine more than the other. Also, it contains charged species available for ionic interactions with the material passing over (see Figure 6). These are characteristics of a brush type stationary phase[7]. Other similar research has studied the efficiency of amino acids separation over cellulose on a TLC plate. A molecule of cellulose consists of a long chain of repeating (+)-D-glucose units, which act the same way modified silica gel does, however it doesn't contain ionic species[7]. This is a polysaccharide type stationary phase or simply polymers of sugars.

Chiral separation was also achieved by creating large cavities on a chiral stationary phase where certain stereoselective interactions of the materials entering and existing cavities would promote the resolution[7]. This type of stationary phase is called inclusion CSP's. A stereoselective reaction is one that selects for one of the enantiomers that enters and exits the cavities in the stationary phase. The most common stationary

phases for this type of separation are cyclodextrins, crown ethers, polyacrylates, and polyacrylamides[7].

In another interesting research paper, Davankov and Kurganov have achieved chiral separation through ligand exchange. They have indicated that complexes containing copper (II) and fixed ligands promote efficient separation of alanine, serine, and leucine. When such a complex is used as a stationary phase, it exchanges one ligand with one enantiomer in the racemic mixture that passes over the stationary phase[7]. The enantiomer that provides the complex with the most stability is favored and thus separated. These types of stationary phases are commonly called ligand exchangers.

In a recent study, a polymer of chiral (±)-2-sec-butylaniline was successfully synthesized according to the FTIR, UV-Vis, and ^{1}H and ^{13}C NMR spectroscopy. The synthesis was accomplished via two distinct methods. The first method is a classical chemical oxidative polymerization of (±)-2-sec-butylaniline with an initiator such as ammonium peroxydisulfate in an acidic aqueous medium[21]. The second method involves an interfacial polymerization that occurs between two immiscible phases of liquids. In the described study the liquids were hexane and methanesulfonic acid. Either of these methods would produce a polymer of aniline with a chiral alkyl substituent[21]. However, this research did not take a step further and see how the modification of SIG with a chiral polymer of aniline would give it the functionality to separate a racemic mixture of enantiomers. The possible applications for this polymer are overarching. It can be applied in areas including chiral stationary phase for HPLC, biological sensors, electrodes for enantioselective recognition, membrane separation technology, microwave absorbents[21].

For our purpose, we want to integrate this research to modify the SIG with a chiral polymer of aniline and investigate how well it can separate enantiomers of carnitine.

Procedure

A brush-type stationary phase was achieved through one method mentioned in the literature review section. We modified the silica gel by a simple oxidative polymerization of aniline in the presence of SIG[19]. To initiate the polymerization, a strong oxidizing reagent was added to the slurry dropwise such as ammonium peroxydisulfate[19]. During the initiation, many positively charged aniline radicals are produced[22]. Radicals are atoms with unpaired electrons in a solution. Unpaired electrons make atoms very unstable. Thus, one radical of aniline will attack another aniline creating a new bond between two chiral anilines. This new species can get oxidized again and attack another chiral aniline, a process we call propagation in organic chemistry[22]. Once all the oxidizing reagent is depleted, the reaction terminates. The addition of HCl is necessary after the termination is completed in order to protonate the nitrogen atom and give it a positive charge[19].

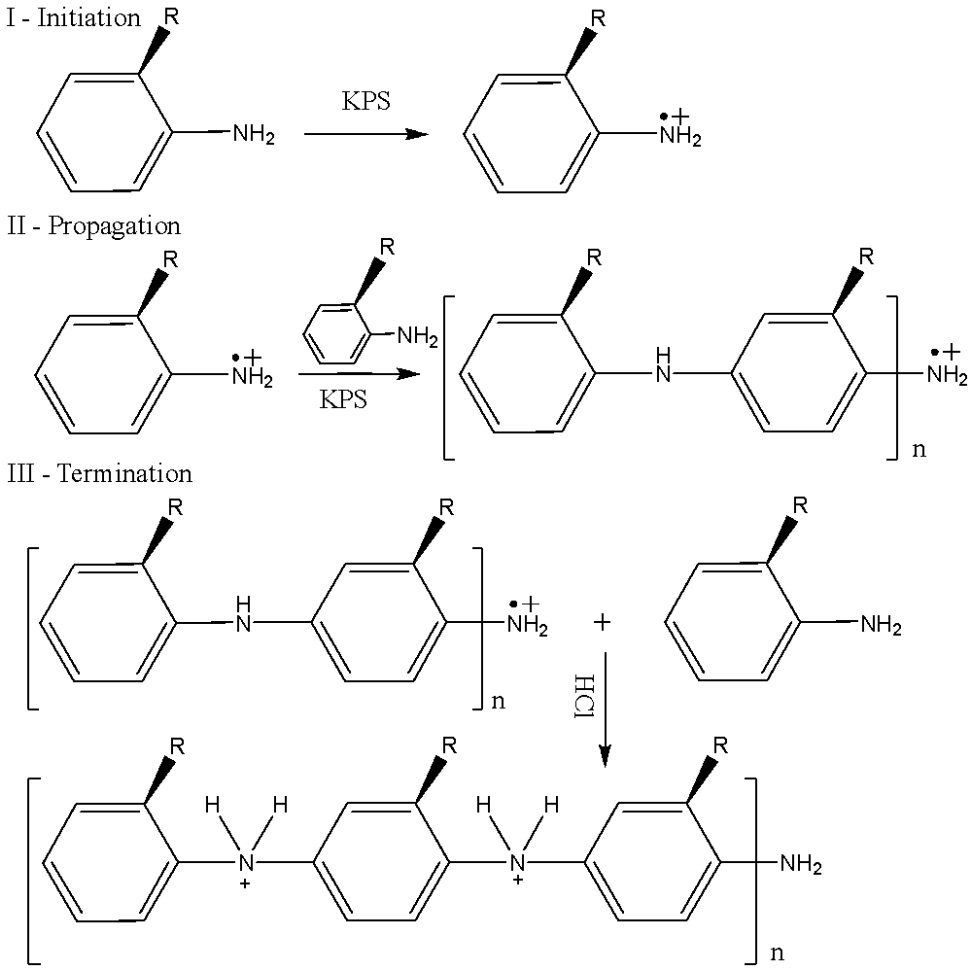


Figure 5. Oxidative polymerization of chiral aniline with potassium persulfate (KPS)[22].

This scheme represents the generation of a polymer consisting of a long chain of aniline monomers. The chain is kept in proximity to the silica via electrostatic interaction between the positive charge on the nitrogen and the negative charge on the SIG. The free silanol groups on the surface of silica gel are capable of forming hydrogen bonds with electron rich species[19]. Only then, can the polymer of chiral aniline and the silica gel be kept in proximity via electrostatic forces.

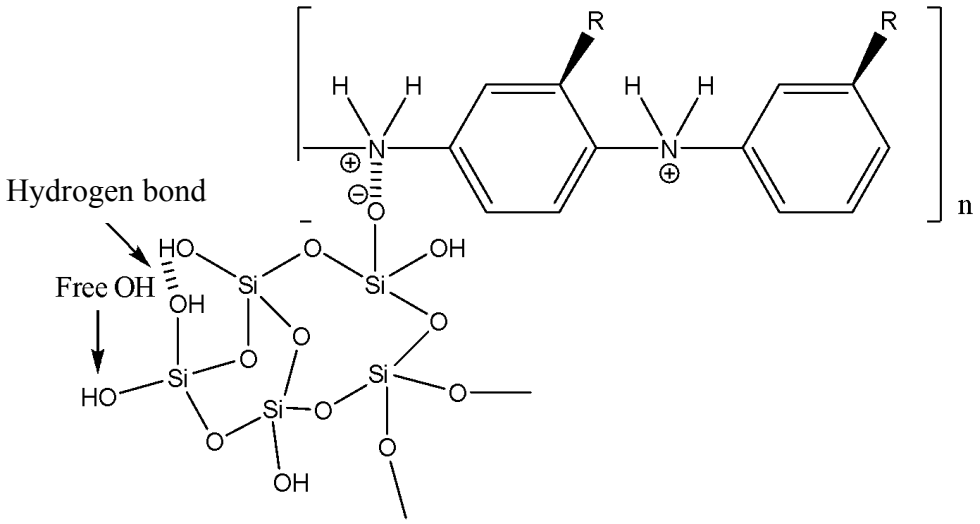


Figure 6. The electrostatic forces holding the chiral aniline polymer and the silica gel in proximity[19].

For training purposes, we produced a polymer of achiral aniline and bound it to SIG. The research has been conducted by Siddiq and it was the same in terms of procedure and mechanism of reaction. The amounts of reagents were used from a different paper. Before handling dangerous chemicals, proper safety guidelines were used. We began the procedure by dissolving 2.59g of aniline hydrochloride in 50 mL of water and a similar solution of 5.71g of ammonium peroxydisulfate. Solutions were kept at room temperature. In a 400 mL free standing beaker was added 10g of silica gel with a particle size of 60-200 μm and a magnetic stirrer. A total of 100 mL of the prepped solutions was poured over 10g of silica gel.[23] The silica gel suspension was stirred for 24 hours at 150 rpm[23]. The initial color of the solution was black and by the end of the reaction it was slightly green. The product was filtered with a Buchner funnel via vacuum filtration and saved in a moisture free environment.

The next obstacle was to apply the newly modified silica gel on a blank TLC. The glass was purchased from Lowe's and cut in the Art & Design Department. We want the slurry to bind well to the glass and prevent it from sloughing off the glass slides. Therefore, a mixture containing 1g of $CaSO_4$ and 4g of silica gel was made in 10 mL of water. The resulting slurry was loaded into a syringe and the tip of the syringe was moved back and forth to make sure we delivered even amounts of silica on the glass slides. The thickness of the layer must not exceed 0.25 mm[24]. This sample size was enough to make four TLC plates. Once the slurry was applied, the plates were left to dry for 1 hour. Further on, the plates were heated in the oven at 176°F for 45 min. This gets rid of any remaining water and makes the -OH groups available for electrostatic interactions with the polymer of aniline.

Once the functionalized TLC plate was prepared, we moved on to preparing the test solutions. Here we strictly followed the methods described in Siddiq's research. At this point we simply wanted to improve our skills at making the TLC plates, thus no racemic carnitine was used. Instead, known amino acids were used one per plate. We were going to measure the distance they would travel with the mobile phase and compare this to the literature value. If the amino acids travel relatively slower compared to the literature value, then we know that our functionalized silica gel stationary phase acts like a brush. The four chosen amino acids were leucine, isoleucine, norleucine, and phenyl-alanine. Solutions of amino acids were prepared by dissolving 1g of each in 100 mL of distilled water. Four developing chambers were set up with the mobile phase being a mixture of n-butyl alcohol–70% aqueous ethylene glycol–ethyl acetate ratio 5:3:2 by

volume[24]. Enough solvent was delivered to be right between the pencil line and the bottom of the TLC. In our case we used 200 mL of solvent per 800 mL beaker to make the developing chamber. Each chamber was covered with aluminum foil to let the vapor pressure of the solvent to build up inside the chamber. A capillary tube was held and drawn above a Bunsen burner and made thin enough to spot the TLC. We have spotted the TLC plates three times with the same amino acids per each plate. The TLC plates were put inside the chamber until the mobile phase reached ¾ of the height of the TLC plates. This took 2 hours and 30 min. Finally, the TLC were sprayed with ninhydrin solution in butanol and infrared light was applied to detect the how far the amino acids have traveled[24]. In chemistry we use retention factor values (Rf) to show the distance traveled by the solute relative to the distance traveled by the solvent. The equation for Rf is $\frac{Distance\ traveled\ by\ the\ solute\ (cm)}{Distance\ traveled\ by\ the\ solvent\ (cm)}$

Results

We've successfully synthesized the functionalized the stationary phase polymer of aniline by carefully following literature procedure. Once the oxidizing agent was added, the color of the reaction mixture has turned into a dark brown, which is due to the formation of radical-cations, and oligomers[25]. Farther on, around 8 a.m., the color of the reaction was dark green, which was due to the growth of hydrochloric acid within the polyaniline matrix[25]. The reaction has been completed around 2:30 p.m. and the final color was black. A similar research study performed by Seddique has conducted an oxidative polymerization of the protonated form of aniline, and their observations included that the initial color of the reaction was dark brown (λ_{max} ~520 nm), and it has changed to a dark green (λ_{max} ~720 nm)[25]. What is different about our research is that the oxidative polymerization of protonated aniline has taken place in the presence of silica gel, which could be why it farther produced a black color. The mass of the slurry was 11.7205g. Also, the slurry was easily dried and dissolved in water for application on the glass TLC. As previously mentioned, we bought the glass from Lowe's and cut it in the Art & Design Department. Below is a representation of the blank TLC plates.

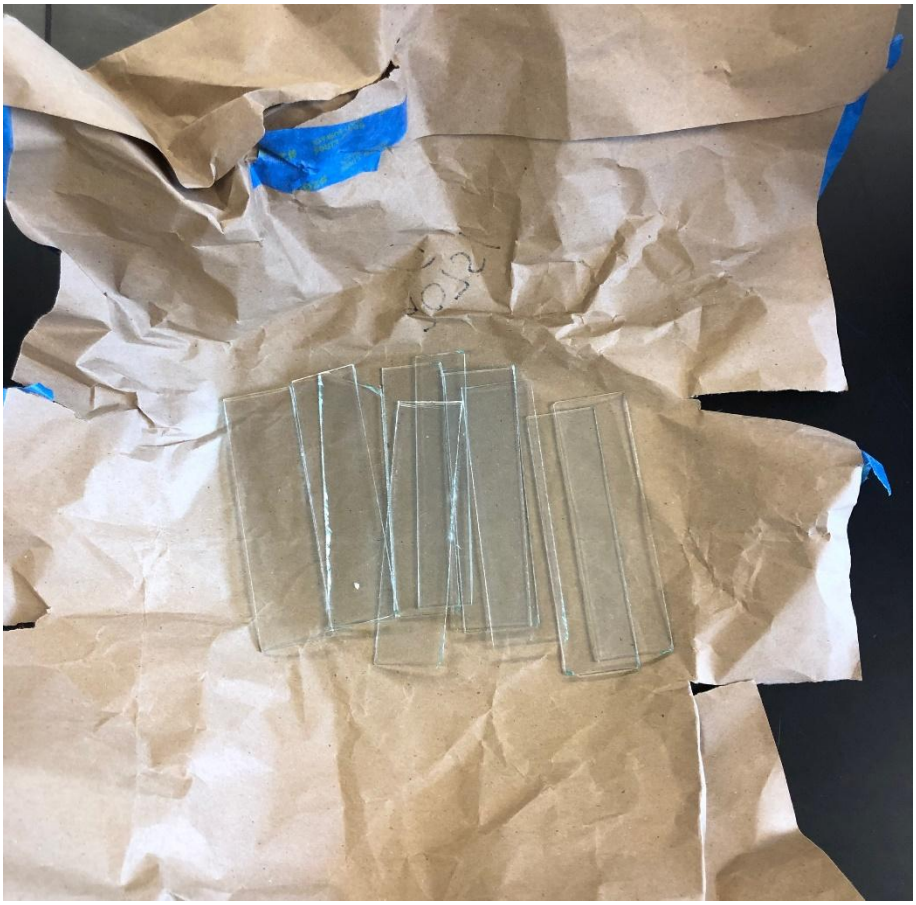


Figure 7. A visual representation of the blank glass TLC plates.

We have succeeded at making a set of TLC plates with the functionalized silica gel stationary phase. The slurry was applied on the blank TLC with a syringe and spread as thinly and evenly as possible. The stationary phase was well boned to the glass TLC with the help of calcium sulfate. Below is a picture of the TLC plates before and after the modification.

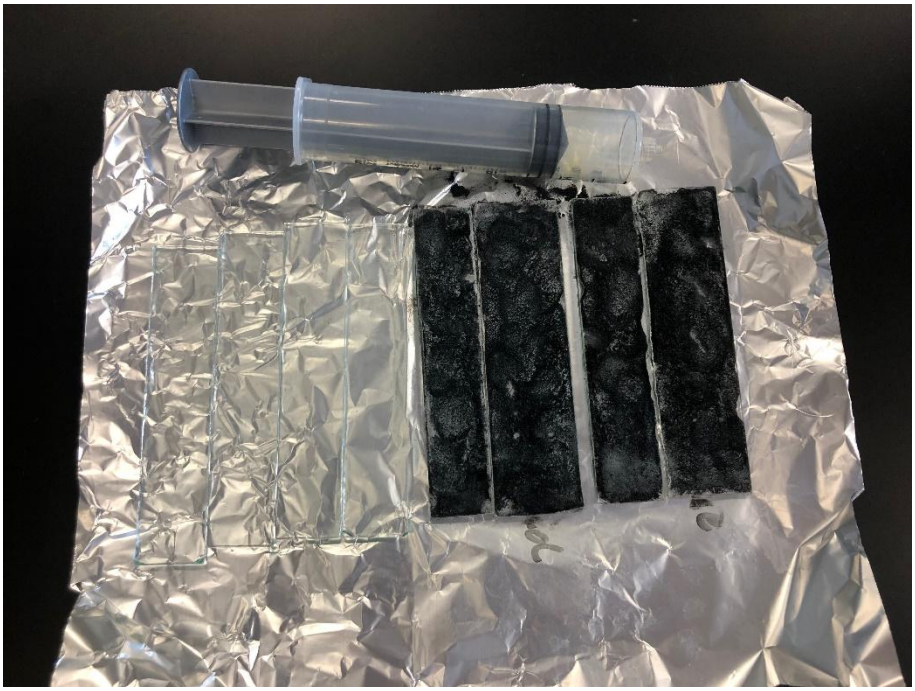


Figure 8. Applied functionalized SIG stationary phase on a glass.

We've successfully spotted the functionalized TLC plates with four different amino acids including leucine, isoleucine, norleucine, and phenyl alanine. Initially, we couldn't see the individual amino acids traveling upwards, but this was due to the functionalized silica gel layer being too thick. We decided to take a set of four unfunctionalized silica gel TLC plates and spot them with the same set of amino acids. They were sprayed with ninhydrin and under infrared light it was visible the trail of individual solutes could be visualized under infrared light. Below is a representation of the unfunctionalized blank TLC, however you can't see the solutes because of the absence of infrared light.

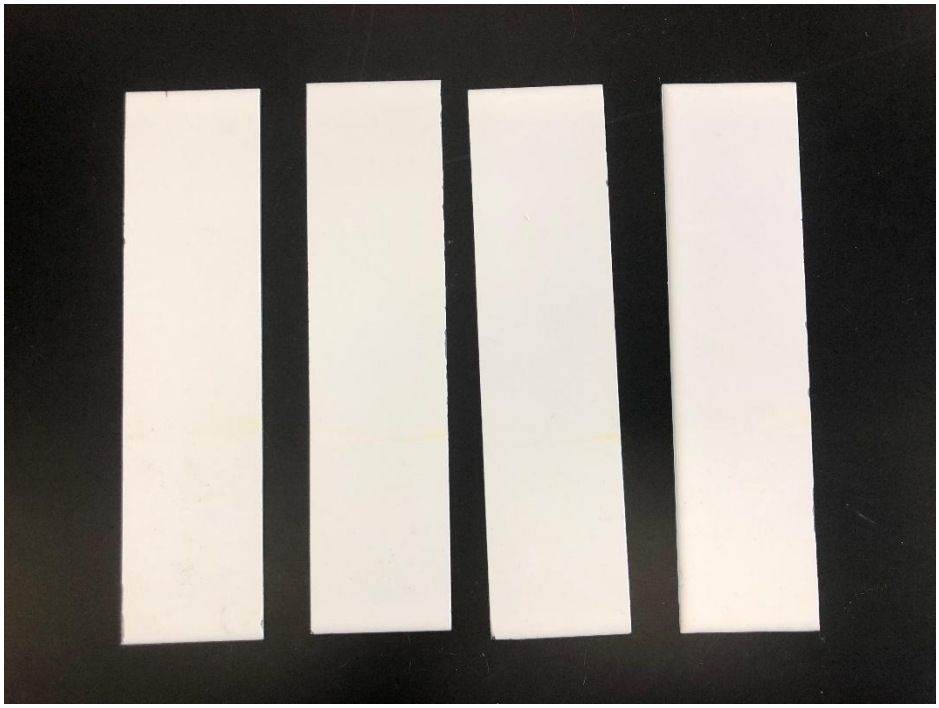


Figure 9. The unfunctionalized silica gel TLC plates spotted with the same set of four amino acids.

We also had success at finding out how separation of a racemic mixture of carnitine works in literature examples. The higher goals that were set up for the project weren't achieved, however the learning of the process that leads to those high-end goals was a success.

Conclusion

Since this attempt was just a training experiment, the greatest challenge was to find a way to apply the modified silica gel slurry as thin as possible and evenly on the glass TLC plates. Our applied slurry was so thick that we couldn't see how far individual amino acids have traveled. Ideally, we need a TLC applicator that is proficient at applying stationary phases on blank glass TLC plates. If such instrument was available, the rest of the research would be doable, and we could get data measurements. Our main goal was to functionalize the silica gel with a chiral polymer of aniline, which was determined to be (±)-2-sec-butylaniline as it was used in a similar study. If our training attempt was entirely successful, then the same protocol was to be applied for the main experiment with the substitution of chiral aniline. The main problem that needs to be addressed to move forward is an efficient way to apply the slurry evenly and thin enough (0.25mm). This will prevent the solute from being simply absorbed and it will move up the functionalized silica gel TLC plate. A successful assembly of a chirally functionalized silica gel TLC plate can promote the separation of a racemic mixture of carnitine amino acids. Moreover, this new silica gel can be packed into an HPLC column to separate larger quantities of material. The stationary phase is not selective for any particular compound; therefore, the array of possibilities is very large.

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