Thin Layer Chromatography (TLC)  
and Column Chromatography

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**Abstract**:

In this experiment, a Column Chromatography and a Thin Layer Chromatography (TLC) was performed to separate out a mixture containing vanillin (C8H8O3) and 9-fluorenone (C13H8O). A 25% mixture of ethyl acetate and hexane was used as the eluent for the column and as the mobile phase for the TLC. Silica was used the stationary phase for both the column and the TLC. The 50:50 mixture of vanillin and 9-fluorenone was initially separated out in the column. Samples collected from it were tested to determine which of the fractions of the samples contained the two chemicals. The chemicals were then separated and isolated, resulting in respectable percent yields.

**Introduction:**

Column Chromatography uses the basic principles of chromatography with the addition of a few more techniques. The basic definition of chromatography is a technique used to separate out a mixture by taking advantage of the different molecular characteristics of the components.[3] There are many types of chromatography, such as High-Pressure Liquid Chromatography, Column Chromatography, Paper Chromatography, and many others.[3] Different types of chromatography techniques are needed to separate out different types of mixtures, especially if they share many molecular characteristics. All chromatography’s have the same two parts: a stationary phase and a mobile phase.[3] Column chromatography utilizes characteristics such as size, net charge, and binding capacity to separate out the mixture, and assigns specific stationary and mobile phases depending on these characteristics.[3] The column itself has a particular setup which we will discuss in depth later, but it can be broadly defined. The general arrangement includes the stationary phase at the bottom of the column, the mixture above, and the mobile phase at the top.[5] As the mobile phase, or eluent for column chromatography, is added to the top of the column, the more polar of the mixture will get more “stuck” to the stationary phase and will hence move slower than the less polar compound of the mixture. This will cause a separation of the mixture in the stationary phase. Fractions of what comes out are to be taken and then analyzed by TLC. Thin Layer Chromatography uses the same principles, except that the mobile phase is at the bottom of a developing chamber, and the sample to be chromatographed and the stationary phase are both on a thin plate.[1] By using TLC, the fractions containing the specific compounds can be located, combined, and isolated using a rotary evaporator. This process can be used on a large scale to purify mixtures or isolate compounds, making it a very useful technique to understand.[2]

**Materials and Methods:**

The 0.14g mixture used was composed of a 50:50 mix of 9-fluorenone and vanillin. Initially, I put enough cotton at the bottom of the column to fully cover the opening. Next, the sand was put above the cotton up until the top of the funnel shaped portion of the column. After making the sand layer flat, about 8cm, or 15g, of silica was added to the column, which acted as our stationary phase. This was then dry packed using nitrogen and the eluent to get rid of any air pockets that may have formed when adding the silica. Dry packing is a technique in which the eluent is allowed to flow through the silica and out the bottom, thus revealing any cracks or pockets that need to be compressed. An additional 3g of silica was then combined with our mixture, and then added to the column, along with a 2cm layer of sand above it. With the column ready to go, the eluent was added again and let to drain, where it was collected in several test tubes. Each test tube was considered a fraction, and each fraction needed to be evaluated to check to see which of them had which chemical. The evaluation of the fractions was done by TLC, using a small sample from each fraction, and then comparing the resulting retention factor values (R­f values) to the known chemical Rf’s. The Rf for 9-fluorenone in 25% Ethyl acetate with hexane was 0.692, and the Rf for vanillin was 0.282. Once the fractions containing similar chemicals were located, each chemical containing fraction was combined into a round bottom flask. These round bottom flasks were weighed prior to the fractions being combined. These two round bottom flasks were then put in the rotary evaporator until all that was left was the chemical expected. The round bottom flasks were then weighed and then subtracted from the original round bottom flask weight, resulting in just the chemical weight. 0.087g of vanillin was collected, resulting in a 124.3% yield, and .059g 9-fluorenone was collected, resulting in a yield of 84.3%.

**Results:**

\*To scale by about 1.47x. Rf =   
9-fluorenone (in 25% Ethyl Acetate with Hexane) Rf = .692 Vanillin (in 25% Ethyl Acetate with Hexane) Rf = .282  
Fractions 2-5: Rf = - 9-fluorenone Fraction 10: Rf = - Vanillin

9 10

5 6 7 8

1 2 3 4

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Chemical | Empty RBF | RBF | Recovered | Expected | Percent Recovered |
| Vanillin | 56.481 | 56.568 | .087 | .07 | =124.3% |
| 9-fluorenone | 136.124 | 136.138 | .059 | .07 | =84.3% |

**Discussion:**

This experiment allowed us to separate a mixture of vanillin and 9-fluorenone through the use of column chromatography and thin layer chromatography. Both techniques can be used to separate out mixture by polarity.[2] In this procedure, 25% Ethyl Acetate with Hexane was used as the eluent. This was because, due to a previous experiment, it was shown to best separate out compounds. I began by making the column, starting off by putting a piece of cotton at the bottom, followed by a layer of sand. Making sure the sand was flat, I then added 8cm, or 15g, of silica, which acted as our stationary phase in this experiment. I then dry packed the column by adding the eluent in and letting it drain out, with a little help from nitrogen. This was necessary to make sure that no air pockets existed in this layer, of which mine did not contain any. To the side, 0.14g of the mixture was combined with 3g of silica, and then added to the top of the column. Finally, the column was topped with another thin, flat layer of sand. I then prepared 10 test tubes to collect the fractions and made sure I had a 50mL of the eluent. The eluent was necessary to drain out all of the chemicals while the stationary phase would separate them out. Once all this was prepared, the eluent was added to the top of the column and let to drain into a test tube, again with the help of nitrogen. Continuous addition of the eluent was necessary to drained out all of the mixture. I attempted to fill each fraction to approximately ¾ of its length, though I did not do this very well. At around my third fraction, I needed to get more eluent, of which I made by hand by adding 25mL of Ethyl Acetate with 75mL of Hexane. When I had collected 10 fractions, I then did a TLC on samples of each of the fractions. This produced the TLC plates represented in the ‘Results’. As shown by the TLC plates, the 9-fluorenone came out faster than the Vanillin. In the context of this experiment, this means that the vanillin had a higher affinity for the silica than the 9-fluorenone, so the vanillin got “stuck” more in the silica and therefore came out last.[3] When considering the TLC plates, the vanillin came out with the lowest Rf. The same principal of affinity is used for TLC, especially since the same mobile phase and stationary phase were used.[5] Preceding this step, we then had to isolate each of chemicals. To do so, I combined the fractions containing 9-fluorenone into a round bottom flask. I initially chose a round bottom flask to small and poured test tubes 2 and 3 into it, resulting in a spill of some of the solution. I quickly transferred the solution in the small round bottom flask to a bigger one and added test tubes 4 and 5. I then went and put the round bottom flask in the rotary evaporator to isolate the chemical and evaporate off the excess eluent. Both prior and after using the round bottom flask, it was weighed to see the mass difference of the chemical isolated. The vanillin was also collected in another round bottom flask, where test tube 10 was added. Since only test tube 10, the last test tube, had shown for vanillin, I added 30mL more of the eluent to the column and let it drain out directly into the round bottom flask. Isolating the chemicals, I ended with a mass recovery of 0.059g of 9-fluorenone, which was an 84.3% recovery. This low value was mainly due to the spill when transferring the solutions to the round bottom flask. I also ended with a recovery of 0.087g of Vanillin, which was an 124.3% recovery. This high value might have been due to me not drying the round bottom flask fully before weighing it. Since the expected mass was 0.07g and the mass I recovered was 0.087g, the weight difference might have gone unnoticed while in lab. If noticed, it would have indicated that the round bottom flask needed to be dried more with nitrogen. A melting point analysis was not achieved due to time constraints.

**Conclusion:**

The separation of a mixture containing 0.07g of vanillin and 0.07g of 9-fluorenone took place in 5 steps. The first step was preparing the column, which includes layering the column correctly and making sure there are no air pockets through dry packing. Secondly, the mixture was added as a mixture between 3g of the stationary phase and the 0.14g of the mixture, which was placed on top of the stationary phase layer and below a thin layer of sand. Next, a few test tubes were prepared to collect fractions, and the eluent was added to the column and left to drain into each of the test tubes. A sample was taken of each test tube for a TLC, which would determine which of the test tubes contained which of the test tubes contained which chemical. When the chemical containing test tubes were identified, the similar chemical test tubes were combined into a round bottom flask and rotary evaporated, isolating the chemical. I resulted in a 124.3% recovery yield for vanillin and 84.3% recovery yield for 9-fluorenone. I would consider the isolation of the 9-fluorenone successful due to its mediocre percent recovery, but I would consider the isolation of vanillin a failure due to the impurity of the isolated chemical.

**References:**

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(3)Coskun, Ozlem. “Separation Techniques: Chromatography.” *Northern Clinics of Istanbul*, Kare Publishing, 11 Nov. 2016, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5206469/.

(4)Martin, Christopher B., Thin Layer Chromatography and Column Chromatography. *Organic Chemistry 1-Laboratory*, 2017.

(5)Stone, David. “Chromatography Resources.” *Analytical Science - Chromatography*, 14 July 2020, https://sites.chem.utoronto.ca/chemistry/coursenotes/analsci/chrom/index.html#:~:text=Column%20Chromatography%3A&text=Russian%20botanist%20Mikhail%20Tsvet%20invented,mixtures%20into%20their%20individual%20components.