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**Thin Layer Chromatography and Column Chromatography**

**Purpose:** The purpose of this lab is to utilize thin layer chromatography and column chromatography as a method of separating compounds. By doing this, it will allow for a better understanding regarding various laboratory techniques.

**Reaction(s):** N/A

**Physical Properties of Reagents**:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Reagent | Structure | Molecular Formula | Molecular Weight (g/mol) | Boiling Point (°C) | Density  (g/mL) |
| Fluorene |  | C13H10 | 166.223 | 295 | 1.2 |
| 9-fluorenone |  | C13H8O | 180.206 | 342 | 1.13 |
| Ethyl acetate |  | C4H8O2 | 88.11 | 77.1 | 0.902 |
| Hexane |  | C6H14 | 86.18 | 68 | 0.655 |

**Procedure & Observation:**

To begin this experiment, begin by dressing in the proper lab attire and gathering all of the required materials that would be necessary. For this experiment you will utilize beakers ranging from 50-150ml, 10ml graduated cylinder, hexane, ethyl acetate, fluorine, 9-fluorenone, silica TLC papers, 10 test tubes, 250ml beakers, analytical balance, and a column chromatography apparatus consisting of a glass column, funnel, sand, and absorbent.

Next, start by conducting a sample TLC analysis of fluorene and 9-fluorenone. This will allow for a base reading regarding the polarity and intermolecular forces acting upon the given reagents within the given eluents. To do this, begin by collecting a silica gel TLC plate and make the appropriate markings using a pencil. Next, utilize a glass capillary tube to conduct the appropriate spotting technique of the reagents onto the silica TLC plates. One will need to place both fluorene and 9-fluorenone together on three sets of TLC plates. Be sure to place an appropriate distance apart from each other and from the base of the plate. Next, place an appropriate amount of eluent into a 50ml beaker using a pair of tweezers so that it will not surpass the spotted reagents prior to the spread of the eluent along TLC plate. Next place a watch glass on top of the beaker to allow for a saturated atmosphere and allow for the eluent to proceed until it reaches the solvent front. Remove the plate immediately once it reaches the front to prevent a botched reading. You will conduct this using a collection of different eluents including 100% hexane, 5% ethyl acetate/hexane mixture, and 10% ethyl acetate/hexane mixture. Record your results of the given TLC with the help of a UV light and utilize this information to choose the best eluent while conducting your experiment.

Next you will need to prepare the glass column for the column chromatography. Set up the column using a ring stand and a clean glass column. Pack the column by adding an absorbent composed of alumina using the slurry method. It is important to ensure that there are no bubbles present within the column to ensure a desired outcome. Next, a thin and even layer of sand is added on the top of the absorbent layer. While packing the column, be sure to rinse the sides of the column to remove debris from the side using the solvent. Next, add the solvent chosen from the initial TLC readings (5% ethyl acetate). Run this through the column until it reaches the top of the sand but DO NOT allow it to surpass the sand to ensure that the absorbent does dry up and crack. Next add a mixture of fluorene and 9-fluorenone. This is created using an analytical balance to measure 100 mg of each of the reagents. You will need to dissolve this mixture using a few drops of dichloromethane. Add this mixture to the column carefully to ensure not to disturb the layer of sand within the column. Next run the column into a test tube until the mixture has made its way into the absorbent layer. Once this has been completed, continue to add solvent into the column as it progresses to prevent it from drying. Collect your samples into labeled test tubes as it makes its way through the column. After the mixture has made its way through the column, spot TLC plates containing samples from each test tube. This will allow for the determination of which test tubes yielded fluorene and 9-fluorenone. After conducting the TLC procedure, place the test tubes that yielded the respective compounds into separate beakers and place under the fume hood to allow for the solvent to evaporate. You will then use the finished product to determine the percent recovery of each compound by deducting the mass of the clean beaker from the mass of the beaker with the compound. Be sure to clean up your lab station.

**Results:**

* **TLC**

|  |  |
| --- | --- |
| **TLC Plate 1 (100% Hexane)** | **TLC Rf values calculation**  **Spot #1: cm**  **Spot #2: 1.002.80=0.36 cm**  **Spot#3: 1.100.39 cm**  **Average: 0.38cm**  **It is assumed that the nonpolar compound flourene exhibited no movement on the nonpolar solvent and remained at the start point.** |
| **TLC Plate 2**  **(5% Ethyl Acetate/Hexane)** | **TLC Rf values calculation**  **Spot #1: 1.15cm**  **2.10cm**  **Spot #2: 1.102.80=0.39cm**  **2.052.80=0.73cm**  **Spot #3: 1.102.80=0.39 cm**  **2.00cm**  **Average:0.39cm**  **0.73cm** |
| **TLC plate 3**  **(10% Ethyl Acetate/Hexane)** | **TLC Rf values calculation**  **Spot #1: 1.10cm**  **2.10cm**  **Spot #2: 1.122.70=0.44cm**  **2.102.70=0.77cm**  **Spot #3: 1.102.70=0.40 cm**  **2.10cm**  **Average:0.41cm**  **0.77cm** |

* Column Chromatography

We collected a total of 10 fractions during the column chromatography.

The following TLC plates and calculations were collected from the fractions collected throughout the column chromatography.

|  |  |
| --- | --- |
| **TLC Plate 1: #’s 1-5 (100% Hexane)** | **TLC Rf values calculation**  **Fraction 1: N/A**  **Fraction 2: N/A**  **Fraction 3:**  **Fraction 4: 0.852.8=0.30**  **Fraction 5: 0.852.8=0.30** |
| **TLC Plate 2: #’s 6-10 (100% Hexane)** | **TLC Rf values calculation**  **Fraction 6: No movement**  **Fraction 7: No movement**  **Fraction 8: No movement**  **Fraction 9: No movement**  **Fraction 10: No movement** |

* Theoretical yield
  + 100 mg 9-fluorenone
  + 100 mg fluorene
* Actual yield
  + 0.079 g 9-fluorenone
  + 0.053 g fluorene
* % recovery of fluorene and 9-fluorenone.
  + 53% fluorene recovered
  + 79% 9-fluorenone recovered

**Discussion:**

After conducting this experiment, it may be concluded that the necessary techniques and procedures were performed in conducting both TLC and Column Chromatography. After conducting the initial TLC readings, it was concluded that 5% Ethyl Acetate was deemed to most appropriate solvent due to the Rf values that were received and the moderate spreading between the to compounds. It received an average Rf value of 0.39cm and 0.73cm. Additionally, the results that were yielded following the column chromatography confirm that we were successfully able to separate the two compounds using these methods. After analysis of the TLC papers spotted with the ascending fractions using 100% Hexane, we were able to see the emergence of two opposing TLC results. Fractions 3, 4, and 5 contained an Rf value on average of 30.66. However, fractions 6-10 possessed no movement as they remained stationary on the TLC start line. This allowed us to conclude that we were successful able to separate the two samples using column chromatography. Upon using 5% Ethyl Acetate/Hexane mixture within the column, we can state that the nonpolar compound, fluorene, came out of the column first and denoted by fractions 3-5. However, fractions 6-10 signify the more polar compound, 9-fluoronone, came out last. This is due to the IMFs acting upon the compounds as they ascend the column. As a result of this, fractions 3-5 and fractions 6-10 were placed into separate beakers to determine the percent mass recovered. After the solvent evaporated we were able to determine that the percentage mass recovered for the 53% fluorene was 53% while, the percent mass recovered for 9-fluorenone was 79%. This disparity amongst the two masses recovered may have been the result of repeated transfer throughout the course of the experiment.

Throughout the course of this experiment, it may be stated that there were some possible sources of errors that occurred. For one, it would have been better to have taken more fractions instead of stopping at 10. This would have allowed us to ensure that the compounds completely made their way through the column. Additionally, there were some problems with spotting which may have occurred upon placing the samples on TLC plate which may have resulted in undesirable results. As a result of this, it is believed that conducting more fractions and improved spotting methods would have yielded better results. Additionally, using various solvents with higher polarity would have allowed for a wider experience regarding how polarity affects column chromatography.