PROGRAMMING THE "RINSE ROBOT"
INTRODUCTION

Description

During the initial part of this exercise, students will attach a chemical group to a solid material inside a reaction tube. Not all of this chemical group will become attached to the solid material. This unattached chemical group is "free" and if not removed will cause unwanted reactions when other chemical groups are added to the reaction tube. This exercise uses thin layer chromatography to determine if any of the free chemical compound is present in the reaction tube. If the free chemical compound is present, TLC is used to determine the minimum number of rinse cycles necessary to completely remove the free chemical compound from the reaction tube.

Goals for This Experiment

The goals for this experiment are to have students:

1. discover that chromatography is primarily a separation technique,
2. learn the techniques associated with thin layer chromatography, and
3. use the technique of thin layer chromatography to determine if a specific compound is present in a solution.

Recommended Placement in the Curriculum

This laboratory exercise introduces students to some of the newest medical drug development technology and the testing done to reach efficiency. It is an introductory chemistry exercise that would be most relevant during a section on drugs or drug testing.

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PURPOSE

The purpose of this lab is to investigate the role of thin layer chromatography in the development of medicinal drugs.

SCENARIO

Doctors and medicine usually get credit for making us well; however, chemists and chemistry are largely responsible for producing today's miracle drugs. Consider a model hypothetical drug, ABCDE. It is made by attaching chemical group A to group B. Group C is attached to AB, Group D to ABC and finally group E to ABCD. Suppose a chemical group A' exists which is just a little different from A, but is similar to A in how it reacts with group B. It could form the hypothetical drug A'BCDE. Since many potential drugs can be quickly screened in small amounts against many biological targets, it is economically advantageous to make A'BCDE at the same time as ABCDE. Further advantage will be realized if A"BCDE, AB'CDE, AB"CDE, and other permutations are also made for simultaneous screening. One approach to discovering the "ABCDE drug group" is illustrated in the video produced by Parke-Davis Pharmaceutical Research, entitled "Automated Synthesis and Diversomer Technology®."

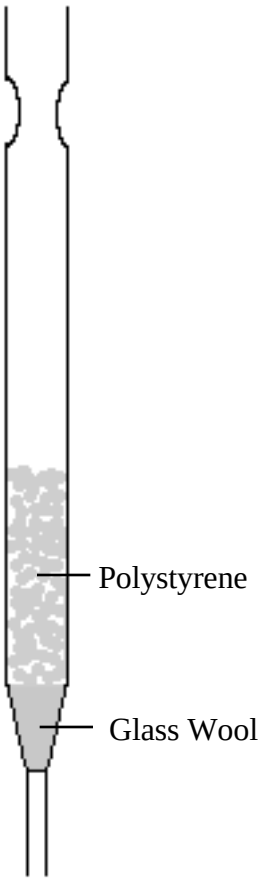
[The video "Automated Synthesis and Diversomer Technology®" might be viewed at this time.]

In setting up the robotic synthesis of the "ABCDE drug group," rinsing the reaction tubes is important. Ideally, the chemical group A (A', A", etc.) is completely attached to the solid material inside the reaction tubes before B, B', B", etc. are added; otherwise some AB, AB', etc. will be free to cause a mess such as solid-ABA, solid-AB'A, and so on. Sufficient rinsing of the tubes will eliminate free A. In a similar manner, the solid material must be cleared of free B after it forms solid-AB, so that it doesn't form BC when chemical group C is added (which could possibly form solid-ABCD and other undesirables). Again, sufficient rinsing of B is required. Therefore, in setting up the robotic synthesis, the required number of rinses, to remove the groups A, B, C, D, and E from the solid on which the drug is formed, must be known.

BACKGROUND

Thin layer chromatography (TLC) is a chromatographic separation technique in which a chemical substance or mixture of substances is applied to a layer of absorptive material on a solid support (in this case, silica on a plastic strip). The strip is exposed to an eluting solvent (in this case a small amount of ethyl acetate in the bottom of a beaker). The eluting solvent moves up the strip by capillary action, and components of the mixture are separated by differing affinities for the absorptive material versus the eluting solvent. (The eluting solvent may be a mixture of solvents as well as a single substance.)

When elution is complete, the solvent front is marked on the strip with pencil. (The strip should be removed from the developing chamber before the solvent reaches the very top of the strip.)



After evaporation of the eluting solvent from the strip, the separated mixture may be viewed directly on the strip if the materials are colored. If the materials are colorless, special methods are necessary. A simple viewing method for colorless materials is ultraviolet light detection of a silica gel strip with an ultraviolet light indicator already on the strip. In this case, the components will appear as dark spots on the strip under UV light. Another method is to expose the strip to iodine vapor (a crystal or two of iodine in a jar): the organic components then show up as orange spots on the strip. Other chemical indicators may be employed as well.

YOUR TASK

In this laboratory exercise, your task is to determine the number of 0.5 mL solvent rinses needed to rinse two model components, A and B, from the solid in a model tube. (Extra rinses, while ensuring a neat product, are not profitable since it costs extra for the extra rinse, as well as extra disposal or recycling cost of the waste solvent.) One general technique that can be used to accomplish this task is thin layer chromatography, TLC.

In today's exercise, the Diversomer technology reaction tubes are modeled by using a disposable pipet plugged with glass wool and partially filled (1-2 cm) with swelled macroreticular polystyrene. The solvent to be used is ethyl acetate, and model compounds, A and B, are benzoic acid and benzophenone.

SAFETY AND HANDLING

Ethyl Acetate:

Hazard Alert: Dangerous fire hazard and explosion risk; irritating to skin and eyes; mildly toxic by inhalation and skin absorption. LD_{50} 6100 mg/kg TLV 1440 mg/m^3.

Benzoic Acid:

Hazard Alert: Moderately toxic by ingestion; irritates eyes, skin and respiratory tract; combustible, LD_{50} 2530 mg/kg.

Benzophenone:

Hazard Alert: none

All rinsings should be disposed of into a waste container provided by your instructor.

MATERIALS

- ultraviolet light source
- spotter (micropipets or capillary tubes)
- Pasteur pipet (5.75 mL)

- TLC strips (silica coated, 4 cm x 10 cm, which can be cut from TLC sheets)
- 50 mL beaker
- 150 mL or 250 mL beaker
- watch glass
- glass wool
- benzoic acid
- benzophenone
- ethyl acetate
- dropper

PROCEDURE

Part I

1. Apply 0.5 mL (10 drops) of solution A (benzoic acid in ethyl acetate) or B (benzophenone in ethyl acetate) to the top of a model tube.

2. Allow the solvent to drip through the pipet into a small beaker or test tube. Collect and label this solvent "rinse 0" or something else appropriate.

3. For each rinse, add another 0.5 mL, or 10 drops of ethyl acetate to the top of the tube. Collect the solvent, which is dripping from the tip of the pipet, in another appropriately labeled beaker or test tube.

4. Repeat this rinsing procedure as needed.

Part II: Analyze the Solvent with Thin Layer Chromatography (TLC)

NOTE: The TLC strips used in this investigation are a plastic sheet that has a thin, even coating of adsorbent silica on it. The silica also contains an ultraviolet indicator. Handle the strips lightly and only on the edges, because your fingers contain natural oils and dirt that will contaminate the TLC strip. Mark on the strip with pencil only since ink contains many components that will dissolve in the ethyl acetate.

1. Prepare a developing chamber by pouring about 0.5 cm of ethyl acetate into a 150-mL or 250-mL beaker. For best results, the developing chamber's atmosphere must be saturated with the solvent. This can be accomplished by lining the developing chamber with filter paper. Place a square of filter paper (10-cm x 10-cm) in the beaker. Cover the top with a watch glass.

2. Measure up 1.5-cm from the bottom of the TLC strip. Using a #2 pencil, draw a line across the width of strip. This line is the starting point and marks where a drop of the rinse solvent is applied with a spotter. See Figure 1.

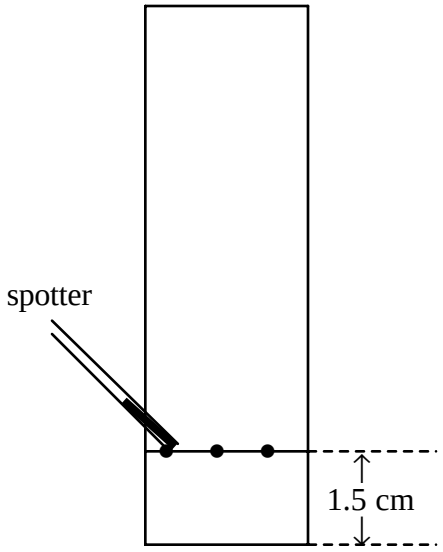


Figure 1: Spotting a TLC strip **Figure 2: Developing a TLC strip**

1. A spotter is a micropipet, or a glass capillary tube which has been heated and pulled to a thin point. Put the thinnest end in the rinse solvent until a small amount of solvent enters the tube by capillary action. The tip of the spotter is gently touched to one of the marked spots on the TLC strip. Gently push down on the spotter until a small portion of the rinse solvent transfers to the TLC strip. Push the spotter down at least three more times to achieve a concentrated spot. Use the spotter to apply a second dot of the rinse solution onto the TLC strip. (Note: Spotters should be lightly touched to the TLC strip to allow small amounts of materials to be applied; otherwise, the strip will be overloaded.)

2. Stand the thin layer strip on end in the developing chamber as illustrated. Take care to ensure that the spotted portion is above the solvent level. Replace the watch glass on top of the beaker to promote even movement. The solvent moves up the strip by capillary action. (See Figure 2.)

   Q1: What happens to the TLC strip and the developing solvent if the spotted portion is submerged in the developing solvent?

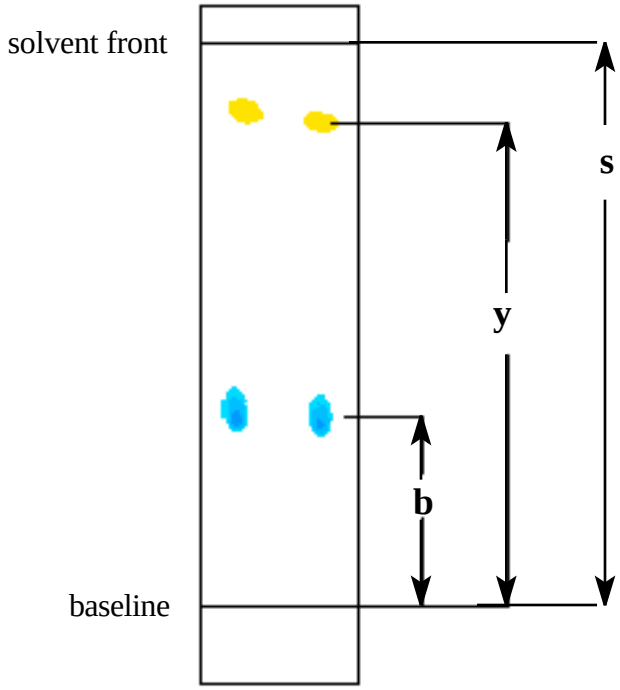
3. Observe that the solvent moves up the strip. Remove the strip when the solvent is about 1 to 2 cm from the top of the strip. Using a pencil, mark the level to which the developing solvent traveled.

   Q2: What would happen if the solvent were to reach the top of the strip before the strip was removed?

4. Allow the developing solvent to evaporate. Can you see any spots? To help see the spots, The strip may now be viewed under an ultraviolet light. With this viewing method, the spots are visible only under the ultraviolet light, so draw around them with a pencil.
   Caution: Do not look directly at the ultraviolet light source; it can hurt your eyes.

5. Report the results of the TLC for the various rinses by calculating the retention factor for any spots that show up under the UV light. See Figure 3 for an example of how to calculate retention factors. Tape the TLC strips into your notebook.

Figure 3: Calculation of R_f values



$$R_f = \frac{\text{distance component travels}}{\text{distance solvent travels}}$$

For the highest component $R_f = \frac{y}{s}$

For the lowest component $R_f = \frac{b}{s}$

Part III: Completing the Task

Do not lose sight of the original problem: How many 0.5 mL rinses should the robot be programmed to do in order to rinse the model components (both A and B) from the solid in the tubes?

PROGRAMMING THE "RINSE ROBOT"
INSTRUCTOR NOTES

Time Required

This laboratory experiment should take between 2–3 hours to complete.

Group Size

Students can work in pairs.

Materials Needed

- ultraviolet light source
- spotter (micropipets or capillary tubes)
- Pasteur pipet (5.75 mL)
- TLC strips (silica coated, 4 cm x 10 cm, which can be cut from TLC sheets)
- 50 mL beaker
- 150 mL or 250 mL beaker
- watch glass
- glass wool
- benzoic acid
- benzophenone
- ethyl acetate
- dropper

Safety, Disposal, and Special Handling

Review the Material Safety Data Sheets of any chemical used in the experiment for information regarding safety and handling. Dispose of waste according to your local ordinances.

Points to Cover in Pre-Lab

The concept of chromatography should be explained. A background is written at the beginning of the procedure. This background could be presented as an introduction to the scenario.

Sample Results

See page nine.

Possible Answers to Questions

Q1: What happens to the TLC strip and the developing solvent if the spotted portion is submerged in the developing solvent?

The substance that was spotted onto the TLC strip might dissolve in the solvent, and it would then appear, after developing the TLC strip, that neither benzoic acid nor benzophenone was present in the rinse.

Q2: What would happen if the solvent were to reach the top of the strip before the strip was removed?

The spot would continue to migrate up the TLC strip and might appear at the top of the strip. Retention factors, R_f, could not be accurately determined.

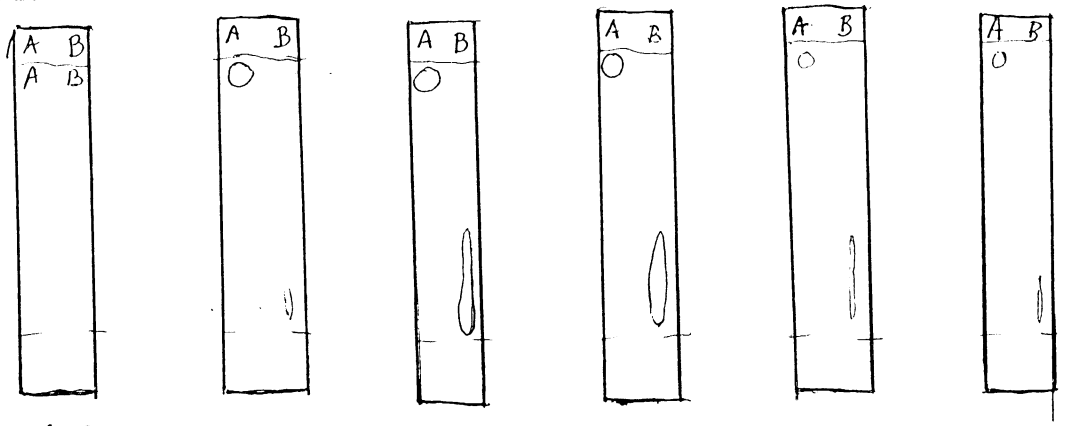
Reference

Automated Synthesis and DIVERSOMER Technology®, **1996**, PARKE-DAVIS Pharmaceutical Research, DIVERSOMER Technologies, Inc.

Sample Results:

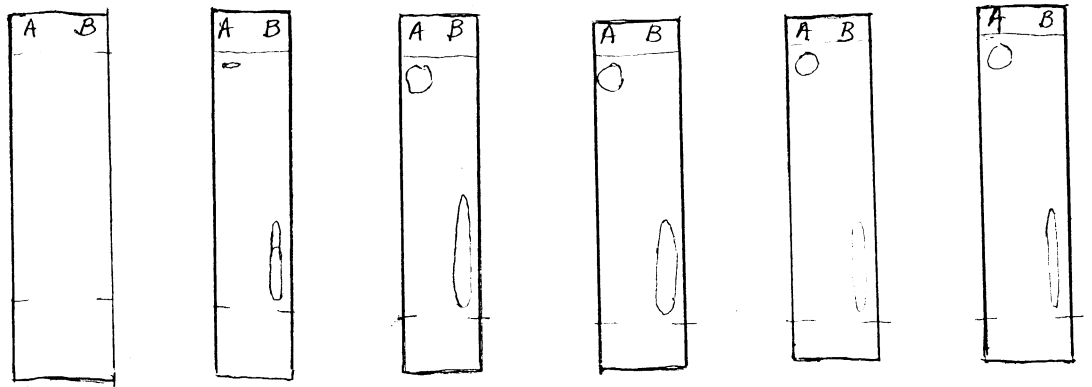
Trial 1

Rinse 0 *Rinse 1* *Rinse 2* *Rinse 3* *Rinse 4* *Rinse 5*

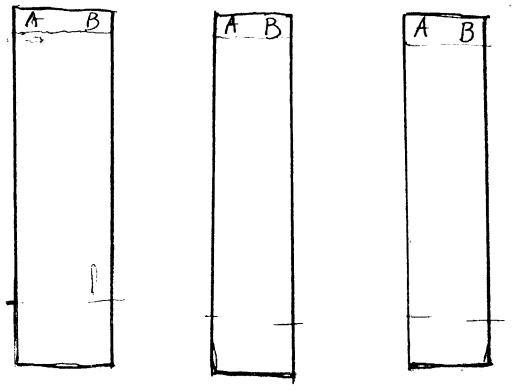


Trial 2

Rinse 0 *Rinse 1* *Rinse 2* *Rinse 3* *Rinse 4* *Rinse 5*



Rinse 6 *Rinse 7* *Rinse 8* *Rinse 9*



Key: A = Benzophenone, B = Benzoic Acid
The eluting solvent is ethyl acetate.
The silica gel strips are Kodak.
The macroreticular polystyrene is available from Aldrich.