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Thin-Layer Chromatography: The "Eyes" of the Organic Chemist

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The general aspects and practical applications of thin-layer chromatography (TLC) have been discussed thoroughly in the literature *(1, 2)*. TLC methods are successfully used in many fields of research and development such as clinical medicine, forensic chemistry, biochemistry, and pharmaceutical analysis *(3)*. This chromatographic technique is relatively inexpensive and has found widespread application as a simple, reliable, and quick analytic tool.

In this inquiry-based activity the usefulness of TLC in the organic laboratory is explored. The experiment demonstrates simple aspects of chemistry (hydrogen bonding and polarity) and applications of TLC. The activity follows the backward design described by Wiggins and McTighe *(4)*. This design is an assessment of students' proficiency and understanding of hydrogen bonding and polarity as applied to TLC. The goal is that students will understand TLC and be able to apply it to an unknown situation. This is accomplished by a simulated industrial application at the end of the experiment. Initially students develop a uniform method to distinguish individual compounds and then apply this method to an unknown sample. Students are not able to tackle the application without understanding the principles covered earlier in the activity.

Procedure and Results

Demonstration

Since this is the first time many students will be exposed to TLC, the instructor demonstrates the proper setup and execution of a TLC experiment during the prelab lecture. The activity is not intended for students to perfect the technique, so the instructor includes important details such as keeping the level of the mobile phase in the chamber lower than the spots on the plates. If this occurs the sample leaches into the eluent (solvent) and faulty results are obtained. Students are also cautioned not to leave plates in the chamber after the solvent front has reached the top edge. Neglecting to remove the plates will lead to diffusion of the sample in all directions, which will cause the spots to appear broad and ill-defined.

Part 1: Understanding R_f

The experiment is divided into four parts. In the first part, students spot three plates of different lengths with the same compound and use the same eluent. The plates are developed (development is stopped when the eluent has almost reached the top of the plate) and the distances traveled are determined. The students discover that the longer the plate, the further the spot travels. The fact that the same compound is used in all three runs should spur the students to see the need for determining a systematic relationship comparing the distances traveled for the different plate lengths. Comparison of the ratios of the distance the analyte travels to the distance the eluent (solvent) travels for each plates regardless of plate size leads to the discovery of retention factor, R_f (Figure 1 and eq 1):

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

Not all students are able to identify this important relationship, and it is important that the instructor participates in guiding them to this discovery.

Part 2: Examining Hydrogen Bonding

During the second part of the experiment students discover that separation in TLC arises from the sample's ability to hydrogen bond to the silica gel on the plate. Six compounds of comparable molar mass, each containing either an ester, ketone, ether, alcohol, or carboxylic acid functional group, are developed on the same plate using eluent A (Table 1). Students' results show that the ketone, ether, and ester

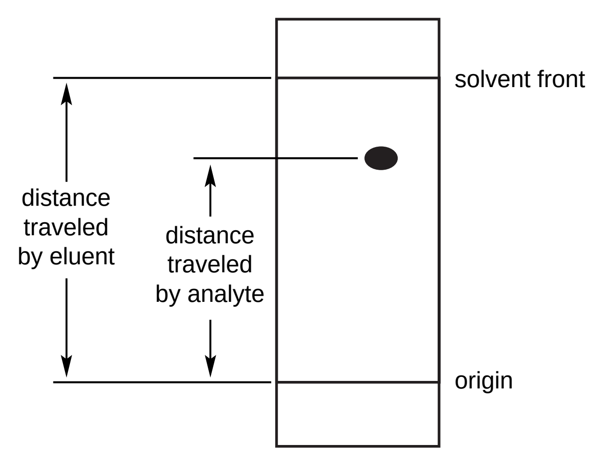

Figure 1. Determination of the retention factor, R_f.

Table 1. Percentages of Ethyl Acetate and Hexane in Eluents A, B, and C

Eluent	% Ethyl Acetate	% Hexanes
A	15	85
B	30	70
C	60	40

travel further up the plate, while the alcohol and carboxylic acid stay near the bottom. Ketones, ethers, and esters are only H-bond acceptors and are less strongly bound to the silica gel surface than alcohols and carboxylic acids, which are both H-bond acceptors and donors (Figure 2).

Part 3: Examining Polarity

Students discover that not only hydrogen bonding affects separation in TLC but polarity of the eluent plays a large role as well. The students are introduced to the concept of polarity by arranging eluents A, B, and C (Table 1) in order of increasing polarity. Students initially evaluate their relative polarities conceptually then determine these experimentally. Using phenol as the analyte, the distances traveled and the R_f values using eluents A, B, and C are determined. Then the students compare their experimental results to the conceptual results. Students find that the R_f values increase as:

Eluent A < Eluent B < Eluent C

This should be easily recognized since the concentration of the more polar ethyl acetate increases in similar fashion.

Students then conduct a qualitative TLC study with compounds containing various functional groups and arrange them in order of increasing polarity. From their experimental findings (various TLC experiments comparing two or three compounds), they are able to establish a trend in functional group polarity as follows (order of increasing polarity):

ether < ester < ketone < aldehyde < amine < alcohol < carboxylic acid

Students can explain this trend by discussing the effects of H-bonding between the molecule and silica gel.

Part 4: Application

The concluding section of the inquiry incorporates what has been learned and applies it to a simulated problem that an organic chemist could encounter. Students are told that two research groups are competing to synthesize a much anticipated anesthetic drug. The group who completes the synthesis first will receive a standing ovation and patent. Both groups are at the final step of the synthesis and the tension mounts as they wait for the completion of a two-day reaction. A member of one group runs a TLC experiment during the first day of the reaction and finds that the reaction is complete. They quietly stop the reaction, isolate the product, and are the first to complete the synthesis.

This is the stage the instructor sets for the students. The students are provided with a prepared solution of the reaction's starting material, a reaction mixture after 10 hours, a reaction mixture after 15 hours, and a reaction mixture after 48 hours (final product). These solutions are pre-prepared by the instructor simulating the course of a reaction. After ten hours a TLC shows some product formation but starting material is still present in the reaction mixture. At 15 hours only product is seen by TLC and the reaction is complete. Students should realize that there is no need to keep the reaction going and it may be stopped.

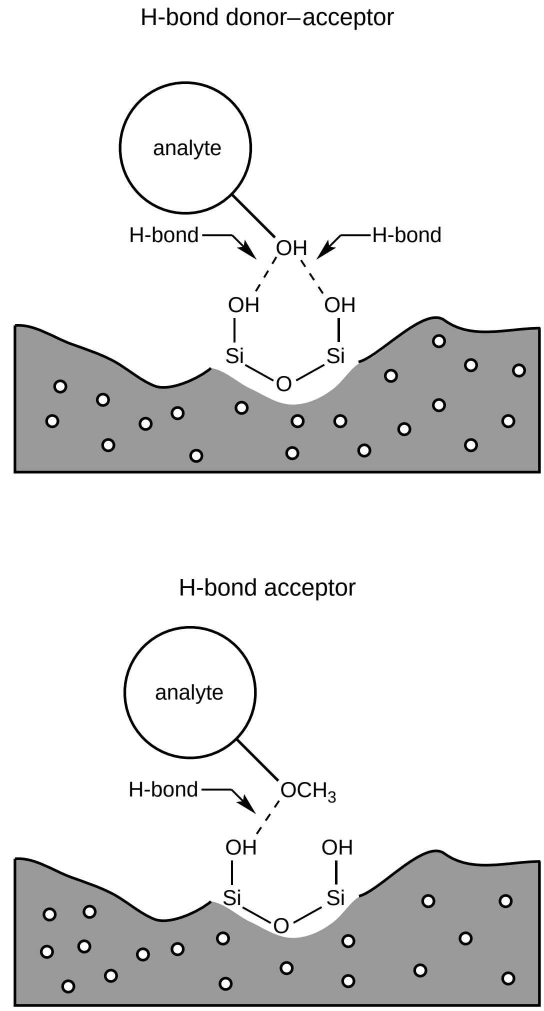

Figure 2. H-Bonding interaction of H-bond donor–acceptor and H-bond acceptor with silica gel.

Hazards

Direct viewing of the UV lamp is harmful to the eyes and should be avoided. The organic chemicals used in this activity are also hazardous to human health. Contact with the skin or inhalation should be avoided. Ethyl acetate and hexanes are flammable and irritants. Silica gel should not be inhaled. Acetophenone, anisole, benzyl alcohol, benzylamine, benzaldehyde, benzoic acid, cinnamaldehyde, cinnamyl alcohol, phenol, phenylacetic acid, and phenylacetate are irritants. Students should wear safety goggles and gloves.

Conclusion

Students are exposed to the principles and common lab practices in TLC and how organic chemists utilize this important technique. They work their way through an inquiry-based set of experiments to understand the theory behind TLC. First, spotting and developing plates of different sizes with the same compound encourages students to search for a systematic comparison, in this case a ratio that leads to the retention factor. Second, students discover the importance of hydrogen bonding in TLC separations. Students analyze a group of structurally different compounds that are either H-bond acceptors or donor–acceptor molecules. These structural differences display different behavior in TLC and students observe patterns. Hydrogen bond donor–acceptor molecules are the most strongly retained by the silica gel surface and have the smallest R_f values, whereas hydrogen bond acceptors have less interaction with the silica surface and have the largest R_f values. Students then correlate these patterns with functional group polarity. These trends show that the more polar functional groups have a greater extent of interaction with the protic silica surface. Finally, students demonstrate they have achieved the desired level of understanding of the TLC technique by solving a simulated application. At the conclusion of this last exercise, the backward design described by Wiggins and McTighe is accomplished. Students have mastered the desired results and have demonstrated this by successful application to an unknown situation *(4)*. Students learn the concepts of polarity and hydrogen bonding and then apply these principles to an unknown situation.

Acknowledgment

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[w]Supplementary Material

Student and instructor notes are available in this issue of *JCE Online*.

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