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Radial Chromatography for the Separation of Nitroaniline Isomers

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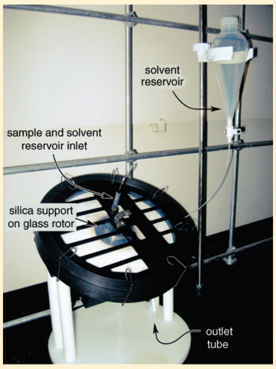
S *Supporting Information*

ABSTRACT: Separation techniques are usually presented in the undergraduate organic laboratory to teach students how to purify and isolate compounds. Often the concept of liquid chromatography is introduced by having students create "silica gel columns" to separate components of a reaction mixture. Although useful, column chromatography can be a laborious technique for students to perform. The separation efficiency can be compromised if students do not prepare the column correctly, and the time required to separate substances can extend beyond a typical laboratory period. Herein, the use of radial chromatography (centrifugal thin-layer chromatography) as an alternative to the traditional column chromatography technique utilized in many teaching laboratories is discussed. Radial chromatography can be used to introduce the principles that underlie liquid chromatography, with the added benefit of providing a relatively fast and highly efficient separation. An experiment for the efficient separation of a mixture containing 2-nitroaniline and 3-nitroanilne using radial chromatography is presented. Typical student results show that each substance can be collected with a high percent recovery (~70−80%). Furthermore, the melting range of each isolated substance is found to be in agreement with literature values. The separation can be completed in 40−50 min once the sample has been loaded.



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Separation techniques play an important role in the undergraduate organic laboratory as students must purify or isolate the compounds they synthesize. Many organic chemistry laboratory manuals detail experiments that involve the use of various types of chromatography,[1−3] with particular emphasis on what is commonly known as "column chromatography". In this technique, the stationary phase often consists of a silica slurry or a dry-packed column filled with silica. The slurry is typically poured into a column and allowed to settle before being used. Separations involving column chromatography can be slow processes (on the order of a few hours) and difficult to complete within a laboratory period since the separation is driven by gravity. In addition, the separation efficiency is largely dependent upon how well the column is prepared, which can be a difficult task for undergraduate students. Although flash chromatography can provide a faster separation, many commercial systems utilize stainless steel housings for columns, thus, making it impossible to actually see the components eluting from the column. This is a disadvantage when separating colored substances. The use of an alternative technique that could address the limitations of column chromatography, yet still provide students with an introduction to the theory and practice of liquid chromatography, would be valuable in an undergraduate organic laboratory setting. The experiment described here uses radial chromatography as a viable alternative to traditional column-based separations.

Radial chromatography (also known as centrifugal thin-layer chromatography) has found some, but not widespread, application in organic chemistry laboratory curricula.[4−8] The technique offers several advantages over traditional column chromatography including ease of operation, low solvent consumption, good reproducibility, high separation efficiency, and a reusable stationary phase (silica plates can be used multiple times). In addition, the technique is automated and allows for rapid separations when compared to traditional column experiments.

Students in our first-semester organic chemistry laboratory perform radial chromatography to separate a mixture of 2-nitroaniline and 3-nitroaniline (100−150 mg per isomer).[9] These constitutional isomers can be difficult to separate in column chromatography given their similarity in structure and the length of time required to achieve adequate resolution. These isomers are well suited for radial chromatography because they produce colored bands that can be easily identified. In our experiment, the analytes are loaded onto a 30 cm circular silica plate. The plate is rotated, and each component separates into a distinct band as the mobile phase passes through the spinning plate. As each substance elutes, students perform thin-layer chromatography to determine the relative purity of each fraction. Once the separation is complete, students combine similar fractions and remove the mobile phase through rotary evaporation. The purity of the resulting isomers is assessed by determining the melting range of each substance and comparing the values with those reported in the literature. At the end of the experiment, students can calculate the percent recovery for each substance to judge the quantitative nature of the technique.

MATERIALS

A Harrison Research Chromatotron (model 7924T) was used in this experiment. The rotor stationary phase was made of silica gel type GF with an average particle size of 15 μm, a thickness of 1

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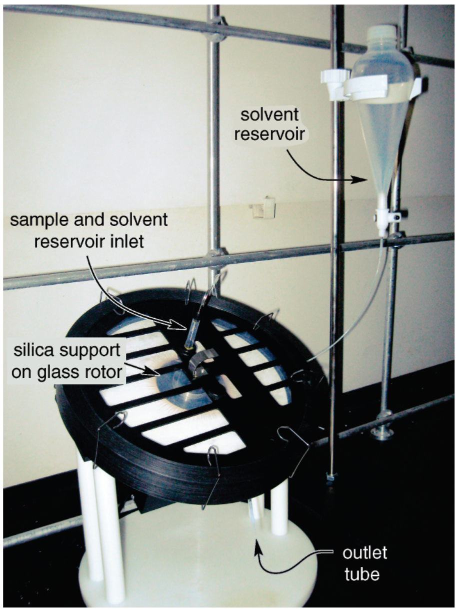

Figure 1. Schematic of the experimental setup.

mm, and a diameter of 24 cm. The rotation rate was 750 rpm.[10] Solvent was delivered from a reservoir at a rate of 7 mL/min. The solvent was delivered from a reservoir at height of 64 cm above the benchtop (Figure 1). Additional information (pricing, models) regarding the Chromatotron can be found at the Harrison Research Corporation Web site.[11]

2-Nitroaniline and 3-nitroaniline were purchased separately from Sigma-Aldrich. Thin-layer chromatography (TLC) plates were purchased from J.T. Baker, Inc. (specifically sold as "Bakerflex Silica gel IB2-F"). The TLC plate dimensions were 10 cm × 4 cm (L × W). UV-lamps were manufactured by Mineralight (Upland, CA; model UVGL-58). Melting points were measured using a Mel-Temp II melting point apparatus.

EXPERIMENT

Before using the Chromatotron, thin-layer chromatography (TLC) is performed to determine the R_f values for standards of each individual aniline isomer. These values will be used to assess the relative purity of each fraction collected from the Chromatotron. A 50:50 (v/v) mixture of ethyl acetate and hexane can be used as the developing solvent for these preliminary studies.

The Chromatotron is first fitted with a 1 mm silica gel plate and 50 mL of hexane is placed in the solvent reservoir. The plate is allowed to rotate while equilibrating with 30 mL of hexane. A 250 mL Erlenmeyer flask is temporarily used as a collection flask. 2-Nitroaniline (122 mg), 3-nitroaniline (107 mg), and 1.5 mL of ethyl acetate are combined in a small glass vial. Once the plate is equilibrated, the sample mixture is loaded onto the plate via the reservoir inlet. The plate is eluted with the remaining 20 mL of hexane, and then the solvent reservoir is filled with 100 mL of a 10:90 (v/v) mixture of ethyl acetate and hexane. The solvent reservoir is filled as needed with the same 10:90 solvent mixture until both aniline isomers have eluted. Roughly 180−200 mL of the solvent mixture was required for complete elution of the isomers.

As the silica plate rotates, each isomer is visible as a distinct colored, circular band. When the leading band is roughly 2−3 cm from the edge of the plate, the collection flask is replaced with a 16 × 150 mm test tube to begin fraction collection. Each test tube is filled to within 2−3 cm of the top. The collected fractions are analyzed by TLC to assess relative purity (using the previously determined R_f values to identity the isomers). Fractions containing the same isomer are combined and solvent is removed through rotary evaporation. The resulting solids are allowed to dry for one week before the percent recovery and melting point are determined.

At the end of the experiment, the silica plate is prepared for use in subsequent experiments by washing with 100 mL of ethyl acetate and then with 100 mL of hexane.

HAZARDS

2-Nitroaniline and 3-nitroaniline are toxic and can cause irritation to the skin, eyes, and respiratory tract. Hexane and ethyl acetate are extremely flammable. All reagents and materials (including the Chromatotron) should be kept in a well-ventilated fume hood to minimize exposure. Nitrile gloves and safety glasses should be worn at all times when performing this experiment. Students should wash their hands thoroughly before leaving the laboratory. Students should be advised not to look directly into a UV lamp and to avoid exposing the light to skin.

RESULTS

Typically, 15 fractions are collected during the course of the separation. TLC analysis revealed that early fractions (∼1−4) contained 2-nitroaniline (R_f = 0.75) whereas later fractions (∼7−14) contained 3-nitroaniline (R_f = 0.65). The relative elution order is consistent with the polarity of each substance (2-nitroaniline is less polar than the corresponding isomer so it elutes first). Once solvent was removed, 100 mg (81% recovery) of 2-nitroaniline and 76 mg (71% recovery) were obtained. The melting ranges of 2-nitroaniline and 3-nitroaniline were 65−69 °C (Lit: 71 °C) and 109−111 °C (Lit: 114 °C),[12] respectively. Separation was completed in 37 min. A table of typical student results can be found in the Supporting Information.

CONCLUSION

This experiment highlights the use of radial chromatography as an alternative separation technique for use in an undergraduate organic laboratory. Although the separation of aniline isomers is discussed here, the technique can be used to effectively separate a myriad of other organic compounds assuming the substances are colored (or visible under a UV light). Currently, continuing efforts are underway to apply radial chromatography to the separation of other substances in our organic chemistry laboratory.

ASSOCIATED CONTENT

Supporting Information

Written directions for students, instructor notes, and sample data. This material is available via the Internet at http://pubs.acs.org.

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