

A Modern Apparatus for Performing Flash Chromatography: An Experiment for the Organic Laboratory

Gregory R. Naumiec,[†] Angela N. Del Padre,[†] Matthew M. Hooper,^{†,‡} Alison St. Germaine,[†] and Brenton DeBoef^{‡,*}

[†]Department of Chemistry, University of Rhode Island, Kingston, Rhode Island 0288, United States

[‡]Westerly High School, 23 Ward Avenue, Westerly, Rhode Island 02891, United States

S Supporting Information

ABSTRACT: A modern apparatus for performing flash chromatography using commercially available, prepacked silica cartridges has been developed. The key advantage of this system, when compared to traditional flash chromatography, is its use of commercially available silica cartridges, which obviates the need for students to handle silica gel. The apparatus has been tested for its ability to perform separations that are commonly found in organic chemistry teaching laboratories, and a laboratory module that combines the techniques of thin-layer chromatography, gas chromatography, and flash chromatography is described. The performance of this new chromatography apparatus was comparable to a traditional flash chromatography column.



KEYWORDS: Second-Year Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Chromatography, Gas Chromatography, Laboratory Equipment/Apparatus, Separation Science, Thin Layer Chromatography

Column chromatography is a key technique that is used ubiquitously by synthetic organic chemists to purify small quantities of fine chemicals. The most common method for performing column chromatography for macroscale purifications in both academic and research and development laboratories is called flash chromatography and was described by W. Clark Still in 1978;¹ however, the first educational application of column chromatography was published in this *Journal* in 1956, as a method for separating three different dyes from McCormick food coloring.² Since then, the experiment has become a mainstay in introductory organic teaching laboratories, though it is now commonly performed using a microscale column or pipet to minimize waste.³

In recent years, the art of performing chromatographic separations has changed dramatically in professional laboratories, but has remained largely unchanged in the organic laboratory curriculum. The advent of automated chromatography systems, consisting of pumps, disposable columns, detectors, and fraction collectors, has streamlined the process for professional synthetic chemists. Unfortunately, these instruments, such as the Isolera, Reveleris, and Combiflash systems, which are sold by Biotage,⁴ Grace,⁵ and Teledyne-Isco,⁶ respectively, are far too costly to be used by individual students in a large, teaching laboratory. Consequently, there now exists a disconnect between the teaching of synthetic procedures and the actual techniques that are used in the chemical and pharmaceutical industries.

Additionally, the traditional chromatography methods used in teaching laboratories require that students handle silica gel, which is an inhalation hazard.⁷ Though the fine dust is often handled in a fume hood, some inevitably escapes. Silica dust also clogs the filters in ductless fume hoods, thus minimizing their ability to provide a safe working environment for students. Through the years, many modifications to Still's original procedure have been proposed in this *Journal*, mainly consisting of various methods to pressurize the chromatography column, but no methods have been proposed to avoid students handling potentially harmful silica.⁸

Additionally, the common laboratory procedures that are often used to teach column chromatography do not give students a research-like experience because they avoid the problem of selecting a solvent system for the chromatography procedure. Rather, students often separate a mixture of compounds (e.g., ferrocene and acetylferrocene) by sequentially passing a nonpolar solvent and a polar solvent (e.g., hexanes and methyl *tert*-butyl ether) down the column. This process is rarely used in a professional or academic research laboratory. More commonly, mixtures of polar and nonpolar organic solvents are used as eluents, either in an isocratic or gradient fashion, and the optimal ratio of the two (or occasionally three) solvents is determined experimentally by thin-layer chromatography (TLC).

Published: January 23, 2013

To remedy these issues and to provide a safe alternative to the traditional procedure for flash chromatography, a two-part experiment combining TLC, gas chromatography (GC), and flash chromatography was developed. Moreover, to enhance the latter portion of the experiment, a low-cost apparatus for performing flash chromatography with prepacked silica or alumina cartridges was designed. Although there is no way to economically reproduce the automated systems that are now prevalent in professional laboratories, the chromatography apparatus that is described herein mimics these systems but relies on "student automation". The makers of the automated purification systems, and nearly all silica gel companies and laboratory supply companies (e.g., Silicycle, Dynamic Adsorbents, Fisher Scientific, and Sigma-Aldrich) sell disposable cartridges that are compatible with the apparatus described herein. The silica cartridges, when used in a well-defined teaching laboratory setting, can be stored in students' drawers and reused several times before appropriate disposal.⁹

■ EXPERIMENTAL OVERVIEW

The experiment commenced with the distribution of five TLC plates, a mixture of ferrocene and acetyl ferrocene, and both a polar and nonpolar solvent (EtOAc and hexanes) to each student. The class was then shown how to perform a TLC and were directed to find the optimal solvent system for separating the mixture by trial and error (R_f of the lower spot should be approximately 0.3).¹ During the first 3-h laboratory period, the students worked in pairs to distribute the work load. Each student performed five TLC experiments using mixtures of EtOAc and hexanes in varying ratios. In the next laboratory period, the students worked independently to separate the two compounds using the apparatus shown in Figure 1 and the

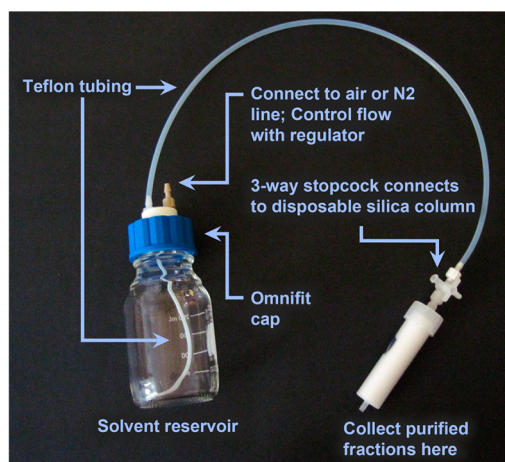


Figure 1. Flash chromatography apparatus.

solvent system that was determined in the first laboratory period. After separating the ferrocene and acetylferrocene, the purity of the isolated fractions was determined using GC. Finally, the purified fractions were rotavaped to ascertain the percent recovery of both the ferrocene and acetylferrocene. Students were graded on both the purity and percent recovery of each compound.

■ HAZARDS

Ferrocene [CAS# 102-54-5] is an irritant, is hazardous to the eyes and skin, and is hazardous if ingested. Acetylferrocene

[CAS# 1271-55-2] may be fatal if swallowed, is toxic if absorbed through the skin, and may cause eye, skin, and respiratory tract irritation. Common solvents for normal-phase flash chromatography, such as ethyl acetate [CAS# 141-78-6] and hexanes [CAS# 92112-69-1], are flammable, and hexanes are a neurotoxin. The procedure described above involves a pressurized glass bottle; consequently, all glassware should be inspected for cracks prior to use to prevent a dangerous rupture. Appropriate personal protective equipment should be used at all times, and the reagents should only be handled in a well-ventilated fume hood. MSDS sheets are freely available from Sigma-Aldrich.¹⁰

■ FLASH CHROMATOGRAPHY APPARATUS

The chromatography apparatus was constructed of a high-performance liquid chromatography (HPLC) reservoir bottle, a polytetrafluoroethylene (PTFE) stopcock, and an Omnifit cap containing two threaded ports (Figure 1). Plastic fittings were used to connect the components with Teflon tubing, as Tygon tubing was found to leach plasticizers into the eluent. Air pressure was used to push the eluent from the reservoir to wet the column, and the three-way stopcock was used to purge air bubbles from the system. The crude sample was loaded onto the column as a concentrated solution, by first removing the column from the system and then injecting it with the sample using a 1 mL Luer-Lok syringe. The cartridge was then reattached, and eluent was again pushed through the system by adjusting the air pressure. Following the collection of fractions, the used silica column was safely discarded or flushed with a polar organic solvent (e.g., EtOAc or acetone), dried with air pressure, and then stored for reuse. Importantly, throughout this entire procedure, students were able to quickly perform preparatory silica gel chromatography without having to manipulate loose silica gel.

■ RESULTS

On the first day of the experiment, the students typically coalesced on a solvent system of either 15% or 20% EtOAc in hexanes. Either solvent system worked well for the subsequent flash chromatography. During the purification, both the ferrocene and acetylferrocene were typically eluted within 10–12 test tubes (13 × 100 mm). A separation of 50 mg of ferrocene and acetylferrocene on a 4 g silica column used approximately 75 mL of the eluent, which is comparable to traditional flash chromatography but is a significant increase from the pipet columns that are commonly used in organic teaching laboratories. As with conventional flash chromatography, the fractions could be collected quickly: a 10 mL test tube could be filled in approximately 5 s. The end point was easily determined, as the acetylferrocene fractions were red-orange. Students usually had no more than two tubes of overlapping spots, as analyzed by TLC, and often had no tubes with overlapping spots (ideal separation). Poor separation was observed if the students failed to correctly prepare the column or if they loaded their mixture onto the column in pure EtOAc. The typical percent recoveries ranged from 40% to 80%.

A simple GC method was developed to analyze the purity of either a ferrocene or acetylferrocene sample in less than 5 min. One of the most time-consuming steps in the two-day experiment was the rotary evaporation. This step could easily be omitted to save time, though it is necessary to obtain percent recovery data.

■ DISCUSSION

The cost of this chromatography apparatus was comparable to that of a glass chromatography column (~\$100). Additionally, this apparatus was much more durable than glass chromatography columns and pipets which are often broken by students, resulting in replacement costs and broken glass hazards. The air pressures required to perform the analysis was comparable to that used to perform a traditional flash chromatography. Typical silica gel cartridges containing 40–63 μm amorphous silica could be reused six times. More expensive columns containing spherical silica particles could be reused ten times without a loss in performance.

Over 300 undergraduate students have performed the two-day TLC and flash chromatography experiment during the past three years. The experimental results obtained using the apparatus shown in Figure 1 were virtually identical to those obtained in previous years using conventional flash chromatography.

■ CONCLUSION

In summary, the apparatus presented herein provides organic laboratory students with a viable alternative to conventional flash chromatography using loose silica or alumina. A two-day laboratory exercise that teaches introductory organic chemistry students to use thin-layer chromatography, gas chromatography, and flash chromatography to separate ferrocene from acetylferrocene using the aforementioned apparatus may be found in the Supporting Information associated with this article. Additionally, two short videos demonstrating the construction of the flash chromatography apparatus shown in Figure 1 and its operation have been developed to facilitate the incorporation of this apparatus into the organic laboratory curricula at other colleges and universities.

■ ASSOCIATED CONTENT

📄 Supporting Information

Student instructions, instructor notes, and two videos demonstrating the construction and operation of the apparatus. This material is available via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: bdeboef@chm.uri.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.M.H. is a recipient of a generous scholarship for aspiring high school chemistry teachers from the Hach Scientific Foundation. The DeBoef group is funded by the National Science Foundation (CAREER 0847222), the National Institutes of Health (NIGMS, 1R15GM097708-01) and a Pfizer Green Chemistry Grant.

■ REFERENCES

- (1) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
- (2) Tracy, M. J.; Sueltenfuss, E. A. *J. Chem. Educ.* **1956**, *33*, 281.
- (3) Svoronos, P.; Sarlo, E. *J. Chem. Educ.* **1993**, *70*, A158.
- (4) Flash Purification Systems, Biotage AB, Uppsala, Sweden, 2011. <http://www.biotage.com/DynPage.aspx?id=101917> (accessed Jan 2013).

(5) Grace Discovery Sciences—The Reveleris Flash System. <http://www.discoverysciences.com/product.aspx?id=6234> (accessed Jan 2013).

(6) Teledyne Isco – Organic Purification Systems. <http://www.isco.com/products/products2.asp?PL=10110> (accessed Jan 2013).

(7) Husgafvel-Pursiainen, K.; Kannio, A.-M.; Oksa, P.; Suitala, T.; Koskinen, H.; Partanen, R.; Hemminki, K.; Smith, S.; Rosenstock-Leiby, R.; Brandt-Rauf, P. W. *Environ. Mol. Mutagen.* **1997**, *30*, 224–230.

(8) For examples, see: (a) Feigenbaum, A. *J. Chem. Educ.* **1984**, *61*, 649. (b) Thompson, W. J.; Hanson, B. A. *J. Chem. Educ.* **1984**, *61*, 645. (c) Bell, W. L.; Edmondson, R. D. *J. Chem. Educ.* **1986**, *63*, 361. (d) Jacobson, B. M. *J. Chem. Educ.* **1988**, *65*, 459. (e) Krause, J. G. *J. Chem. Educ.* **1991**, *68*, 790. (f) Deal, S. T. *J. Chem. Educ.* **1992**, *69*, 939. (g) Davis, J.; Vaughan, D. H. *J. Chem. Educ.* **1995**, *72*, 266–267. (h) Horowitz, G. *J. Chem. Educ.* **2000**, *77*, 263–264. (i) Pontén, F.; Ellervik, U. *J. Chem. Educ.* **2001**, *78*, 363. (j) Butler, J. D.; Choung, W.; Kurth, M. *J. Chem. Educ.* **2010**, *87*, 1265.

(9) Sereda, G. *Chem. Educator* **2005**, *10*, 306–307.

(10) Sigma-Aldrich Home Page. <http://www.sigmaaldrich.com> (accessed Jan 2013).