JOURNAL OF CHEMICAL EDUCATION

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Laboratory Experiment

Quinone Synthesis and a Visual Introduction to Column Chromatography: An Undergraduate Experiment

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Cite This: *J. Chem. Educ.* 2022, 99, 3731−3734

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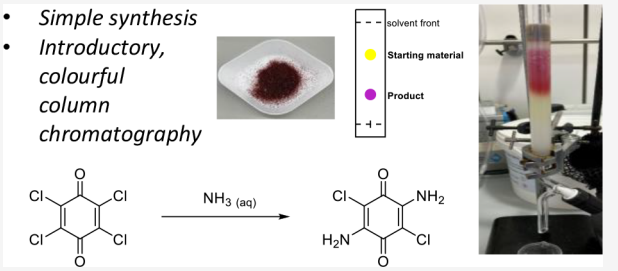
ABSTRACT: An introductory normal phase column chromatography experiment is described and evaluated for second year undergraduate chemistry laboratory class. The experiment involves a nucleophilic substitution yielding a crude mixture of reactant and one product, both of which are brightly colored and contrasting, creating a clearly visual separation when purified using column chromatography. A student survey was conducted which showed that 75.4% of students (n = 53, approximately 50% of year group) agreed or strongly agreed that the experiment worked well for them, and 88.7% agreed or strongly agreed that the experiment was enjoyable. Every student was also assessed in the lab on their practical technique, with the year group achieving an average of 72.1% in this assessment.

- *Simple synthesis*
- *Introductory, colourful column chromatography*



KEYWORDS: *Second-Year Undergraduate, Laboratory Instruction, Chromatography, Thin Layer Chromatography, Nucleophilic Substitution, Organic Chemistry*

BACKGROUND

Column chromatography is a technique which has remained generally much unchanged since its inception. However, this technique is still commonly used in research laboratories as a purification process and so is, therefore, generally taught in the undergraduate (UG) chemistry laboratory curriculum. There have been several publications describing visual, colorful banded columns for an introduction to column chromatography,[1−4] and there have been publications involving column chromatography and synthesis;[5−7] however, there are currently no publications combining both a simple synthesis and visually indicative column chromatography separation. Here, we describe an undergraduate experiment which combines the context of a simple organic synthesis of a quinone derivative (quinones are known for being highly colored),[8] and subsequent purification using a simple example of column chromatography, which features the important colorful bands needed for a visually accessible, first introduction to this challenging but important technique.

EXPERIMENTAL SECTION

All materials were purchased from Sigma-Aldrich with no further purification.

p-Chloranil (0.1 g) was dissolved in ethyl acetate (1 mL). Concentrated aqueous ammonia was added to this mixture (3 drops via Pasteur pipet, roughly 0.2 mL). This was then put to the side while the column was set up (ca. 20−30 min). A silica gel column was set up employing the "slurry pack" method using petroleum ether and ethyl acetate (4:1) as eluent. Petroleum ether (2 mL) was added to the reaction mixture, and this was wet loaded onto the column, before the crude substance was purified by column chromatography. Students collected a crude thin layer chromatography (TLC) plate using the same solvent mixture and performed TLC analysis of every fraction from the column. Product can be isolated as a dark purple solid (0.02 g, 23%, >300 °C).[9] δH (500 MHz, DMSO-d_6) 8.1 ppm (s). δC (125 MHz, DMSO-d_6) 99.98, 149.33, 170.63 ppm.[12]

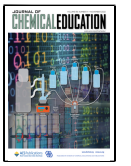
HAZARDS

p-Chloranil is an irritant and is toxic to aquatic life. Concentrated ammonium hydroxide (ammonia solution) is corrosive, causes severe burns, and gives off noxious fumes. Ethyl acetate is flammable and an irritant. 60−80 °C petroleum ether is flammable, is a potential carcinogen, and is a potential mutagen. Silica gel is a respiratory hazard. All work should be conducted in a fume hood, wearing appropriate PPE.

DISCUSSION

This experiment begins with the short synthesis of 2,5-diamino-3,6 dichloro-1,4-benzoquinone. The starting material, p-chloranil, a yellow solid, is reacted in the presence of concentrated aqueous ammonia. When left for a short time

Received: September 2, 2021
Revised: September 27, 2022
Published: October 10, 2022



https://doi.org/10.1021/acs.jchemed.1c00940
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(approximately 20−30 min in our UG laboratories while students setup the column), this produces a crude mixture of starting material and product. While it was convenient for us to leave the reaction for 20−30 min in a teaching setting, we have found it can be left for as little as 2−3 min to the same effect. This incomplete conversion is beneficial in this context as it allows a simple mixture for students to work with for an introductory column. If desired, the reaction could be pushed to completion by heating the reaction under reflux and monitoring progression using TLC. Students were encouraged to obtain TLC of this crude mixture and use this to refer to throughout the column purification (Figure 1). When

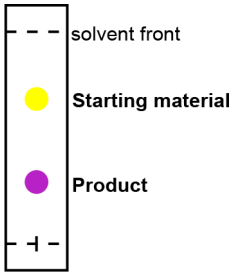
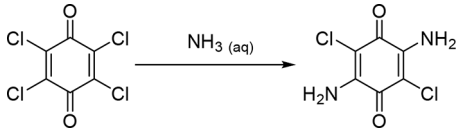


Figure 1. Crude TLC as viewed under UV light (ChemDraw).

performing TLC analysis in lab, it was noted that, despite the vivid colors clear in the column, UV light was required to view the spots on the TLC plates due to the dilution of the compounds in the mixture.

The reaction itself is an interesting example of nucleophilic substitution, the theory of which could be explored in the laboratory session, albeit not the focus of the introductory experiment in this case (Scheme 1).[10,11]

Scheme 1. Reaction of p-Chloranil with Concentrated Aqueous Ammonia to Synthesize 2,5-Diamino-3,6 dichloro-1,4-benzoquinone



Once loaded onto the column, students can clearly see the two bands moving through the silica and separating due to the vivid colors of the starting material (yellow) and product (purple), as depicted in Figure 2.

Students were encouraged to watch a technique video of column chromatography before beginning the practical. This technique video was filmed in the teaching laboratories by laboratory teaching staff, so it provided an authentic example of column chromatography. In fact, when asked, students all agreed or strongly agreed that watching this video was a useful exercise before attempting the technique. Students set up the column using the slurry pack method, using a sintered glass column. For this experiment, it was optimal to use a column of ca. 2.5 cm in diameter, and to fill this to around a third with silica slurry (ca. 6−7 cm). Once the students had added enough silica gel slurry to fill the column halfway, they added a little more solvent mixture and packed the silica using rubber bellows. A cautious approach to loading was taken in view of the inexperience of the students having a greater chance of disturbing the top of the silica column. Therefore, once

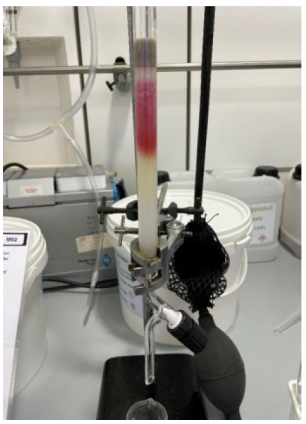


Figure 2. Typical column chromatography setup showing yellow and purple band separation.

packed, students added a thin layer of sand and allowed the solvent to pass down to the top of the sand before loading the crude sample. This crude reaction mixture (reaction in approximately 1 mL of ethyl acetate) was diluted with petroleum ether and then transferred to the column (not adding the petrol led to a poor separation). This sample was then also allowed to flow down to the top of the sand before adding more sand and then topping up with the solvent mixture. The solvent mixture for this column was 4:1 (petroleum ether:ethyl acetate) which was premixed for students to save time. Under this solvent mixture, the two bands separate into two very clearly visible bands as shown in Figure 2. Students monitored the progress of the column using TLC and typically collected 12 fractions, each being roughly 10 mL. If time permitted, the students could isolate the more polar purple band resulting from amino-substitution.

Students performed TLC analysis of each fraction while running the column using the same solvent mixture and visualized it using UV light. Figure 3 is representative of typical TLC analysis from this column.

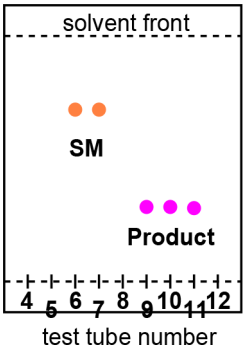


Figure 3. Typical TLC analysis of fractions from column chromatography.

EVALUATION

In a typical lab class of 3 h, students performed the short reaction, set up the column, and confirmed isolation of both product and reactant by TLC analysis. Students performed TLC analysis of each fraction, and the importance of doing so, even when the bands are clearly visual, was emphasized. In this short lab session, the main objective was to introduce students

https://doi.org/10.1021/acs.jchemed.1c00940
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to this complex technique and develop student confidence in this area; as such, there was not sufficient time for students to remove the solvent and analyze fractions during this short lab session. However, copies of IR and NMR spectra of product are included in the Supporting Information, both of which are consistent with data from the literature.[12] The melting point of this compound was also found to be over 300 °C; the literature melting point value is 360 °C.[9] In a longer lab session, the experiment could be extended to include isolating the fractions containing the product and a discussion of the nucleophilic substitution mechanism. It is recommended that the reaction be pushed to completion or conducted on a larger scale if isolating the product for analysis, as the yield for the reaction and purification on the scale suggested here is very small (typical mass of product is 0.02 g). For more advanced second year students, it could also be possible to determine the appropriate solvent mixture through TLC solvent tests and to perform suitable analysis on each isolated compound.

In an evaluation of this experiment (n = 53), students were shown the following five statements and asked to respond on the 5-point scale:

- Question 1, I found the column chromatography experiment enjoyable.
- Question 2, I felt the column chromatography experiment was pitched at the correct level.
- Question 3, The column chromatography experiment worked well for me.
- Question 4, The column chromatography technique video was useful to watch before attempting the experiment.
- Question 5, I learnt new things about column chromatography during this experiment.

Figure 4 shows the summary of the findings from this survey:

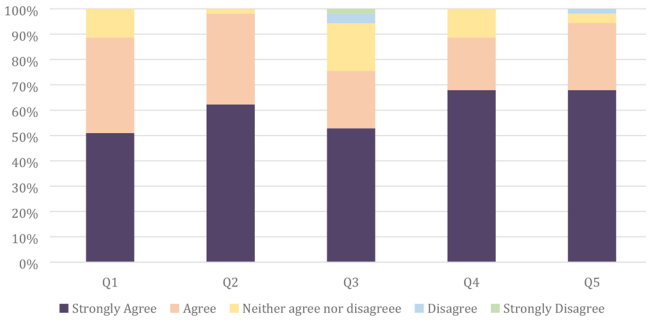

Figure 4. Graphical summary showing student evaluation of the column chromatography experiment.

A significant challenge in teaching column chromatography is that it is very process-heavy, with many sequential instructions that need to be performed in the correct order in a timely fashion. This, coupled with the multitasking aspect of running the column and doing TLC analysis, is a recipe for an overall unenjoyable student experience. In this survey, 89% of students surveyed agreed or strongly agreed that this experiment was enjoyable to do. There are many fine details that come with designing an "enjoyable" experiment from a student's point of view, but something that could be rationalized as connected is the student performing an experiment which is pitched at the right level and doing an experiment that works. In this synthesis and introductory column experiment, 98% of students agreed or strongly agreed that the experiment was both pitched at the right level, and 75% agreed or strongly agreed that the experiment worked for them.

From an instructor's perspective, it is clear that this experiment succeeds in providing students with a gentle introduction to column chromatography. One instructor saying "this experiment worked well to show students the principles of column chromatography clearly. It was also helpful to be able to contextualize this technique using a reaction and explore the purpose of column chromatography with students". Students mostly struggled with the multitasking aspect of performing column chromatography, particularly setting up the column. However, students did report that they found the experiment a sufficient introduction to the technique, with one student saying, "Since it was a new technique, I was quite nervous for this lab. However, now that I have completed it, I feel a lot more confident in that technique." Students are also subject to in-lab assessment for this experiment which focuses on practical technique, laboratory skills, and professional skills. For this experiment, the cohort average was 73.8%; this grade (1st) suggests that, overall, students were competent at performing this technique effectively and safely.

CONCLUSIONS

A new experiment for introduction to column chromatography has been designed and evaluated which combines both the visual separation of bands and contextualization in a simple synthesis. On evaluation, it was found that a significant proportion of students thought:

- The experiment was enjoyable.
- The experiment worked.
- New things were learnt about column chromatography.
- The experiment was pitched at the correct level (for a first introduction to column chromatography technique).
- Watching a technique video before the session was useful before attempting the experiment.

There are opportunities to expand this for a lengthier laboratory session. The nucleophilic substitution could be explored in more detail. Isolation of the purple product resulting from amino-substitution could also be analyzed using IR spectroscopy or NMR spectroscopy.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00940.

Student protocol (PDF, DOCX)
IR and NMR data (PDF)

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https://doi.org/10.1021/acs.jchemed.1c00940
J. Chem. Educ. 2022, 99, 3731−3734

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank the University of Warwick, Department of Chemistry, for financial support.

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https://doi.org/10.1021/acs.jchemed.1c00940
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