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A Guided-Inquiry Experiment: Separation of a Five-Component Mixture by Liquid-Liquid Extraction and Column Chromatography

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Abstract In this article we present a guided-inquiry based approach to separation of a five-component mixture using acid-base chemistry and column chromatography. In this approach students are required to come up with a viable protocol to successfully separate a five-component mixture of unknown compounds and use ^{1}H NMR spectroscopy and melting point determination to identify the compounds. In guided-inquiry based approach presented here the students are assigned an unknown mixture containing a strongly acidic, weakly acidic, basic, polar-neutral and nonpolar-neutral components to separate and solutions to be used for extraction and the students are asked to draw a flow-chart outlining their separation protocol. This approach challenges the students to apply fundamental knowledge gained in lecture of acid-base chemistry and intermolecular forces to come up with a separation protocol.

Keywords Guided-inquiry based liquid-liquid extraction, Acid-base extraction/column chromatography, Organic chemistry separation laboratory experiment

1. Introduction

Separation is one the fundamental techniques used for purification and isolation of compounds in the laboratory. Most separation techniques used in synthetic organic chemistry laboratories rely on acid-base chemistry and intramolecular and intermolecular forces. Acid-base chemistry allow for separation of mixtures via liquid-liquid extraction techniques. Intramolecular forces allow for separation of mixtures via distillation techniques and intermolecular forces allow for separation of mixtures via chromatography techniques and recrystallization techniques. The understanding of the fundamental principles behind these techniques is therefore crucial in training of organic chemists. In the late 1980s a laboratory experiment on separation of a five-component mixture was reported. This experiment was designed as "recipe-based" approach in which students follow a given procedure to achieve the separation. [1] Most recently, "recipe-based" approaches have been developed for a laboratory experiment on the separation of the components of a commercial analgesic tablet [2], and separation of two mixtures of unknown organic compounds [3]. Also, a guided-inquiry experiment in which students were assigned a mixture of compounds and are required to design their own separation procedures has been reported even though the mixture was of only three-components. [4]

The guided-inquiry separation and characterization of the five-components in the unknown mixtures we report here will take a total of three laboratory meetings. In the first meeting the students are assigned unknown mixture containing a nonpolar-neutral component, a polar-neutral component, a basic component, a weakly acidic component and a strongly acidic component. Representative structures of compounds in the unknown mixtures are shown in table 1.

Additionally, the students are provided with a list of reagents that they will use to perform the separation. The students are then required to design a separation protocol and draw a flow-chart outlining how the separation will be achieved.

The acidic nature of the strongly acidic (benzoic acid derivatives, pK_a ≈ 4) and the weakly acidic (phenol derivatives, pK_a ≈ 10) components will allow their separation from the mixture and from each other. Similarly, the basic nature of the basic component (aniline derivatives) will allow for its separation from the mixture. The neural components will be separated from each other via column chromatography based on their polarity. Each component in the mixture will then be identified based on its characteristic ^{1}H NMR spectra and melting point.

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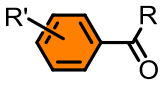
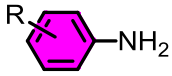
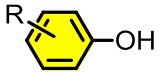
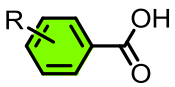
Table 1. Components in the Unknown Mixture

Components	
	Nonpolar neutral
	Polar neutral
	Basic
	Weakly acidic
	Strongly acidic

2. Method

Experimental Procedure

In this experiment students may work in pairs or individually. On the first laboratory meeting students are assigned an unknown mixture containing the five components. Also, students are provided with solutions and solvents to be used in the experiment (table 2). Students are then required to design a procedure in the form of a flow chart (see example illustrated in figure 1) outlining how they intend to achieve separation of the mixture. The instructor has to approve the student's suggested procedure before they can proceed with the experiment.

Table 2. Other Reagents Used in this Experiment

Organic Solvents	Extraction Solutions
- Ethyl acetate	- 1M HCl - 1M KOH - Sat. $NaHCO_3$
For chromatography - 100% hexanes - 20% ethyl acetate in hexanes	

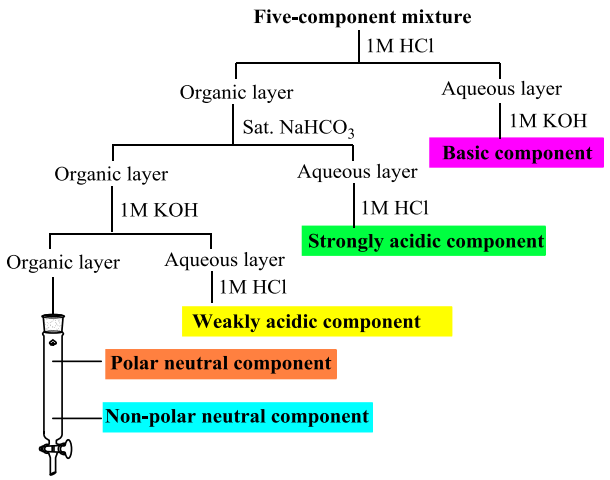


Figure 1. An example of a flow chart outlining the separation procedure for a five-component mixture

During the first laboratory meeting the students will successfully separate three of the five components via liquid-liquid extraction. During the second laboratory meeting the students will successfully separate the two remaining components using column chromatography. During the third laboratory meeting the students will characterize the separated five components via ^{1}H NMR and melting point determination and identify them from a list of possible compounds in the unknown mixture whose Chemical Abstracts Service (CAS) numbers were provided.

Format of the Classroom

The structural chemistry course (CHEM3600L) at Georgia College (GC), where this laboratory experience was implemented, is a 2 credit hour course with 4 contact hrs. per week. The class meets once a week. This class can be considered an extension of Organic I and II laboratory that is traditionally taught at the undergraduate level, but with an emphasis in spectroscopy. While the designation of the course is a laboratory course, instructors of record may use part of this class time to teach students different spectroscopic methods in a lecture style/active learning environment.

The course contains at least 4 experiments which are all guided inquiry, including the one presented here. The instructor typically provides general guidelines about the experiment of the week, but students are asked to design their own experimental procedures prior to coming to class. A typical class size for CHEM3600L at GC is 20 students. The students are mostly chemistry or biology majors looking to fulfill their chemistry requirements. A pre-requisite of Organic Chemistry I, II, and their associated laboratory components is required to enroll in the course. This laboratory, therefore, works well for a small class size. The laboratory experience would work well for larger class sizes if it is divided into two sections per week.

3. Results

Most preliminary procedures were approved by the instructor of record on the first day of the experiment with minimal changes. Students were also asked to provide references to any source that they might have used for their work in proper ACS format.

The majority of instructor changes to student led procedures included: wrong order of addition of reagents, wrong glassware usage, and wrong terminology. Upon the completion of the 3 weeks, ~70% of student groups could successfully isolate the five components.

During the experiment, it was observed that most students had acquired proper liquid-liquid separation techniques from previous organic chemistry laboratory courses. In the event that separations would not work, rotovap-evaporation procedures were implemented to "check" for substances and progress. Some groups even implemented solid-liquid separation techniques *via* filtration of precipitates.

After the isolation process, students successfully characterized their components using traditional spectroscopy techniques such as IR and NMR. In addition to providing melting points for each substance. The substances were therefore identified *via* many reproducible methods through a straight comparison to literature published spectra and melting points. The low risk high learning potential of this experiment, makes it ideal for any class that involves upper level majors in spectroscopy.

4. Conclusions

This experiment was successfully performed by students in structural chemistry course, an advance laboratory course for students who had already taken two semesters of organic chemistry. The students were delighted to know that no matter which extraction solvent they chose to start their separation protocol they achieved separation. We are currently developing a guided-inquiry experiment for separation of a six-component mixture.

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