Exp 2 – Acid-Base Extraction and Isolation of Excedrin Components

The solubility of organic compounds is primarily dependent on polarity. You may recall "*like dissolves like,*" meaning polar compounds dissolve in polar solvents and non-polar compounds dissolve in non-polar solvents. It is safe to assume that most organic compounds of medium to low polarity have limited solubility in water. More polar compounds like alcohols are more likely to be soluble in water, but are only sparingly soluble when there are six or more carbons present in the molecule. In this lab, students will utilize acid-base chemistry to separate a mixture based on preferential solubility in water or ethyl acetate, a polar organic solvent. Excedrin is over-the-counter analgesic containing the active ingredients aspirin (ASP), caffeine (CAF), and acetaminophen (ACE) that can be separated through **acid-base extraction**.

Acids (HA) react with bases (B) to form a conjugate base (A^-) and a conjugate acid (^+BH). It is likely that one or both of the products are ionic compounds, making them significantly more soluble in water than their non-charged counterparts. In this experiment, we will learn how to take advantage of this change in solubility for the separation of a mixture of acids and bases.

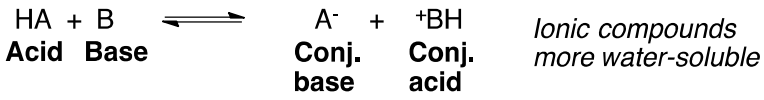


Figure 1. A general acid-base reaction.

The functional groups of interest in organic acid-base chemistry are strongly acidic carboxylic acids, weakly acidic phenols, and basic amines. Carboxylic acids are deprotonated equally well by weak and strong bases such as $NaHCO_3$ and NaOH, respectively. The by-products are different but both reactions form a **sodium carboxylate salt**, which is likely water-soluble (**Figure 1a**). Phenols do not react with $NaHCO_3$ and instead require a strong base for reaction to occur, resulting in a water-soluble **sodium phenoxide salt** (**Figure 1b**). Amines react with strong acids to form water-soluble **ammonium chloride salts** (**Figure 1c**).

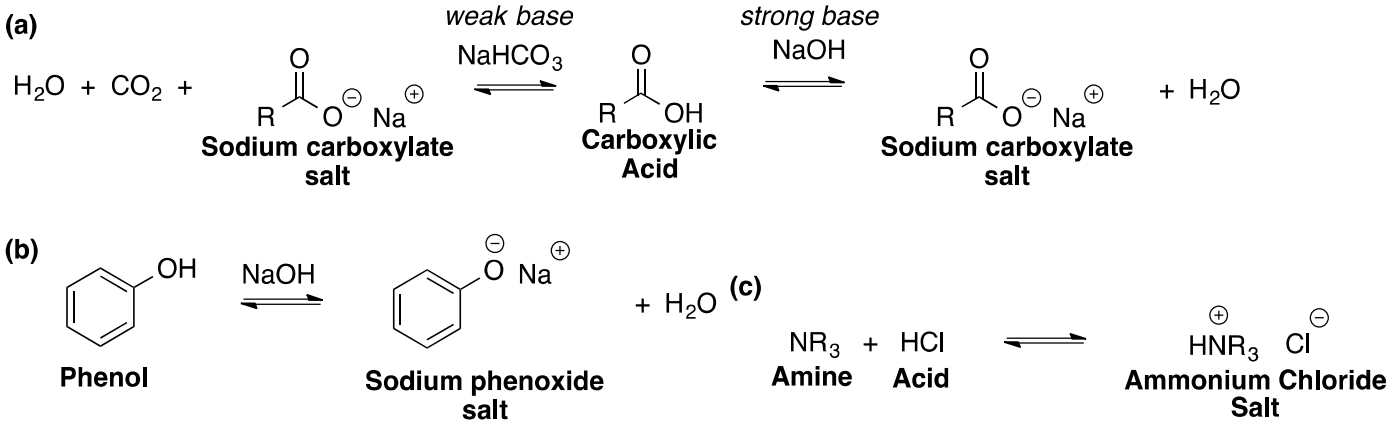


Figure 2. Reactions between **(a)** carboxylic acids with a weak and strong base; **(b)** phenols with a strong base; and **(c)** amines with a strong acid.

When both acids and bases are present in a mixture, a liquid-liquid extraction is carried out and one of the reactions above is performed. The mixture is dissolved in an organic solvent and a solution of either acid or base is added. The unreacted component is extracted in the organic layer and the reacted component, a salt, is transferred to the aqueous layer.

Suppose you're separating a mixture containing a carboxylic acid, phenol, and an amine. The extraction can be started in one of two ways: (1) react the carboxylic acid with a weak base or (2) react the amine with an acid. Either way will theoretically work, but let's work through the example that starts with a mildly basic extraction (**Figure 3**).

The mixture is dissolved in an appropriate organic solvent, in this case ethyl acetate (EtOAc), and this solution is extracted with a weak base (ex. Sodium bicarbonate or dibasic potassium phosphate). The organic layer (**ORG**) contains unreacted phenol and amine. The mildly basic aqueous layer (**AQ_{basic}**) contains the carboxylate salt (the conjugate base of a carboxylic acid). The carboxylic acid is protonated with acid, thus precipitating from the solution, and permitting isolation *via* filtration. The remaining organic layer is extracted with a strong hydroxide base to deprotonate the phenol, leaving the phenoxide salt (conjugate base of phenol) in the basic aqueous layer and the unreacted amine in the organic layer. The amine can be isolated by drying (ex. Sodium or magnesium sulfate), filtering off the drying agent, then evaporating the solvent in a rotary evaporator (rota-vap). The phenoxide salt must be acidified (reprotonated) before being filtered and isolated, as was done with the carboxylic acid.

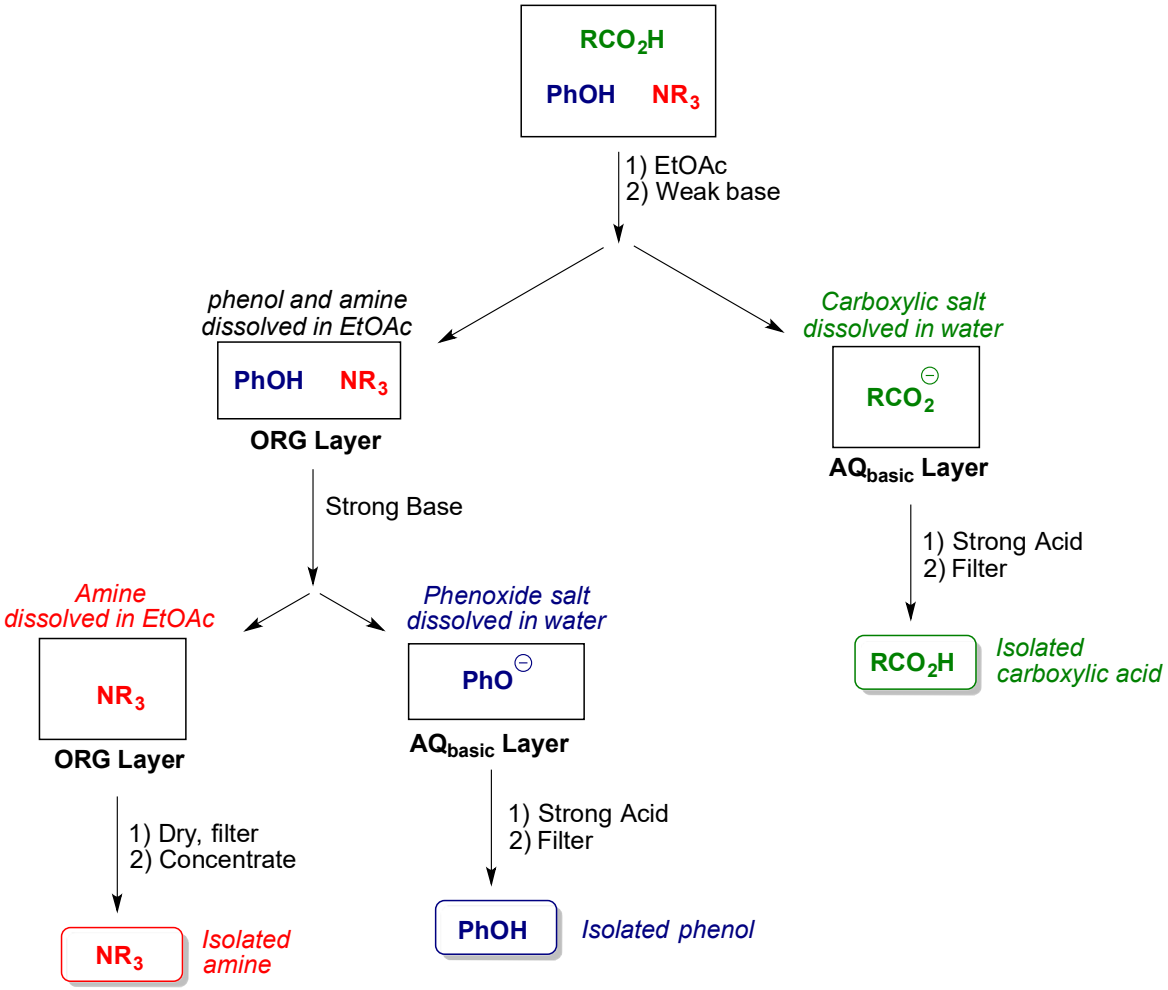
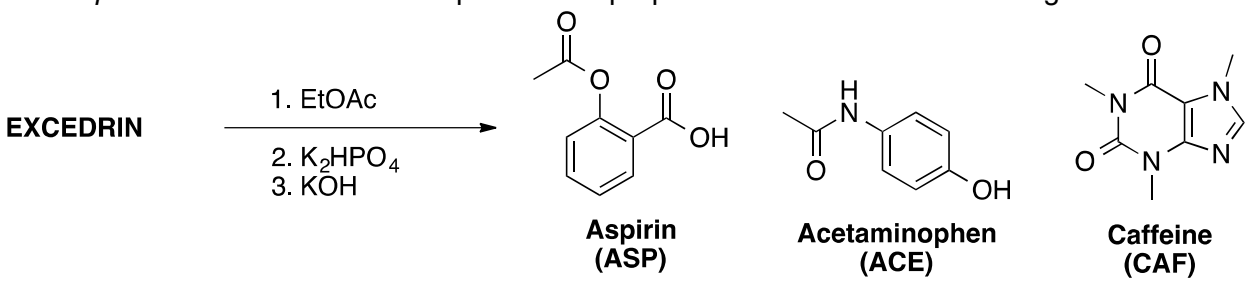


Figure 3. Flow chart for the acid-base extraction of a carboxylic acid from an amine.

A similar procedure will be carried out in the separation of the three active ingredients in Excedrin. Keep in mind that in each extraction, there is no guarantee that 100% of the compounds end up in the expected layer (refer to the reading on Partition Coefficients). TLC will be used to determine the effectiveness of the separation of each component. IR spectroscopy will be used to confirm the identity of each compound.

Notebook Preparation – Turn in with lab report (after 'experimentation' not before). *See sample notebook page online for purpose / table format*

- *Purpose* – one-sentence description of the purpose in addition to the following scheme:



- *Reagent table* – Amount (mg or mL), MW, bp or mp, density, and one-word hazards found in the clean-up/safety table for each of the chemicals in the scheme above. For Excedrin, list only the amount to be used and leave space to write down the actual mass used in lab.
- *Procedure:* hand-drawn 'comic strip' with diagrams of all equipment and chemicals with amounts. Include pertinent notes from the Clean-up & Safety Table. **This should include the completed flow charts from lecture.**
- *Safety & Clean-up* – (incorporate pertinent notes at appropriate stages within the procedure above)

EXPERIMENTAL PROCEDURE – Students work in pairs

**This experiment includes many different clear, colorless liquids. Containers must be labeled with the contents, your initials, and experiment date before the material is inside.

ACID-BASE EXTRACTION ***Frequently refer to flow charts along with written procedure.***

All steps involving ethyl acetate (EtOAc) must be performed in the fume hood. Only one student from each pair should be at the fume hood at a time – rotate different parts of the experiment. Your TA must approve your separatory funnel technique with water before you begin the experiment.

Separation of the active ingredients in Excedrin - Obtain the mass of a single tablet of Excedrin® and crush using a mortar and pestle. Add 20 mL of EtOAc to the mortar and mix with a stir rod in the fume hood for 5 minutes. The three active components will dissolve and the starch binder (inactive ingredients) will not. Decant the solution into a small glass funnel with a small piece of cotton using a glass stir rod to aid in the transfer. Collect the filtrate directly in a separatory funnel secured on a support ring on a ring stand.

Extraction with weak base - Add 10 mL of $K_2HPO_{4\,(aq)}$ (aqueous dibasic potassium phosphate) to the separatory funnel. Cap then invert the funnel twice, holding onto the cap. Vent into the fume hood by holding the funnel upside-down and open the stop cock with the tip pointing *away from your face*. Continue to mix and vent frequently for at least 3 minutes. A chemical reaction is taking place and proper time must be given for components to travel to the preferred layer. Drain the mildly basic aqueous layer containing deprotonated aspirin into a labeled scintillation vial (**K_2HPO_4 – ASP AQ**) and set aside. The organic layer will remain in the separatory funnel. Extract the organic layer with an additional 3 mL of K_2HPO_4 (add 3 mL of $K_2HPO_{4(aq)}$, mix and vent for several minutes, then drain into the **K_2HPO_4 – ASP AQ** vial). The organic layer remains in the funnel. One student in the pair should move onto "**Isolation of Aspirin**" using the combined **K_2HPO_4 – ASP AQ** extracts.

Extraction with strong base - Add 10 mL of 1 M KOH to the separatory funnel. Mix the layers for 3 minutes (vent early and often into the fume hood). Drain the aqueous layer containing deprotonated acetaminophen into a second small, labeled container (**KOH – ACE AQ**) and set aside. Extract the organic layer with an additional 3 mL of KOH (add 3 mL of KOH, mix & vent for a few minutes, then drain the aqueous layer into the **KOH – ACE AQ** vial). Keep the organic layer in the funnel.

Isolation of caffeine - Wash* the remaining organic layer with 10 mL of *aq*. NaCl (brine). Separate the layers, draining the organic layer into a small, labeled Erlenmeyer flask. The brine wash (aqueous) should be kept in a separate container labeled "waste" and transferred into the liquid waste at the end experiment. Use an additional 2 mL of EtOAc to rinse any residual caffeine from the walls of the separatory funnel. Remove any visible water from the bottom of the Erlenmeyer using a pipet. Dry the organic layer by adding two spatula tips of anhydrous sodium sulfate (Na_2SO_4). Allow the capped organic layer to sit with occasional swirling for 5 minutes (move onto one of the isolation steps below while waiting). Decant the organic layer using a small glass funnel with loosely packed cotton into a pre-weighed 50 mL RBF. Concentrate the dried organic extracts using a rotary evaporator (rota-vap). This concentrated caffeine extract may either be a liquid or solid, depending on purity. Obtain the <u>mass of caffeine</u> by difference with the original flask then transfer into a labeled vial.

Isolation of Aspirin – This step may be performed on the benchtop. Tear one 2-inch piece of pH paper into many small squares to conserve and put the pH paper back where it belongs. Determine the pH of the **K_2HPO_4 – ASP AQ** solution by dipping a stir rod into the solution then touching to a small piece of pH paper on a watch glass. Obtain 10 mL of 6 M HCl in a labeled test tube. Slowly add 6 M HCl drop-wise to the **ASP AQ** solution, swirling and taking pH readings after every 5-10 drops, until the solution is acidic (pH 2 or less). Do not rush this process! Re-label the vial "**Acidic ASP AQ.**" It may be necessary to get additional 10 mL portions of HCl. Please conserve and take only small amounts at a time (see pre-lab #4).

Isolation of Acetaminophen – Carry out the same acidification procedure used to isolate aspirin. Label the vial "**Acidic ACE AQ**."

Acidic aqueous extraction – The neutral protonated compound, either aspirin or acetaminophen, is in the aqueous solution and will be extracted with EtOAc. *If the solution contains a significant amount of precipitate, skip this step and move to the next paragraph.* Transfer the acidic aqueous solution to the separatory funnel and add 15 mL of EtOAc. Mix and vent for 3 minutes, then drain the aqueous and organic layers into separate flasks. Extract the aqueous layer with an additional 15 mL of EtOAc (add 15 mL EtOAc to the aqueous layer, mix & vent for several minutes, then remove the aqueous layer). Wash the combined organic extracts with 10 mL of brine. Separate the layers and dry the organic layer over anhydrous Na_2SO_4 for 5 minutes (remove visible water from the organic layer by pipet, add the drying, and allow to sit with occasional swirling). Filter into a pre-weighed 50-mL round-bottom flask then concentrate using a rota-vap. The concentrated extracts may either be a liquid or solid, depending on purity. Obtain the <u>mass of product</u> by difference, transfer to a labeled vial, and proceed to analysis.

If a significant amount of either ASP or ACE precipitated out of acidic solution, save this in a vial in your drawer until the next lab to allow the crystals to form completely. The solution needs to be opaque to qualify. In the next lab period, collect the product by vacuum filtration and allow to air dry for 10-15 minutes. Obtain the <u>mass of the solid</u> then transfer into a capped vial labeled "**ASP or ACE + (initials)**."

<u>ANALYSIS</u>

Pay close attention to timing and stop wet lab work at least 20 minutes early to leave time for clean-up. No students should be present in the lab after the scheduled lab period. Analysis (IR and TLC) will be completed in the second week of lab after all three products are isolated.

TLC (week 2, fume hood only) - TLC standard R_f values were obtained in Exp 1 and can be referred to without repeating this part of the experiment. Dilute a small amount (microspatula tip) of each component isolated in this experiment with 1 mL of acetone in a test tube. Analyze by TLC using 1:2 hexanes / ethyl acetate with 1% acetic acid as the mobile phase. Visualize the plates under a UV lamp, circle the spots, and calculate all R_f values. Repeat as necessary to obtain optimal results (Ex. if spots are too large / smeared - dilute your samples; if lanes are slanted - be more careful when placing the plate in the jar and do not move the jar).

* Wash = add brine to the funnel and mix for 1 minute before draining the aqueous layer

IR – After TLC analysis, determine whether each of the isolated components are pure (1 spot). Do not attempt to take an IR spectrum of contaminated samples. Instead, take the IR of a standard. Follow TA instructions on facilitating a proper rotation for students using the IR spectrometer (ex. sign-up on the board). Obtain the IR of each pure compound using a Nujol mull (grind the mull for at least one minute). Honest self-assessment – ask the TA for a refresher on how to use the IR if needed.

Before leaving week 2: Check with your TA to be sure you've completed your analysis.

References & Supplemental Reading

Mohrig 4th ed. Chapter 10.1-10.5 (Extraction), Drying agents (Chapter 11), TLC (Chapter 18)

Revell, K. D. *J. Chem. Ed.* **2011**, 88, 1413.

Table 1. Clean-up and Safety

Clean-up – leave the lab as you found it!	**Safety**
Glass waste: uncontaminated pipets only	HCl and KOH are *corrosive & toxic*.
Liquid waste: contents of rota-vap trap, TLC solutions	Acetone and ethyl acetate are *flammable*. Caffeine is a *stimulant* and is NOT to be ingested or taken home.
Solid waste: filter paper, used pipets	
Product waste bag: product vials	Do not look directly into the UV lamp.

Introduction: Pre-Lab Questions, Week 1

1. Classify ACE, ASP, and CAF as acidic, basic, or neutral. Indicate which functional group determines the acid-base properties of each.

2. What reaction takes place in the addition of dibasic potassium phosphate (K_2HPO_4) to the mixture of ASP, ACE, and CAF? Show the chemical equation with full structures in support of your answer. Indicate whether each component (ASP, ACE, and CAF) should be in the aqueous or organic layer after the reaction.

3. What reaction takes place in the addition of potassium hydroxide (KOH) to the organic layer (after pre-lab #2)? Show the chemical equation with full structures in support of your answer. Indicate where each component would be after the reaction.

4. What volume of 6 M HCl is required to react with (neutralize) 13 mL of 1 M K_2HPO_4? …to neutralize 13 mL of 1 M KOH? Show your work.

Pre-Lab Questions, Week 2 ***Re-produce 3 copies of the following table either by hand or typed** - one per compound (ASP, ACE, CAF) - and *fill in all but the observed stretches*. ~~You will not be permitted into the lab without these tables!~~

Table x. IR Analysis of (Compound Name)

Functional Group	**Bond**	**Expected (cm^{-1})**	**Observed (cm^{-1})**

(add more rows as needed)

Results: In-Lab Questions

1. Report the mass recoveries of ACE, ASP, and CAF after isolation, including estimated uncertainty in balance measurements (ILE). Calculate the % recovery of each component from the initial amount of Excedrin used. Show your work.

2. Compare the recoveries above to the theoretical recoveries (Exp 1, pre-lab #1) and list the specific part(s) of the procedure where product may have been lost.

3. Report and discuss the TLC results: Make a table with the R_f values for each spot in each sample (4 columns – sample, ACE, ASP, CAF). Identify each spot as ACE, ASP, or CAF by entering the R_f value in the appropriate column for each sample. Include standard R_f values from Exp 1. Explain whether or not the separation was effective.

4. Interpret the IR spectra of ACE, ASP, and CAF. Reproduce the IR tables into the word processing document (no hand-written tables in the results section). Use three sentences to describe how the IR spectra can be used to positively identify each individual compound (unique stretches).

5. Compare the results of Excedrin separation *via* column chromatography (Exp 1) and acid-base extraction (Exp 2) as follows. While one method may not be generally *better* than the other, there should be some differences in the points below.

(a) Restate the recoveries of each component by each method.

(b) Which method produced greater amounts of ASP, ACE, and CAF? Note that different methods could be more ideal for isolating different components.

(c) Which method yielded higher purity of each component as determined by TLC?

(d) Based on your results and discussion above, was column chromatography or acid-base extraction more effective for separation of Excedrin components?

CHEM 8M, Binder UCSC
Exp 2 - Acid-Base Extraction (Excedrin) Name _____
Due Date in Syllabus

Section Day _____ Time _____ TA Name _____

RUBRIC & STUDENT CHECKLIST (DO NOT TURN IN)

SECTION	INSTRUCTOR COMMENTS	POINTS ASSIGNED
IN-LAB QUIZ		**/ 5**
LAB REPORT		
INTRODUCTION Each pre-lab question is addressed in its own paragraph using complete sentences. Structures and calculations are hand-written, where appropriate.		/ **35**
RESULTS The main results are stated, as outlined in the in-lab questions, using complete sentences.		/ **60**
NOTEBOOK PAGES Proper format: reaction scheme, chemical info table, 'comic book' procedure including flow charts, waste and clean-up procedure.		/ **40**
NEATNESS AND ORGANIZATION Proper grammar and format per instructions in syllabus and writing guidelines		**/ 10**
REMOTE LAB ATTENDANCE Registration in Zoom meeting for live or enrolled lab section and participation in meeting.		/ **10**
TOTAL		**/ 160**