Tools of the trade: Thin-Layer Chromatography (TLC) and Liquid-Liquid Extractions (LLE)

François Magnan

Thin-layer chromatography and liquid-liquid extractions are very common organic laboratory techniques that are practiced by synthetic chemists on a daily basis. Whereas the first one provides qualitative data on a sample's purity, the second is a purification technique for eliminating ionic species.

As you have seen both techniques previously in CHM1321, this experiment is intended to be a review of said techniques and the theory behind this; as such, it aims to test your understanding of the techniques by giving you broader experimental freedom. For this reason, no overly specific lab procedure is given to you, and you are instead in charge of planning your own steps based on your results and observations.

OBJECTIVES

1. PEDAGOGICAL

- Understand the role and purpose of liquid-liquid extractions.
- Identify the aqueous and organic phases.
- Understand the relationship between a solution's pH and a molecule's form, based on the molecule's pK_a.
- Predict the partition of a molecule between aqueous and organic phases, depending on pH.
- Understand the role and purpose of thin-layer chromatography.
- Evaluate and compare the relative polarities of molecules, and predict their relative rates of elution by TLC.

2. EXPERIMENTAL

- Identify the partitioning of various dyes between organic and aqueous phases as a function of pH.
- Plan and execute a liquid-liquid extraction step to isolate dyes from a provided mixture.
- Apply TLC to assess the success of the extraction steps.

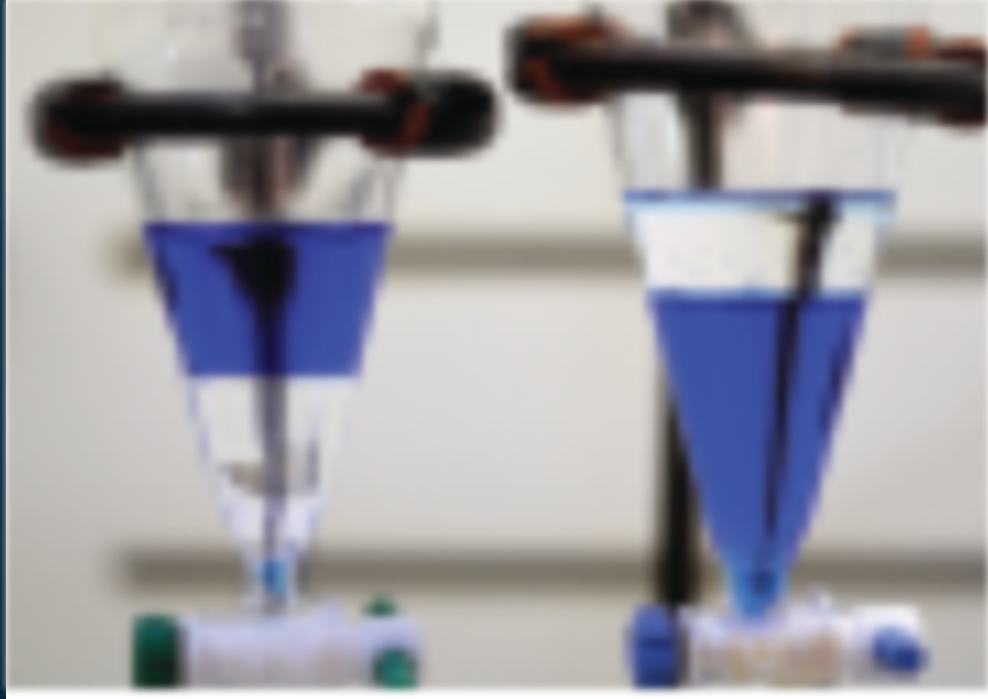
ALSO AVAILABLE...

KEY TERMS

- **Chromatography**
- **Hydrogen Bonds**
- **Ions**
- **Miscibility**
- **Polarity**
- **Phase**
- **Partitioning**
- **pK_a**

KEY VIDEOS

- **Liquid-Liquid Extraction** (Theory and Technical)
- **Thin-Layer Chromatography** (Theory and Technical)



THIN-LAYER CHROMATOGRAPHY

Chromatography is a technique that allows the separation of the various components in a mixture. The molecules are carried by a fluid (eluent / mobile phase), which is allowed to migrate through (or on) a specific material (substrate / stationary phase). The ease (speed) of migration of each molecule will depend on their relative affinities for both the stationary and mobile phases. A molecule that has a higher affinity for the stationary phase will migrate through more slowly than another molecule with a lower affinity for it.

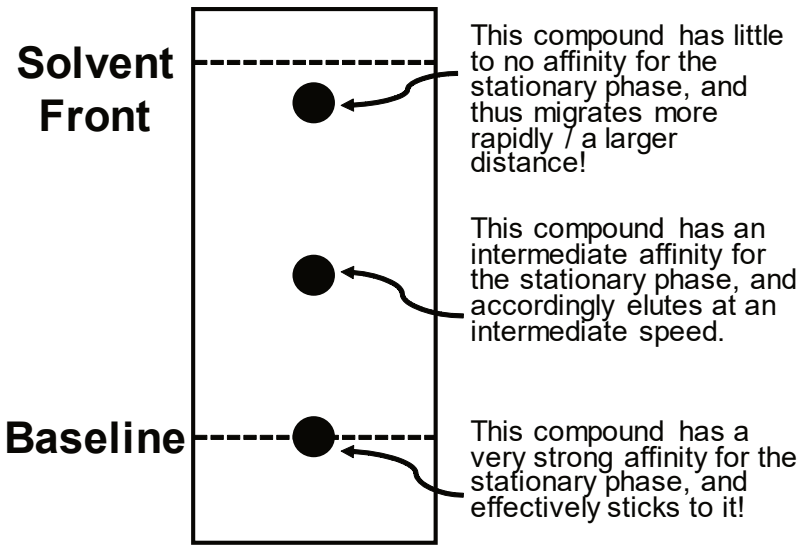


Figure 1 – Reprensentation of a developed TLC plate with compounds of various relative affinities for the mobile and stationary phases.

Of particular interest for CHM2123 is a specific subset of chromatography known as thin-layer chromatography (TLC). Here, a mixture of molecules is deposited (spotted) at the bottom of a thin layer of silica powder coated on a metallic plate and eluted using a solvent which is allowed to climb along the plate through capillary action. The bottom of the plate, where the initial mixture is spotted, is labelled the baseline. Conversely, the maximal height the solvent was allowed to reach from the baseline is referred to as the solvent front.

Based on each molecule's respective affinities for the mobile and stationary phases, they will migrate up along the plate at varying rates (Fig. 1). Each compound thus moves from the baseline by a specific distance, obviously inferior to the distance travelled by the solvent (the molecules can't travel faster than the solvent carrying them!) Because the migration is a result of an equilibrium of the interactions between the molecules and both the stationary and mobile phases, the distance travelled along the TLC plate will always be a specific ratio (or fraction) of the total distance travelled by the solvent. This is defined as the Retardation Factor (Rf), which is a number between zero and one and that can be calculated as such:

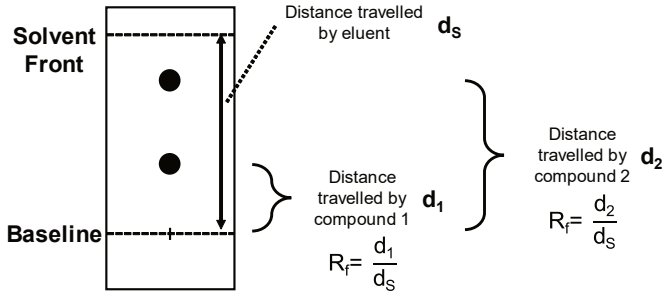


Figure 2 – Analyzing an eluted TLC plate. Under ideal conditions, each compound will migrate a certain distance d_x. The ratio of that distance to the distance separating the baseline and the solvent front (d_s) provides the R_f.

This affinity of the molecules for either the eluent or the substrate can be understood in terms of intermolecular interactions. In molecules (including eluent), polarity is the separation of electron density (*i.e.* regions around the molecule that are richer in electrons and regions that are poorer), which originates from differences in electronegativity between the various atoms. Polar molecules tend to have stronger intermolecular interactions, as the region of increased electronic density (negatively charged) of a molecule A is electrostatically attracted to the region of decreased density (positively charged) of molecule B. Similarly, hydrogen atoms bonded to electronegative atoms (namely nitrogen, oxygen, fluorine) lead to particularly polar bonds due to the extreme difference of atomic electronegativity. This results in the formation of hydrogen-bonds, some of the strongest strong intermolecular interactions commonly observed.

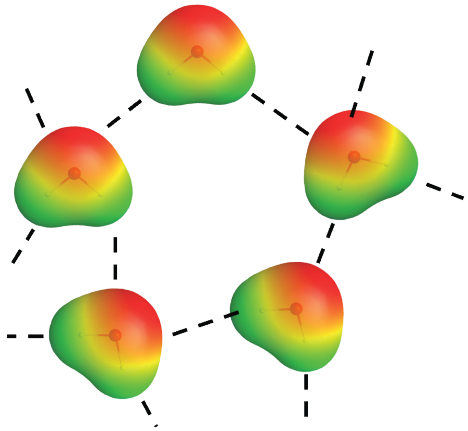


Figure 3 – A cluster of water molecules held together by dipole-dipole interactions and hydrogen bonds (dashed black lines). The color of the molecule's surface contour indicates relative electron density, from regions rich in electron density (red) to regions deficient in electron density (blue).

The capacity of the substrate to form intermolecular interactions will depend on the identity of the substrate itself. The most commonly used substrate (and the only relevant one for CHM2123!) is silica (SiO_2), the main component of glass, which consists of a 3D lattice of silicon and oxygen atoms. More importantly, the surface of silica is terminated by polar Si–OH groups, which can undergo both hydrogen-bonding and dipole-dipole interactions with eluting molecules.

The polarity of a molecule can be guesstimated by considering the functional groups it bears:

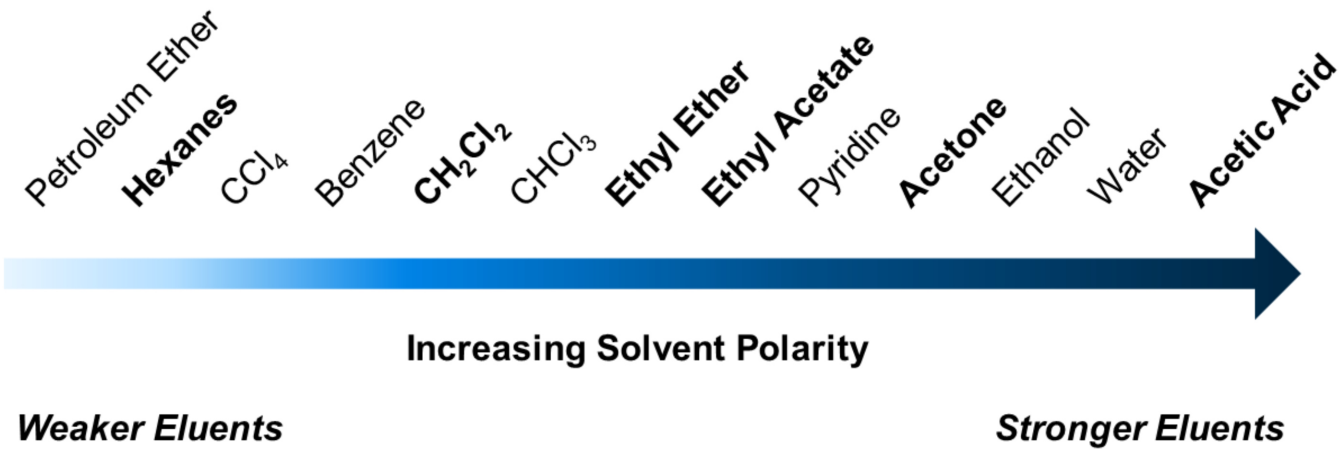


Figure 4 – List of eluents ranked in increasing strength for chromatographic elution. Bolded solvents are the more commonly employed in the laboratory. Note that the same rationale used to explain an analyte's polarity can be used to account for an eluent's strength. Stronger solvents are very polar molecules tha can form hydrogen bonds, while the least polar solvents are simple non-polar hydrocarbons.

- Groups that can undergo hydrogen bonding – particularly hydrogen-bond donors (HBD; –OH and –NH groups) – will adhere more strongly to the silica, while hydrogen-bond acceptors (HBA; nitrogen, oxygen, and fluorine atoms) will adhere to a lesser degree. As a rule of thumb, comparing the number of HBDs first, and then HBAs, per molecule should give you a good idea of how well they will elute.

- Electronegative atoms lead to the presence of permanent dipole moments across a molecule. Favorable interactions between this dipole and the silica's dipole moment slows the migration of molecules. Stronger molecular dipoles lead to better retention on silica.

As a rule of thumb, the ability of a molecule to form hydrogen bonds – particulary donors – will have a larger impact on its elution rate than its simple polarity, although they usually come hand in hand

So, to recap:

- Polar molecules interact more strongly with the stationary silica through dipole-dipole interactions and/or hydrogen-bonds, and will resultingly migrate through at a slower pace (low R_f)
- A non-polar molecule forms little to no intermolecular interactions with the polar silica: as a result, it is barely held back by the silica and will easily migrate through (high R_f).

So how do we force molecules through?

As hinted above, the migration of the molecules through the substrate depends on the relative strength of their interactions with the substrate. More polar compounds interact more strongly with the polar silica and so will be retained on the column longer. Consequently, polar molecules require a stronger solvent – that is, a more polar solvent that can disrupt the analyte-silica interactions by forming stronger analyte-solvent interactions – to force them to elute further up the TLC plate. This can be done by simply changing solvents, but a much better approach is to use *mixtures* of solvent; the combination of a polar solvent with a non-polar one. By varying the relative amounts of each, a mixture of the right overall polarity can be found.

So what?

The proverbial question remains: what is the point of all this talk of polarity, silica, R_f and what not? Well remember: each different molecule will migrate a specific distance under given conditions. If one was to deposit a single spot of a solution containing *n* distinct compounds, one could expect, after elution, to observe *n* distinct

spots along the TLC plates, as all should migrate at different rates. Coupled with the technique's low-cost, rapidity and ease of operation, this makes TLC an excellent frontline tool to both ascertain the progress of a reaction (*i.e*. to track the disappearance of the starting material, the appearance of product and, potentially, side-products) as well as the identity and purity of a compound.

Although simple and efficient, the usage of TLC requires some troubleshooting: since every reaction is different, one must find a suitable eluent mixture to ensure proper and meaningful results. Think of it as the Goldilock rule: the eluent must be neither too weak nor too strong, but just right, to obtain good results:

- If the eluent is too weak (*e.g*. hexanes), none of the compounds will migrate a significant distance on the plate, and it will appear as though a single spot is present, on – or very close to – the baseline. Increasing the polarity of the mobile phase will allow all the spot to migrate at their own pace.

- Conversely, if an eluent is too polar (*e.g*. ethyl acetate), all the compounds might just simply elute all the way to the top, near the solvent front, as they are easily carried by the solvent and aren't allowed to properly equilibrate with the silica. In this case, decreasing the solvent polarity will allow this equilibrium to be achieved and promote a proper separation of the spots.

- In some cases, two compounds might always co-elute for a given pair of solvents, regardless of ratios. Sometimes, simply switching to a different pair of solvent (*e.g*. dichloromethane/hexanes instead of ethyl acetate/hexanes) might do the trick. In many cases, some compounds will simply never cleanly separate. (This won't be the case in CHM2123!)

LIQUID-LIQUID EXTRACTIONS AND REACTIVE EXTRACTIONS

Save for a few very polar organic solvents (*e.g.* acetonitrile, ethanol, methanol, tetrahydrofuran), most solvents are practically immiscible with water. Perhaps the best everyday example would be a salad dressing containing vinegar and olive oil: regardless of how well you shake the dressing, it will always go back to form two distinct layers on top of each other. Much like TLC, this phenomenon can be rationalized with intermolecular interactions.

The water and acetic acid molecules that make up the bulk of vinegar are very small and very polar molecules that can participate in hydrogen bonding. On the other hand, olive oil is mainly comprised of fatty acids (oleic acid being the most abundant one), carboxylic acids with very long (~15-18) carbon chains. This carbon chain is non-polar in nature, and since it accounts for most of the molecule, it lends the molecule an overall non- (or poorly-) polar character. Knowledge of intermolecular interactions tells us that polar molecules tend to group up, whereas non-polar molecules will prefer other non-polar molecules: this effect is commonly referred to as "like dissolves like".

This preferential affinity of molecules can be put to good use in an organic chemistry lab to purify compounds prepared during a reaction. Most organic compounds, being comprised mainly of carbon and hydrogen atoms, are relatively non-polar in nature and will tend to dissolve preferentially in an organic solvent rather than in water. Conversely, ionic compounds (salts) are extremely polar due to the complete charge separation between ions: they will dissolve almost exclusively in water.

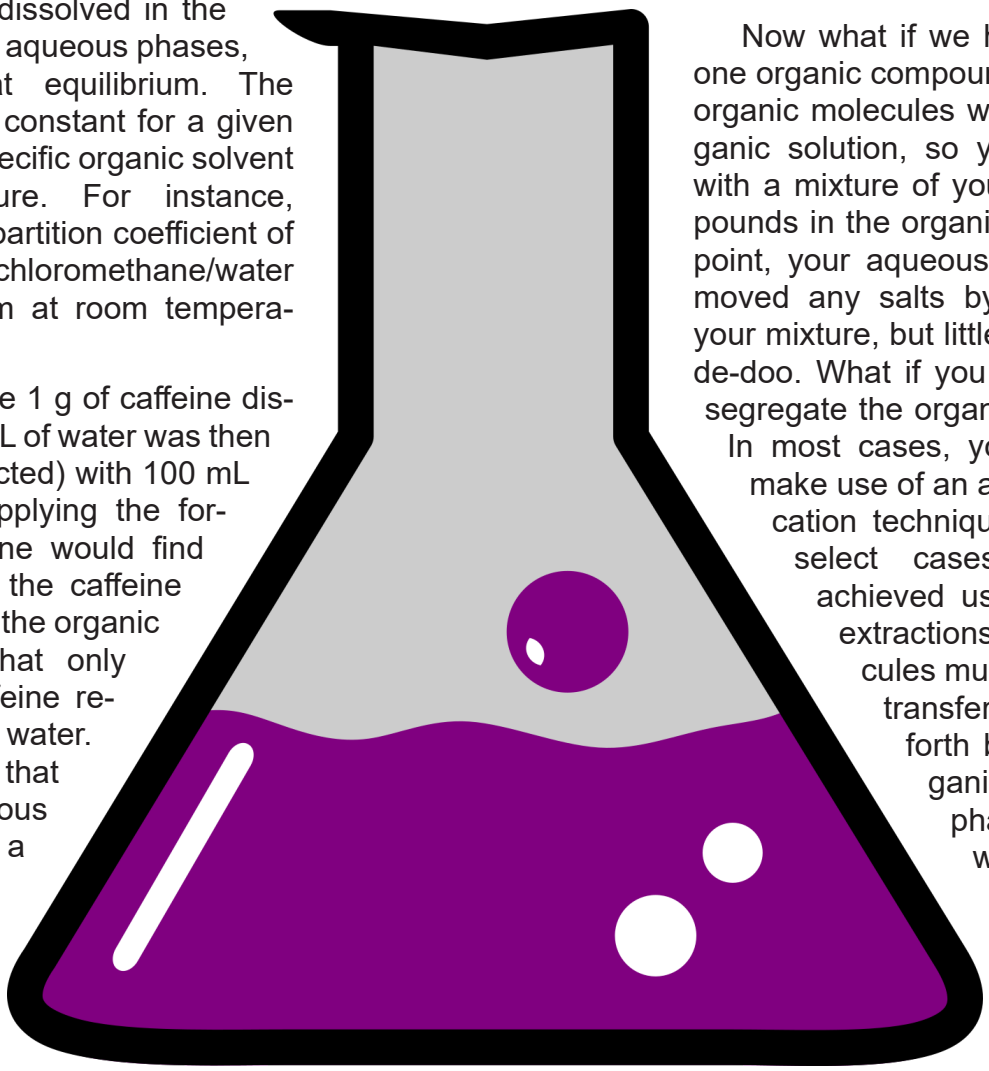
An important distinction here is the use of the word *preferentially*. Many organic molecules are moderately- to well soluble in water; life on Earth wouldn't exist otherwise! In fact, all organic molecules display some solubility in aqueous

media: that solubility is simply extremely low for many of them. This is however an important distinction to make for practical reasons: most organic molecules, if “given the choice” between water and an organic solvent, will preferentially, but not completely, migrate into the solvent. As solubility of a solid solute in a given solvent is an equilibrium, this partition of the solute between the two phases is also an equilibrium, which can be expressed mathematically by the partition coefficient K:

$$K = \frac{C_O}{C_W}$$

Where C_O and C_W are the massic concentrations of solute dissolved in the organic and the aqueous phases, respectively, at equilibrium. The value of K is a constant for a given solute with a specific organic solvent and temperature. For instance, caffeine has a partition coefficient of 4.6 for a dichloromethane/water biphasic system at room temperature.

Let’s assume 1 g of caffeine dissolved in 100 mL of water was then mixed (or extracted) with 100 mL of DCM. By applying the formula above, one would find most (82%) of the caffeine transferred into the organic solvent, and that only 0.178 g of caffeine remained in the water. Re-extracting that leftover aqueous solution with a second 100 mL of solvent would isolate an additional 0.146 g of caffeine (~97% total extraction). Because of the low boiling point of dichloromethane (36°C), the organic solution of caffeine can then be easily evaporated to yield assumingly pure caffeine.



Is that it?

Nope! This is not quite the end of our little story. First, a quick recap: organic solvents and water are, for the most part, immiscible, and will stay segregated. While shaking them hard together will form an emulsion, they will sooner or later re-separate to give two distinct layers. If you throw in some organic molecules in the mixture, they will tend to dissolve preferentially in the organic solvent. Salts, on the other hand, will shoot straight for the aqueous phase.

Now what if we have more than one organic compound? Again, most organic molecules will prefer the organic solution, so you will end up with a mixture of your various compounds in the organic phase. At this point, your aqueous phase has removed any salts by-products from your mixture, but little more. Whoop-de-doo. What if you want to further segregate the organic compounds? In most cases, you will need to make use of an alternative purification technique, but in some select cases, it can be achieved using liquid-liquid extractions. For this, molecules must be selectively transferred back and forth between the organic and aqueous phases. How would we do this? Well, as mentioned

above, salts, ionized compounds bearing electrical charges, dissolve nicely in water. Following that logic, an ionized organic molecule will prefer to dissolve in water than an organic solvent. The easiest way to do in the lab is *via* acid-base chemistry. To do so, however, requires a good understanding of pH and pK_as.

Acid-base chemistry

Any weak acid HA can be described using the following chemical reaction and mathematical equations:

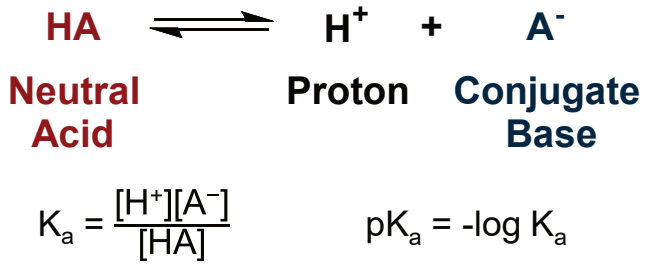


Figure 5 – Generic equation for the dissociation equilibrium of a weak acid into a proton and its conjugate base, along with the mathematical formulas defining K_a (acid dissociation constant) and the corresponding pK_a.

An acid's pK_a provides us with information regarding the strength of the acid: the lower the pK_a, the stronger the acid. The mathematical expression for K_a can be rearranged to provide the Henderson-Hasselbalch equation:

$$\mathbf{pH} = \mathbf{pK_a} + \log \frac{[A^-]}{[HA]}$$

This equation can help us understand and predict the partitioning of an acid's equilibrium reaction (whether the dominant form of the molecule is as the neutral acid or as the ionic conjugate base) as a function of the solution's pH. Let's assume any weak organic acid HA in the presence of an aqueous solution of variable pH: there are three possible scenarios:

1. pH = pK_a: In that case, log([A⁻]/[HA]) must equal zero, which means that [A⁻] = [HA]. If a weak acid is in an aqueous solution whose pH is exactly the value of the acid's pK_a, then exactly half of the molecules are in the neutral HA form, and exactly half of the molecules are in the ionized A⁻ form. If an organic phase was present, the electrically neutral HA molecules would migrate toward it, while the ionized A⁻ form would remain in the aqueous phase. Overall, the weak acid would be evenly distributed between the organic and aqueous phase: not a particularly desirable outcome for purification purposes, but not a bad start.

2. pH < pK_a: In that case, log([A⁻]/[HA]) is negative, which means that [A⁻] < [HA]. Such acidic conditions move the equilibrium to promote the neutral HA form, which again is organic soluble. Most of the weak acid molecules would preferentially dissolve in the organic phase!

3. pH > pK_a: In that case, log([A⁻]/[HA]) is positive, which means that [A⁻] > [HA]. Such basic conditions favor the deprotonation of the acid and move the equilibrium to form the ionized A⁻ ion, which will prefer the aqueous phase. Most of the weak acid molecules would preferentially dissolve in the aqueous phase!

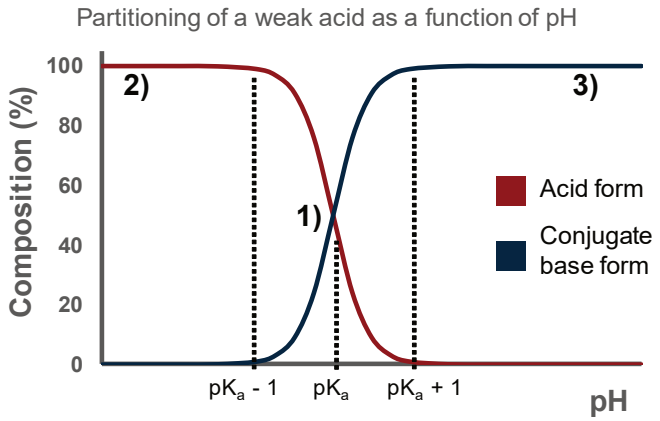


Figure 6 – Graph showing the partitioning of a weak acid as a function of the solution's pH. Acidic solution push the equilibrium toward the neutral acid form, while basic conditions promote the ionic conjugate base form.

This dependence on the speciation of the weak acid as a function of pH can be visualized with the help of graph 6, which models the distribution of molecules as a function of pH

relative to the acid's pK_a value, as calculated using the Henderson-Hasselbalch equation. As you can infer from this graph, changes occur mainly in the pH region that corresponds to the acid's pK_a +- 1; outside of this region, the speciation of the acid remains, for all intents and purposes, constant.

Let's take as an example benzoic acid (which has a pK_a of 4.20) that is dissolved in dichloromethane (or any other appropriate organic solvent). In the presence of strongly acidic water, not much happens, since at acidic pHs (inferior to 3.20 (pK_a – 1)) over 91% of all the molecules are in the neutral HA form. Being a neutral organic molecule, benzoic acid remains in the organic phase. However, if we trade the acidic water for basic water (pH > 5.20), a reaction occurs. The base deprotonates the benzoic acid to form the benzoate anion. Being electrically charged, the anion is particularly polar and is better stabilized by water, and will leave the organic solution for the aqueous one.

Back to the proverbial so what? Why would a chemist bother with this step? Well, let's assume instead that you've prepared benzoic acid by the oxidation of toluene, and that your reaction was incomplete: you still have toluene in your solution. In the presence of neutral water and an organic solvent, no separation will occur: both starting material and product are neutral organic molecules and will preferentially dissolve in the solvent. However, add some base, say sodium hydroxide, to the water and you have a different story. The acid is deprotonated into the benzoate, which migrates to the aqueous phase. The toluene, on the other hand, is inert in these conditions, and remains behind in the organic phase. Using a trusty separatory funnel, you can physically separate your two layers (remember, organic solvents and water don't mix!) to obtain an aqueous solution of sodium benzoate from an organic solution containing the toluene. This said, you need to isolate benzoic acid, not sodium benzoate! Well, acid-base chemistry is reversible: you can neutralize the base by acidifying the reaction mixture sufficiently (below pK_a-1) to regenerate the neutral benzoic acid. Being insoluble in water, the neutral acid in this case will conveniently precipitate as a white solid, which can be filtered and dried.

To summarize these steps, we will make use of flow charts: graphical representations of the various steps taken – and chemical used – during the purification routine. These have the advantage of rapidly showing the various steps taken and their impact on the target product and the various impurities. An sample flow chart is provided below for the scenario described above.

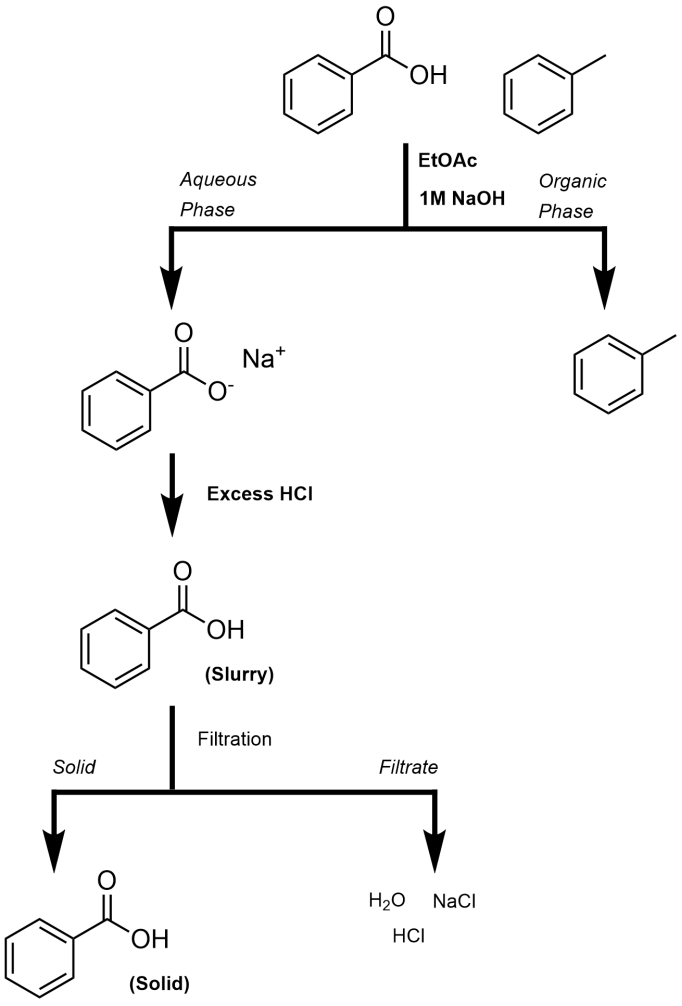


Figure 7 – Sample flowchart describing the purification of benzoic acid from toluene using reactive extractions.

On paper, any organic molecules that possesses an hydrogen atom (so pretty much *all* of them) can participate in acid-base chemistry. However, one needs to consider the pK_a of the molecule, as it might not be amenable to liquid-liquid extractions. Using the case above, toluene *could* be deprotonated at the methyl position with a base to form the benzyl anion. Toluene however is a very poor acid (pK_a of ~40) and consequently the conjugate base is particularly basic: if water was present (pK_a = 15.7), it would immediately protonate this benzyl anion to form instead an hydroxide ion (remember that acid-base reactions always promote the formation of the weakest acid). So, practically speaking, acid-base chemistry can only be exploited if the organic acid's pK_a is less than 15; otherwise, you can safely assume the acid will never be significantly deprotonated in the presence of water, and will always be a neutral molecule, regardless of the water's pH.

This rationale can also be applied to basic molecules. While basic water will nicely dissolve organic acids such as benzoic acid, acidic water will, for similar reasons, dissolve organic bases, such as aniline. The easiest way to consider this scenario is to consider instead the pK_a of the base's conjugate acid. In the case of aniline, (pK_a of anilinium is ~4.6), it will preferentially dissolve in acidic water (pH < 3.6) but will remain in an organic phase if the pH is too high (pH > 5.6).

For this laboratory class, a good understanding of pK_as will be necessary, as they are useful tools for predicting behaviours in liquid-liquid extractions, but also for understanding many organic mechanisms. While you will not be required to memorize major pK_a values (Fig. 8), you need to understand what pK_as are and what information they provide. You should also be able to predict the impact of structural changes on a molecule's pK_a.

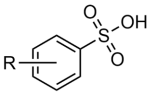
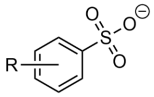
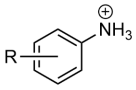
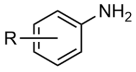
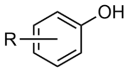
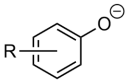
Functional Group	Example	pK_a	Conjugate Base
Hydrochloric acid	HCl	-7	Cl^-
Protonated ketone/ aldehyde		~-6	
Sulfuric acid	H_2SO_4	-3	HSO_4^-
Sulfonic acid		~-1	
Anilinium		~4	
Carboxylic acid		~4.5	
Carbonic acid	H_2CO_3	6.35	HCO_3^-
Iminium		~7	
Phenol		~9	
Ammonium		~10	
Water		14	
Alcohol		~16	
Ketone /Aldehyde		~20	
Alkyne		~25	
Amine		~31	
Hydrogen	H–H	36	H^-
Alkene		~42	
Alkane		~52	

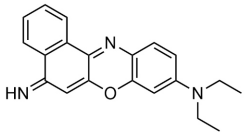
Figure 8 – Table of approximate pK_a values for commonly encountered functional groups in organic chemistry.

SEPARATION OF A DYE MIXTURE *VIA* REACTIVE EXTRACTIONS

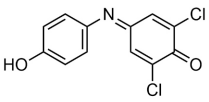
Thin-Layer Chromatograhy

Liquid-Liquid Extractions

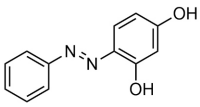
Experiment Time ~ 1h30 min.



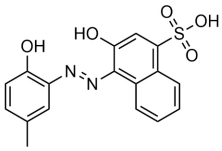
Nile Blue



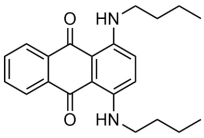
DCIP



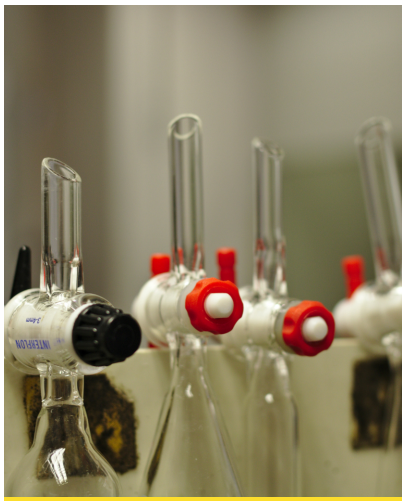
Sudan Orange



Calmagite



Sudan Blue



REAGENTS

Calmagite

DCIP

Nile Blue

Sudan Blue

Sudan Orange

1M Hydrochloric acid

1M Sodium Hydroxide

Demineralized Water

Hexanes

Ethyl Acetate

OVERVIEW

This experiment is intended to be a review of common laboratory techniques that you have already seen in CHM1321, and aims to test your understanding of the techniques by giving you experimental freedom. For this reason, no overly specific lab procedures are given to you. Theory and technical videos on both liquid-liquid extraction and thin-layer chromatography are available online to assist you with the experiment.

For this experiment, you will be working with the five dyes shown above. You will be provided with stock solution of each individual dye, along with a mixture of two of the five dyes. Your goal is to exploit acid-base chemistry to separate the two dyes into two distinct **organic solutions** by liquid-liquid extraction. You will confirm the success of your purification by thin-layer chromatography.

PART A

For this first part, you want to assess the partitioning of each five dyes between two phases as a function of pH. To that effect, you are provided with aqueous solutions of dilute (1 M) HCl and NaOH, as well as demineralized water (neutral), along with either hexanes or ethyl acetate, depending on your assigned dye mixture.

For each of the five dyes, prepare three test tubes, each containing 1–2 mL of your organic solvent, and 1–2 ml of the acidic, neutral and basic aqueous solutions, respectively. To all three tubes, add 2–3 drops of the corresponding pure dye solution, manually

shake for a few seconds and allow the phases to settle. Note the color of each respective phases. Repeat for all dyes.

PART B

For this second part, you need to determine the TLC behaviour of the two dyes found in your assigned mixture. Pick the corresponding three test tubes (acidic, neutral, and basic) for both of those dyes.

Perform TLC on each test tube, sampling in each the phase that preferentially dissolves the dye. Perform this step twice, using two different eluents (1:3 v/v ethyl acetate/hexanes and 1:9 v/v methanol/ethyl acetate). Determine which eluent works best for your pair: you will be using this eluent for the remainder of the experiment.

PART C

Based on the information you collected in **Part A** regarding the relative solubilities of both dyes as a function of pH, you now need to plan a reactive extraction to **selectively** and **completely** extract either one of your dyes into an aqueous solution. To that end, you will have access to the same solutions (1M HCl,1M NaOH and water) and solvents (ethyl acetate *or* hexanes) that you had in **Part A**.

Use 1 mL of the dye mixture provided to you as a starting point. For every step of the extraction, use approximately 3 mL of both organic and aqueous solvent.

Using TLC (and the eluent you chose in **Part B**), analyze both your final organic and your aqueous phases, as well as the starting mixture of dyes. Compare your results to the results obtained in **Part B**: if you were successful in your separation, proceed to **Part D**. Otherwise, resume or re-attempt your reactive extraction.

PART D

The aqueous solution from **Part C** contains the conjugate acid/base form of one of your dyes, rather than its neutral form. Using liquid-liquid extraction and acid-base chemistry again, convert that conjugate form back to its neutral form: to that end, concentrated HCl and NaOH are made available. Again, extract using 3 mL portions of organic solvent.

Confirm the success of this step by running TLC on these final organic and aqueous phases, using the eluent chosen in **Part B**.

TIPS

- Develop your TLC as soon as possible after spotting them. Certain dyes can fade if left for too long on silica.
- Remember that you can run more than one spot per TLC plate!
- Be careful not using too much solvent during the liquid-liquid extraction steps: if too much is used, the resulting solutions are too dilute, and the colors will be difficult to observe.