

## Lab 6B • 10/13/11

{lab report due dates} Lab reports do need to be in some kind of typed format – most likely you’re going to be it computer-generated. If you would like that means you could e-mail me your lab reports. {lab report format} If you do e-mail it, please do give some indication in the title of your e-mail that it is for a lab report. If you are going to submit your lab reports electronically, then I also as that you send me a statement of whether you would like to receive your grades back by e-mail or not. The reason I want you to send me that statement is because of the FIRPA act, which says that I’m not supposed to release grade information to anyone except you, unless you authorize it. E-mail is an insecure format of communication. It’s always possible that after you receive an e-mail, your brother, sister, mother, father, boyfriend, girlfriend, someone might get access to your computer. I don’t want to inadvertently have that grade information slip out. If you send me an e-mail saying please send me my grade by e-mail, you’re also therefore saying that if something happens afterwards, if that grade gets out after I send it, that you won’t hold me responsible for that. If you prefer to keep it secure, I can always give you the comments back in class and the grades back in class. If you send it to me electronically, let me know if you would like to have your grades returned electronically as well.

{reference to syllabus}

A comment on how research is normally published. You do a lot of work and research; usually, it’s not just one person doing a research project; it’s a whole group of people working together on one project over several years. Once you do get some kind of results that you think is worthy of publishing, you then write up your work, and that work is often submitted to what is called a peer-reviewed journal: that means that other chemical researchers across are give your report to evaluate. You send it to the editor of a particular journal, they send it out to other people o review. Those other people will read8 over your research. They’ll see, is the research complete? Do the data make sense? Do your conclusions that you drew from the data make sense? Sometimes they say, “this is fine, publish as is.” More commonly, they’ll say you need to rewrite this section or why don’t you try this one experiment to try such-and -such, or they may come back and say no, this is good research, but more needs to be done before it’s ready to be publication. Then there’s a back and forth that goes on; eventually , the publication might accepted, then it becomes published, then it because part of the chemical literature.

For one of these articles, it might start off like this. You start out with a paragraph-long synopsis, an abstract, so that someone that’s just flipping through one of these journals can get a quick idea of what the article’s about. Then you give an introduction where past experiments have been done, to give some justification to why you’re doing research. To be realistic about it, there’s two versions of justification that we’re doing. One is on the research itself. But, fortunately or unfortunately, because research involves money, you’re also speaking to the grant writers who are funding the research. You then give a complete listing of all of the data for your project, show your calculations, draw your conclusions, and then discuss what the importance of those results is. Often you’ll follow that with a closing statement of what future research the project that you submitted would enable. Why is what you did worthy not just in and of itself, but what does that allowed to happen.

The lab report consists of five sections that I will mention quickly and then go over what goes in each one. There’s the purpose, where you say what you did as an experiment. This whole thong [example] is based off of something you might have heard in one of your speech classes – tell ‘em what you’re going to tell ‘em, tell ‘em, then tell ‘em what you told ‘em. Give a purpose statement first to tell what is the experiment about; you’re going to present your data. There’ll be a few calculations, like percent error and percent yield. You’ll mame some conclusions that’ll parallel your personal statement. If your purpose statement says what the point of an experiment is, then you come back in the conclusion statement and say what did you find out. Then the last section will be a discussion section. For example, if you’re doing a synthesis project, the goal may just be that you synthesized the molecule. if, on the basis of your data that you collect, mainly in the form of spectra, shows that you formed a new molecule, then there’s not a heck of a lot that you need to discuss. In this class, I don’t have you discuss theory on the lab reports; instead, I do that on the lab quizzes.

I need to discuss the philosophy behind which most articles are written. Most of the time in chemistry, we take the view that the report should focus just on the chemistry itself. Most of the time, you never use first or second person in communicating in reports – so no I, we, our, you, yours, y’all – it’s all just it. So you end up writing along of exactly the same kind of constructions that your English teachers probably told you not to write. They used to say things like to don’t say, “the book was red”; “I read the book.” That’s exactly what you don’t do on a lab report. No Is, wes, hes, it’s all its. That’s because your focusing on the science. The idea that what we’re trying to talk about is just the results, without referring to the humans involved. Of course, there are some fields in science where they disagree with that. They think, yes, you should go ahead and honor the researcher. Some journals allow for a first or second-person approach. But since the majority of chemical journals do handle it in the third person, where it’s non-personal, we’re going to follow that format for the lab reports.

What would you says is the purpose statement? As written in many lab texts, it may appear that the purpose of the experiment is to learn something. But that’s focusing on your role as a student being educated on how to do these labs. We don’t want to focus on the learning aspect; we want to focus on what was accomplished during the experiment.

I know that when I presented the experiment, it was entirely from the perspective of you learning how to perform the experiment. {purpose statement for this experiment}.

The purpose statement should be this – it is a concise statement of the qualitative or quantitative goals in an experiment. Qualitative might be to say you did synthesize something, you didn't synthesize something it; there's not a number there, but is something that you can discuss. Quantitative would be some kind of numerical information.

Do not put in procedure. Of course, in any proper article, you're going to put an exhaustive procedure in, but because you already did it for your prelab, and because it's in your textbook, I know it's in the textbook, you know what page, I know what page, we don't need to go through the dance where we all acknowledge that the procedure's there. If you vary from the procedure, however, you might want to mention that as part of your discussion; otherwise, don't write the procedure up, save that time for something else.

Next is a data section. In general, I am concerned about the distinction between data and calculations. A minor point on grammar. What would be wrong with this statement: the data I got from this experiment was good. It's the data were – data is a plural word. Datum is the singular. What are data? How are those different from calculations? Data are anything that you get from the experiment that you don't have to do an interpretation on. So it's direct qualitative or quantitative observations that require no manipulation. {commenting on proper formatting and use of tables}{advise for constructing table} Every number always has to have units if units are not already associated with it. You must format chemical formulas correct. For example, you would not write a chemical formula with the numbers being the same size as the letters, you have to put those numbers in subscript form. It looks uneducated and unprofessional in a chemistry report if you are incorrectly formatting your formulas. A comparatively minor point but one that still comes up frequently these days [, especially since the rise of texting and tweeting as a means to communicate]. We may refer to the formula for water as "aitch-two-o", but that's not the number zero, because there's no element zero; it's o as in oxygen. Because of too many years grading lab reports, yes I do notice such errors. Again, it's a minor error, but if you're trying to look educated, you would never write H<sub>2</sub>O, because there's no such thing. Another point: trail and trial are both perfectly valid English words, so you're spellchecker is not necessarily going to know that you didn't do trail 1 and trail 2 in your experiment; you did trial 1 and trial 2. Be sure to double-check what you're written, and don't just depend on your spell checker or grammar checker. Make sure that every piece of data, you have a label for. Don't assume I know what you're talking about just because I happened to be the one that assigned you the lab to do. {types of data obtained}

Most of the time in this class, I'm not going to be grading on whether or not you got perfect results; I'm grading on whether you know how to express those results. Since you're first-year chemistry students, you're going to make mistakes in synthesis sometimes. You're going to spill your product. Or maybe something will happen that's beyond your control; maybe the experiment won't even work. As long as you commentate and express what happened in your experiment, that's what I care about.

Let's move on to calculations. If the data are the direct observations, then calculations are anything that you do with the data. If we make a graph but we're just plotting the data, that's just another representation of the data. As soon as we break out a calculator or do some kind of addition or subtraction, that's, indeed, a calculation. Any manipulation of data would classify as a calculation. There are three different calculations that you'll need to do for this experiment: % composition, % yield, and % error.

Formatting of calculations – First, write out the equation once, either in word form, or in symbol form. I do this because sometimes a student will perform an experiment beautifully and get fantastic data, but maybe used the wrong equation(s) to do the calculations; even if you had the right equation, you might substitute the wrong value into that equation. Taking the calculations step-by-step, showing me the equation that you're going to use, followed by a substituted equation, it gives me a chance to see where you might have a mistake if you made a mistake in your calculations. What would be the formula for percent composition? How much mass you obtained for a particular sample, versus the total mass of that mixture, then multiplied by 100%. In formatting the calculations, you'd write the equation out like this first, take one of your sets of results, substitute that into the equation, and show me what the value you get is. Once you show me the substituted equation once, I don't need to see it over and over and over again. Don't include a substituted equation for every set of data; it just ends up cluttering your report. What you could do is show the prototype equation, show the substituted equation, then make a table of all of your results. There are times when is fine, even, to blur the line between data and calculations. For example, you might want to have a column and, right after you state what the [blah] are, you might want to calculate [blah] for each set of [blah]. It's technically a calculations, but's makes sense to present it in that way.

% yield – how much did you get out of an experiment, versus how much do you think you should have gotten. Most of the time we're going to be viewing this in terms of a molar calculation – here's the starting material, how much product should you get. If you get less than that, then you express percent yield. {application of % yield to experiment}

% error – measured value minus accepted value, all divided by the accepted value, times 100%.

You might have seen the same formula with absolute signs before, but I prefer to leave the absolute signs out, since, whether or not the error is positive or negative, tells you automatically if your measured value is too big or too small. That's why you start with the measured value and subtract out of that the accepted value; if what you got was too big, you then got a positive value. You then divided by the accepted value to get some sense of proportion, some sense of scale. Any time you perform an experiment where you produce some data that you can compare to the literature value, that you can compare to accepted values, we should always make some kind of comparison; in this case, since it's numbers, we can do percent error. There is one important point: what unit of measure must we use if we're doing percent error for temperature? Kelvin. Why? Imagine that you were trying to do a percent error for the freezing point of water. Water's 0 °C. If you put that into the equation, you would end up dividing by zero, even though that temperature is a perfectly valid temperature. You have to use an absolute scale for temperature, which that's what Kelvin is. Percent error for temperature must always be expressed using Kelvin, because of that case of 0 °C not having a definable meaning.

Conclusion(s) – There's parallelism. What you put in that conclusion statement should match what you had in the purpose statement. You may end up quoting values that you had in your data and calculations, but if you had a really complicated report that had pages worth of data and calculations, someone who's reading it might want to see that nice encapsulated short little sweet ended, to see what really happened. Your conclusion should be a summary of the results of the experiment that addresses the goals as presented in the purpose statement.

Discussion – The discussion section does not need to include a discussion of the theory; that's going to be for the lab quiz. What kind of things would you put in the discussion section? You do not need to think of every possible source of error for an experiment. If you were in a physics class, you would talk about your systematic versus random error. But, so often I end up seeing things like this: the percent composition may be incorrect because the balances were miscalibrated. Yes, that's always true that there's a chance the balances could be miscalibrated, but do you know that it's miscalibrated or not? If you spilled something, you could make a comment in your the data table, maybe put an asterix by whatever value you're quoting, and down below say: this value is artificially low due to loss of product. If you wanted to refer to that in your discussion, you could. Here's another one that would be dangerous out in the real world: the results were off due to human error – in the real world, they'd fire the humans and get new ones. Be a little careful with your wording there.

#### Chromatography

As kids, you might have done an experiment where you take a paper towel and you put a big ink splotch on the paper towel, and then you put the paper towel in a bucket of water and allow the water to flow up through the ink dot. What would you see in that kind of case? The ink runs up with the water, and depending on the type of ink that you use, you end up with the ink dot getting separated. That's an exact parallel to thin layer chromatography, the form of chromatography that you're going to be doing for this next experiment. I want to give a brief introduction to the topic of chromatography in general, then a discussion of what thin layer chromatography is, what we're using TLC for – not tender loving care, in this case – in analyzing this experiment, and then how to properly conduct a TLC plate development.

Let's tackle chromatography in general first. Chromatography is a technique by which a mixture of compounds can be separated primarily on the basis of their polarity. There are other versions of chromatography, such as ion-exchange chromatography – with a more direct involvement of charge than just polarity – and size-exclusion chromatography – based on the size of a molecule and not just on the basis of its polarity. Because the three main forms of chromatography that we are going to do in this class – gas chromatography, column chromatography, and thin layer chromatography – are, in our case, polarity-based, I'm going to limit my discussion, for the moment, to polarity. Chromatography is a technique by which a mixture of compounds can be separated on the basis of their polarity.

Let me give you an example, which is gas chromatography. Imagine that you have some kind of tube, but that the inside of that tube is coated with some kind of material. Just for the sake of argument, in this example, let's say it's some form of polar material; maybe it has alcohol functional groups or something like that. Imagine that we then pass through it nitrogen gas – something that's inert and is not going to interact particularly with this polar group. Imagine that at that same we have a volatilized sample – volatilized is a somewhat fancy way of saying evaporated; that sample might, say, include a mixture of alcohols, which are also polar groups. As the nitrogen gas, which is carrying this vaporized sample of alcohols, passes through the tube, the different components of that mixture are going to interact on the basis of their polarity with the inside coating of this tube. Based on the structure of the molecules involved, maybe some of the alcohols that we're trying to separate, some are more polar than others. The ones that are more polar are going to interact more strongly with this tube and maybe not progress as rapidly through the tube, whereas alcohols that are much more non-polar, maybe big long-chain molecules, wouldn't interact as extensively with the tube and would pass through the tube more quickly. Based on this difference in polarity, and especially if you make the tube long enough, you're eventually going to separate out that mixture of compounds. That's the basis of chromatography, polarity being the key delineating factor.

In this examples, there are two types of materials involved. There's what is known as the stationary phase. It's an immobile support – that what the word stationary means, it doesn't move.

Support means some kind of framework, background, foundation upon which some kind of material is held or placed. It's an immobile support that contains a substance that can separate components of a mixture, based on polarity. This tube with its coating, that's the stationary phase; that's the stuff that doesn't move, the stuff flows through. The stationary phase is an immobile support that contains a material that can separate the components of a mixture passed through it. The other component would therefore be the mobile phase, which is generally a gas or liquid that carries the mixture to be separated.

Now let's see these common forms of chromatography. The one that we've already been discussing is gas chromatography, often shortened to GC. In gas chromatography, a sample is volatilized and passed through a column; this tube generally is called a column in gas chromatography. The tube might be a meter long, it might be multiple meters long, wrapped up into a coil. A sample is volatilized (evaporated) and passed through a column. That column is coated on the inside with some form of separatory material. That means it's the stationary phase. The sample is pushed through the column by some form of inert gas, some non-reactive gas, typically, something like argon or nitrogen. That constitutes the mobile phase.

Color. In that example of a pen splotch being separated out, what you'll see is different colored components that come out of it. Chromatography is this idea that if you have a compound that you're visually separating, you'll separate it into several potentially-colored dots. That doesn't actually happen in a lot of forms of chromatography. For example, when we do TLC, we're not going to be able to see any of the compounds. But, we do have visualization techniques that we could use to figure out where the compounds are.

Column chromatography – Yes, this is the same word column that I used a moment ago to talk about the equipment in a gas chromatograph, but this is a much different approach. In this case, the column is a piece of glassware (usually glass). There's a whole range of sizes this might be. If you're dealing with extremely small samples, the column may only be as big as a pipette. If you're dealing with a gram or so of material, maybe it'll be a few centimeters wide, half a meter tall. On an industrial scale, it may be a meter or two wide and several meters tall. They all work on the same principle – that if you want to separate a massive amount of material, that you pass it through a much larger amount of the separatory material. A column is usually loaded, filled in this way. At the bottom, you'll usually put sand or, if it's not reactive to what you're separating, some cotton – something to make a nice flat layer. The amount of separation that occurs depends on the distance the compound travels through this material. If you have an uneven layer, where that distance therefore varies, that means you could get non-ideal separation; you might end up with things mixing together. So, you start out with a nice flat layer, you fill it up with whatever your separatory material is. The most common material used in general organic applications is silica gel, which you can think of as pulverized glass. Glass is silicon oxide – silicon attached to four oxygens which are attached to silicon which are attached to oxygens, so on and so forth. On the surface, where you might break these silicon oxygen bond, you can end up with the silicon-oxygen-hydrogen as a sequence of bonds, which means the surface of glass can be somewhat polar. We could recognize that by the fact that water crawls up a piece of glass; that meniscus that forms is because of the polar interaction of water with the surface of glass. Once you fill it with the silica gel, then on top of it you'd place the compound to be separated, your mixture. That mixture, you push through the silica gel using some form of solvent. The solvent flows through, the compounds pass through the silica gel, the differentiation between compounds occurs because of polarity, and, if you do it well enough, each compound comes off individually out of the bottom of the column; the technical word is elute, to come out. They will elute at different times; that's how you can separate large amounts of material.

Thin layer chromatography – super thin. It's a piece of plastic; sometimes the backing is glass. That's the support. It's covered with silica gel; it's actually the same material that I said is often used in column chromatography. Because it's coated with silica gel, you've got to be a little bit careful with the way that you're handling these plates. Very soon, we're going to talk about marking the plates. If you were to use too much force when you're marking it, you can just scrape the material off. You've got to be very gentle with it or otherwise you'll ruin the plate. Normally, it's like a race – you put a starting line, where you're going to spot a series of compounds. You then put this into a solvent; the solvent flows up the plate. The plate does the separation, so the different compounds will travel different distances, so you might get something that looks a little bit like this. As an example of why you would use TLC plates, let's say that you're doing a chemical reaction, and you know what the starting material is, and you know what the product is. If you're done the reaction before and you have some sample handy, you might put a little bit of the reactant and a little bit of the product on the plate, and then along with it, the reaction mixture. They'll all start off at the same point, and then as solvent flows, there should be the differentiation. You might observe something like this. This is before development, before processing the plate, and then this would be after development.

If you have the same compounds, and you're using the same form of TLC plate, and you're using the same solvent to do the development, the ratio of distances that these dots travel is consistent, it's predictable. How do we come up with some set of ratios? We look at what's called the solvent front, which is a term used similar to the way that we talk about weather fronts – it's the leading line of the approach of something. The solvent is moving through the plate, so the maximum point that the solvent reaches, that's called the solvent front. We measure the solvent distance; that would be the maximum distance one of these dots could travel, if it got fully dissolved and just crept along only with the solvent. Most compounds don't travel that far; in fact, the separation depends on what is the interplay of the compounds you're trying to separate with the TLC plate versus the solvent. For each one of the spots that you end up with, you measure its distance. From that, we create a value called a retention factor, retention meaning now much did it get stuck on there.

That Rf value is equal to the compound distance divided by the solvent distance. Again, if the TLC plate is prepared and developed in a regulated, systematic way, then you're going to get consistent Rf results.

What does that do for us in this example, where I have a product, a reactant, and a reaction mixture? Here's a dot for the reactant, here's a dot for the product, and notice that both dots are present in the reaction mixture. What that means is that the reaction is working at least partially, because you do have product that's formed. It also means the reaction's not complete, cause you still have reactants present. This is one of the most common things we did with TLC plates back in grad school, is to check the progress of reaction.

What you'll be doing in this experiment is not checking on the progress of reaction, but instead identifying the components of an analgesic table. Analgesic tablets, they tend to contain common pain-killers like aspirin, acetylsalicylic acid, ibuprofen; sometimes there's some extras thrown in there, principally caffeine. We could take an analgesic table, crush it up, dissolve it up, filter out the solids – in most tablets you have something called a binder. That is related to a problem in drug design – you might get some really useful, beneficial chemical, but how do you get it into your system? In the case of analgesics, you wrap it up with a binder that keeps thing together until it gets to your digestive system and then it falls apart. Crush the tablet, dissolve it, filter it, and then spot it onto this TLC plate. Along with it, but some reference compounds. Put pure acetylsalicylic acid, ibuprofen, caffeine, what ever we think might be present. Run the TLC plate. Calculate the Rf values for the individual reference compounds, and all of the spots that you get from your tablet. If you can match any of those up, then you can identify the components of the table. That's the goal of the experiment.

Let's talk about how to do TLC. First, how to make the plates. First off, when writing on TLC plates, do not press too hard, or you will scratch off the silica gel. You must use pencil in marking TLC plates. Why do you think that might be true? There's no ink. The lead – which is not really lead – the material in pencils will not crawl up the plate. Because ink in a pen – that's an organic substance – we're going to use organic solvents – it would just crawl up the plate with your compounds, obscuring your compounds or ruining the plate. You're going to have some form of starting line. That starting line should be about 0.5 – 1.0 cm above the bottom of the plate. Do make sure that your plates have flat bottoms; if this is a separation based on distance, we want to have a consistent starting point, a flat starting point. Make sure that your line is also very parallel with the bottom part of the plate. So draw a line across the bottom of plate parallel to bottom, roughly 0.5 to 1.0 cm up, closer to 1.0 cm. After you've drawn that line, you're going to put spots of your compounds on the plate. To do this, you'll be making a series of micropipettes. Once you've done that much, you've prepared your plate.

Now we move on to development, which is the process of putting in the solvent and letting the solvent flow through. That means we do need to talk about common TLC solvent systems. Let me give you an example. Let's say that we have a mixture of different carboxylic acids. Carboxylic acids are really polar functional groups – compared to other molecules that have the same number of carbons, at least, carboxylic acids are pretty polar. What if you put a bunch of carboxylic acids on a TLC plate? Since TLC plates have a polar substance that coats them, that means carboxylic acids are going to really want to stick to the plates. If you use a non-polar to develop them, then the solvent's going to flow through, but because the solvent is not very polar, there's no reason for the carboxylic acids to want to go along for the ride with the solvent, so they'll stay mostly in place, which means they won't have very good separation. What if you went the opposite extreme, what if you used a solvent that was highly polar. Then, the dots would possibly prefer to move along with that solvent, but if it was too polar, they'd all move along at the same pace. Again you'd end up with poor separation – this time, the dots would all be at the top, though, versus the bottom. You need some kind of solvent system that's in between – polar enough to move the compounds, but non-polar enough that some kind of separation occurs. In general, a solvent or solvent mixture is chosen so that the most effective separation of the components can be achieved.

I'll give to examples of common solvent systems. The system that I used most frequently was a combination of hexanes and ethyl acetate, which are also two common lab solvents. Hexanes, as a reminder, is a mixture of different six-carbon compounds, different isomers; ethyl acetate is an ester, where if you take the hydrogen off of acetic acid and put an ethyl group on, that's ethyl acetate. Hexanes, being just hydrocarbons, are more non-polar. Ethyl acetate's not a highly polar material, but relative to hexanes, at least, it's polar. Depending on the proportion of the two, you can adjust the polarity of your system. In lab, I had five, six, seven different little containers that were set up, each with a different proportion of these solvents. As I did a reaction, I could prepared a set of TLC plates, develop them all at once, and figure out for that reaction: what is the best mixture? Another common one, where you might need more polarity, is a combination of chloroform and acetic acid. Chloroform is more polar than hexanes, so automatically you start with a more polar environment. Acetic acid is water-soluble; it's pretty darn polar. This system also has a funky side-effect: depending on what form of silica gel that you use, some forms of silica gel go translucent when chloroform is passed through them. If you then a had a compound mixture that did have highly-color components, then as those components pass through the gel, you can see their progress through the gel (which is kinda cool). Even if they may not be visible, if you shine a UV light on the compounds, you might get them to respond that way. We won't have to go through the process of choosing solvents for today's lab; it's already been done for you.

Let's talk about the development chamber. It's simply a jar, but it does have a piece of filter paper in it. What is the purpose of the filter paper? To maintain the equilibrium of the solvent in the chamber.

All solvents have vapor pressure, which means some non-zero portion of the solvent is always going to evaporate unless you are in a closed system. If you have a closed system, it does eventually reach a maximum vapor pressure. Imagine that you have this chamber with solvent at the bottom, then your TLC plate. Your TLC plate has a good surface area to it, which provides a place where the solvent can evaporate from. If the whole point is trying to get the solvent to crawl up the plate, we don't want it evaporating off of the plate. What we do is help it out. We put a piece of filter paper in. The solvent crawls up that filter paper, the solvent evaporates off the filter paper, and it assures that you have a saturated environment inside the chamber. It reduces the amount of evaporation that will occur from the TLC plate itself.

The chamber looks like this. Here's our filter paper, which is used to ensure that the interior of the chamber is fully saturated with solvent, to prevent solvent from evaporating off of the TLC plate. Notice that the filter paper does not go all the way around the chamber. Why would you guess that the filter paper is not wrapped all the way around? (It is not out of concern for blocking the ability to see the plate in the chamber). What happens if the TLC plate touches the filter paper? Then you could have solvent coming in from the sides. The whole point is that we're trying to separate this by passing one way through the TLC plate. If you have solvent flowing from the other directions, then it interferes with the development process, your R<sub>f</sub> values are off, you won't get correct results. The TLC plate must not touch the filter paper; otherwise, solvent could flow sideways across the plate.

One more gotcha about the development chambers. Let's say this is our TLC plate. Let's say that we've got the starting line here. Notice where I've drawn the solvent level. The solvent absolutely must be below that starting line; that's why I'm recommending that you have about a 1 cm gap from the bottom of your TLC, to leave you visibly enough room to get some solvent in there. Why do you think is that you can't have a solvent cross that line to begin with? It would just wash the dots away; it would dissolve the dots. Maybe it would crawl up the plate as well, but you'd totally lose that starting point. The solvent level must begin below the starting line, so to prevent dissolving up the dots. You prepare a TLC plate, you'll have solvent in the chamber. Keep the lid closed until you put the TLC plate in, so you can make sure the chamber's saturated; put the TLC plates in; make sure it's standing up in such a way that it's not touching the filter paper; put the lid back on, so you can re-establish that equilibrium.

How do you know you're one developing a TLC plate? That solvent is going to crawl up the plate, and you want to make sure that you stop it before you get to the very, very, very top, because to calculate these R<sub>f</sub> values, we do need to know how far did the solvent travel (as well as how far did the compounds individually travel). You can watch the solvent progress, you can see it'll make the TLC plate look wet. Once you see it approach roughly 0.5 cm from the top, use a pair of forceps and pull the TLC plate out of the chamber. Because you need that solvent front's distance, as soon as you pull it out of the chamber, take a pencil and lightly mark how far did the solvent progress; don't wait any time to do that, because it's going to start evaporating as you pull it out of the chamber. Once the solvent nears the top of the TLC plate, remove the plate from the chamber, and immediately mark the position of the solvent front. That's the process of development.

The last portion is visualization. In this experiment, you will not be able to see your spots, so you will not see spot run. This silica gel has had a UV-reactive dye added to it. Many organic compounds absorb UV light. If you have a compound that was spotted on the plate that absorbs the UV light, then that means the UV light it's not going to make it through to the TLC plate, so it will appear as a dark spot on the TLC plate, a little bit like a sunspot. Once you've taken this out of the development chamber, let the solvent evaporate; shine the light on it, and wherever you see dark spots, circle those. Those will be how you identify most of your compounds. Some forms of silica gel are prepared with a UV-active dye, that causes the plate to glow – green, orange. If a compound on a TLC plate absorbs UV light as well, then that compound will appear as a dark spot, since the light, therefore, will not have hit the TLC plate itself. This is a non-destructive visualization technique [usually], since all you're doing is shining light on it. Quite technically, you might have some type of alkyl iodide, or something that will decompose if you shine light on it too much.

What if your compounds do not absorb UV light? Then this trick is not going to work, and we have to use some other technique. One common technique is to use an iodine chamber. Much like we had a chamber for the TLC solvent, we have a little chamber where you put a few crystals of iodine in. Iodine would preferentially complex the organic compound, versus the TLC plate, so where your compounds are will look like brown spots because of the iodine. I'd like to try to use a chemical stain [instead], since it's more commonly used as a form of visualization. In the chemical stain, the entire TLC plate is normally dipped in some kind of reaction mixture that causes a chemical reaction that therefore colors the different spots. One of the most common visualization agents is the compound p-anisaldehyde. If you know a little something about plants or cooking, anise is a material that has a sort of licorice flavor to it, and this is one of the compounds associated with that plant, which is why it's called anisaldehyde. Take a TLC plate, dunk it in this, and different functional groups will turn different colors. Ketone will often turn a sort of orangey color; a lot of alcohols tend to turn pink; carboxylic acids, often they're purple – point being, they'll show up, they'll be visualized. Because you dipped this in this compound mixture and you have reacted it, it is a destructive technique.

I. Purpose – a concise statement of the qualitative or quantitative goals in an experiment.

II. Data – Direct qualitative or quantitative observations that require no manipulation.

III. Calculations – Any mathematical manipulation of data.

% composition = mass of compound / mass of mixture \* 100%

% yield = mass obtained / theoretical mass \* 100%

% error = measured value – accepted value / accepted value \* 100%

% error for temperature must always be expressed using Kelvin

IV. Conclusions – A summary of the results of an experiment that addresses the goals of the experiment as presented in the purpose statement.

V. Discussion –

Chromatography – A technique by which a mixture of compounds can be separated on the basis of their polarity.

volatilized – evaporated

stationary phase – an immobile support that contains a material that can separate a mixture of compounds passed through it.

mobile phase – a gas or liquid that carries the mixture to be separated.

Gas chromatography (GC) – A sample is volatilized and passed through a column that is coated on the inside with some form of separatory material (stationary phase). The sample is pushed through the column by an inert gas, such as argon or N<sub>2</sub> (mobile phase).

R<sub>f</sub> = retention factor = compound distance / solvent distance

Preparing TLC plates

\*When writing on TLC plate, do not press too hard, or you will scratch off the silica gel

\*TLC plates must only be marked in pencil, since the ink from a pen would potentially obscure the compounds being tested.

– Draw a line across the bottom of the plate parallel to the bottom

– Each compound or mixture to be tested is spotted on the plate using a micropipette.

Development – TLC solvent systems – A solvent or mixture of solvents is chosen so that the most effective separation of the components can be achieved.

– hexanes/ethyl acetate

– chloroform/acetic acid

Development chamber – Filter paper – used to ensure that the interior of the chamber is fully saturated with solvent to prevent the solvent evaporating off the TLC plate.

\*The TLC plate must not touch the filter paper, otherwise solvent could flow across the TLC plate.

The level of the solvent in the chamber must be below the starting line of the TLC plate, otherwise the dots could simply be dissolved.

– Once the solvent nears the top of the TLC plate, remove the plate from the chamber and immediately mark the position of the solvent front.

Visualization – Some forms of silica gel are prepared with a UV-active dye that causes the TLC plate to glow under UV light. If a compound on the plate absorbs UV light, the compound will appear as a dark spot on the plate (since the UV light would not reach the plate) – non-destructive

– chemical stain – the TLC plate is dipped into a reactive mixture that will react with the compounds on the plate and colorize them – destructive

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Structures

Identical to those from lab 6A (10/12/12)