## [SQUEAKING] [RUSTLING] [CLICKING]

SARAH HEWETT: All right. Good afternoon. Today, we are going to start talking about a new lab, the Essential Oil lab. And if we look ahead a little bit in the semester, so we're still working on finishing up the Charles River lab. We have today, then we have day four on Wednesday and Thursday. And then next Monday and Tuesday will be day five of the Charles River lab, and that'll be your guiz day.

And then after that, we're going to start a whole new set of labs. And the last three labs of the semester-- so there are five total labs-- we're going to rotate so not everybody is going to be doing the same lab at the same time. So each bay in each group with your TA, you'll be doing a different lab. So everybody will get a chance to do all three, but you'll just do them in a different order.

So after the Charles River lab, the A prime 2 group, the closest to the middle of the lab, you'll be doing the essential oil lab. The center group will be doing the catalase lab, and then the group closest to the doors, A prime 4, will be doing the ester lab. And your TAs will help make sure that which lab you're supposed to do so you know which pre-lab to write up. And if you any questions, it corresponds to groups A, B, and C in the printed version of the lab manual, and I think the version on Steller has been corrected with the A prime notation for telling you which lab you're going to be doing when so you can keep track of what you need to write and what pre-labs you need to do.

And then if we take a moment and look back a little bit at what we did for the Charles River, I went on to the EPA website, and they have a buoy outside the Museum of Science just down the river a little ways where they keep track of a whole bunch of different metrics in the water. And you can go check out what the buoy has recorded. And so if you're interested, you can go to this website. And they have, also, the Massachusetts standards that you can compare these numbers to for some of them anyway.

And so the most recent reading that they had was from Monday, September 23, which was last week. And you can see that the pH was around eight and a half which is similar to what you guys measured. The dissolved oxygen that they had was around 10 and 1/2, which is a little bit higher than what you guys had, but again, it changes we know based on the temperature and the day. And then this pink line that I added down here at the bottom is the phycocyanin which is indicative of the cyanobacteria that you heard about in lecture before. And so you can see it was it peaked here in July and the beginning of August when we had the cyanobacteria bloom.

So something that you can check out if you want to when you're writing up your lab report if you want some more information about what metrics they look at and keep track of in the Charles River. And now, talking about our next lab, essential oils. So the word essential has a few different definitions depending on how you're using it. And some people will say it's essential, like, it's the most important thing. The definition of essential that we mean when we are talking about an essential oil is that it is a fundamental or central to the nature of something or someone, like the essence of a thing.

And so the essential oils come from plants, typically. And so we are talking about the essence of the plant or something that comes from that plant specifically. A little bit of history of essential oils. Their recorded use is far back in history as ancient Egypt. The Egyptians were big into perfumes and cosmetics and all of that. And they have records of pressing plants to get the oils out to use in different perfumes for the different scents. And also, in the days of the plague in the medieval era, the doctors would wear these masks full of different plants. And they thought that breathing through the plants and the oils would help protect them from disease. And if you look around now at essential oils, you can find them almost anywhere. But you may come across something called thieves' oil, which is a blend of the oils from those different plants.

And the story behind that, which may or may not be a legend, is that there during the plague, everybody would throw all their corpses out into the street and then there would be people who would go and loot the corpses to steal what they could. And there was a group of thieves who was running around stealing stuff off of all these dead bodies but they never got sick. And rumor has it that they concocted this oil, and they would wear that and put that on themselves and it prevented the plague. And so that's what the story of the thieves' oil may or may not be true.

So today, if you look online and you do a search for essential oils, you can find a million different places to buy them. You can find all sorts of information, some true some not, about what essential oils do. So you may see something like this that has different types of essential oils, different plants that they come from, and all of the things that they are rumored to treat.

You can buy the essential oil of almost any plant that you like, depending on where you look. And not all of this is just hearsay. There are people that are actually doing different studies, especially in the veterinary medicine world, of using different essential oils to treat different fungal infections or bacterial infections. A lot of these have antibiotic properties, and they can disrupt some biofilms depending on the different properties which isn't super surprising since a lot of these are just hydrocarbons that you could also synthesize and are similar in structure to some of the drugs and synthetic products that we make to do these same things.

So essential oils, where do they come from? So you ... how do you go from something that looks like a plant from the garden to a little vial of oil? And the ratio of how much plant you need to get a few milliliters of oil is very, very large. So it's not the most efficient process. But the way that it is most commonly done commercially now is through steam distillation. So you can grind up your plant material, chop it up a little bit to increase the surface area. You heat up water, put steam through the material. It helps volatilize the oils. And then the steam and the oils co-distill. You can condense it back down. And then you'll get a mixture of this water with all of the water soluble parts of the plant and then your essential oils, and you can separate them out.

And the reason that you can separate them out is due to their structure. So most essential oils are made up of isoprene units. And so that's the structure of an isoprene. It has five carbons and eight hydrogens. And then when you stick them together, you get a class of molecules called terpenes. And so a terpene is more than one isoprene unit. So the way that you name these things is that if you have two isoprene units, that's the smallest terpene you can have, so it's called the monoterpene. Then sesquiterpene, diterpene is four because it has two of the monoterpenes. Triterpene and tetraterpene is when you have eight.

So if we look at some of the molecules that you may know from everyday life, does anyone know what this is? So that's vitamin A. If you look at the structure of it, we can try and find all of the isoprene units, so these five carbon units. So if you start at the top there is one there. And we have another one, two, three, four, five there. And then you can keep going down the chain. So how many isoprene units do we have here? We have one, two, three, four. So this would be a diterpene.

And then does anyone know what this molecule is? So that's menthol use in minty flavorings. And this has two isoprene units and then this hydroxy group. So you can have these terpene structures that have the isoprene units and then they also can have different functional groups attached. A lot of times it'll be a hydroxyl group or a carbonyl group.

And then this molecule, which you also may have heard of, is camphor. So that's used in lotions a lot of times to treat irritation. And this one's a little bit tricky, but you can find the isoprene unit. So there's one there and then one there. Oh, other way. So those are some common terpenes that are found in essential oils that you may or may not have seen in other contexts.

The structure that we care about, in this particular lab-- yes, Jesse.

STUDENT: I'm a little bit confused. So on the slide said five carbon and eight hydrogens?

SARAH Yes.

**HEWETT:** 

**STUDENT:** So for menthol, for instance, there's five carbons and more than eight hydrogens. Right?

**SARAH** Yes, in this case they would.

HEWETT:

**STUDENT:** So does it require a double bond in order for it to be called-- classified as an isoprene?

SARAH No. I was also kind of curious about that, and I personally didn't find a good answer on the internet. But when I
HEWETT: was looking through the literature and stuff, menthol was referred to as a terpene. So I don't know, if maybe once they get combined together with the double bonds that it's not as strict, or once you start adding extra groups. I'm not sure. That's a good question, and I can get back to you on that and how they actually name and classify these things. Because, yeah, in some of the cases, the double bonds go away, and it doesn't have quite the right ratio.

So in our lab, the essential oil component that we are going to be looking at is carvone. And there are two types of carvone. So carvone is a major component of spearmint oil and is also a major component of carraway oil. And those are two very different things, and if you've smelled either of them, then that they smell different, they taste different. And the reason for that is because we have a chiral center down at the bottom. So there are two different possible isomers of this molecule and they have different properties, some properties are different. So we'll need to take a moment and look at chiral centers. So a chiral center occurs when four different substituents are attached to one carbon center. And you have probably talked about this in your organic chemistry class or in your other chemistry courses. So as a quick refresher to determine, they are labeled the R versus S. And to determine whether it's an R or an S stereocenter, you go through and you look at each group that's attached to the carbon, and then you assign priority to each group. And this is typically done most straightforward is through atomic mass.

So you look at the atoms that are attached to the carbon center. And then the highest atomic mass has the highest priority. The lowest has the lowest priority. And if you have a double bond, it counts as two bonds the atom counts twice.

So if you have longer chains and they look similar at the first point, then you keep going down the chain until the first point of difference and then you can help assign priority. So if we look at our carvone molecule, usually, if your stereocenter has a hydrogen, that's pretty much always going to be your lowest priority group. So I've drawn this particular isomer with the hydrogen pointing away from us so that we can more easily determine R versus S. So can anyone help assign the priority of these three groups?

Does anyone know what the highest priority would be? Alec.

**STUDENT:** It would be the substituent towards the end.

SARAH Yep. Why?

HEWETT:

**STUDENT:** Because it's attached to a carbon that is secondary. It's attached to two more carbons.

SARAH

HEWETT:

**STUDENT:** And a double bond.

Yes.

SARAH Yeah, no, you're saying it right. So this one's attached to two carbons and one of them being a double bonds so
HEWETT: that counts kind of twice. And these carbons are just attached to two hydrogens each, so this has an overall higher atomic mass, so this is going to be our highest priority. Then what? Pointing out this way. So then you go to this carbon, and this one's double bonded to a carbon. But this one's double bonded to an oxygen so that has higher priority and that just leaves our final group. So does this go counterclockwise or clockwise? Counterclockwise? So R or S? S.

So the way that chiral molecules work is if you take the mirror image, then you get the opposite configuration. So if this is our S, if we flip these two substituents on our chiral center, then we get the R. Or if you prefer to look at it as the actual mirror image, if you kind of ignore this, then this molecule and this molecule are the mirror image of each other. So even though this group is pointing forward in both, the oxygen and the double bond have flip sides, so these two are mirror images. So this is also R.

And a big part of organic chemistry and figuring these things out is being able to manipulate the molecules spatially in your head. A lot of times it's easier with models. But does that makes sense how we get those? Excellent. So we can talk about different cultural centers, and we can also talk about chiral molecules as a quick review. So if you have chiral centers in your molecule, then you'll have, potentially, a chiral molecule. So a molecule's chiral if it is not superimposable on its mirror image. And then within that, we have two other ways to name these stereoisomers. One is in enantiomer which is a pair of molecules that are mirror images that are not superimposable on each other. So that would be like this pair of molecules. So these are enantiomers. They are mirror images. And if you try to flip this around and stick it on this one, you'll see that if you flip this over so that the chlorine and the hydroxyl group are on the same side, they'll be pointing back and these are not superimposable.

Same thing with these two. These are enantiomers. They are mirror images, and they are not superimposable on each other. But you can see that all four of these molecules are related and that they are isomers and that they have the same atoms in a very similar configuration. The only thing that differs is the stereochemistry at each of these chiral centers. And so these molecules, say, are related to each other and that they are not mirror images, but they are also not superimposable on each other. And so these are called diastole diastereomers.

These two have the same relationship. These are diastereomers. So are these two and these two. So that's the terminology that we are going to be using when we talk about different molecules that have different numbers of chiral centers. So if you have more than one chiral center, you can get all of these different relationships.

This becomes important in biological systems. So if you have a protein, your amino acids in your protein are chiral. So you end up with protein the protein structure is determined by the chirality of these amino acids. So you can end up with a chiral surface that has a bunch of chiral and individual molecules in it. And then if you have a substrate for your enzyme that is also chiral and you have different enantiomers, one will fit and one will not.

And you can also look at it if you have a cultural center that has three different things to it and a receptor that has three different locations, then there is only one orientation that will match up properly. So if you start rearranging those substituents around that carbon center, then you won't be able to line up with your target properly.

And this comes into play in your eyes and in your smell and in your taste. So these carvone isomers, these enantiomers, one of them taste like mint and the other one tastes like carraway . And most people can tell the difference. There is about 10% of the population that cannot tell the difference between these two isomers. So when you get your oil in lab, if you smell yours and then if you smell your neighbor's, hopefully you should be able to smell a difference. But it'll be interesting to see if anybody can or cannot tell the difference between these two chemicals.

One of the ways that this chiral recognition and this chirality in biology has played out is in the case of thalidomide, which again, is something that you may have heard of in your other chemistry courses. So this is the structure of thalidomide. And there is a chiral center. Where's the chiral center in thalidomide? Right there. You be a little more specific. To the right of the N, yes. So here's a chiral center, so you can have an R and S versions of thalidomide.

And as it turns out, let's see, I don't want to say this wrong. The r version is used to treat nausea and is used uses a sleeping aid. And the S version causes birth defects. And so this drug was given in the '70s and the '80s to a lot of women who had morning sickness. And they took it while they were pregnant and their children ended up with a lot of birth defects because they were given both enantiomers of this drug.

And even if they had been given enantiomerically pure thalidomide, your body has enzymes and you can do reactions that will convert in between the stereoisomers. So there is no safe way to give thalidomide to a pregnant woman. In a less traumatic case, ibuprofen, this is the structure of ibuprofen. Where's our chiral center?

**STUDENT:** That one.

SARAHThat one? Excellent. So you can have R and S versions of ibuprofen as well, and only the S version of this drugHEWETT:works in your body as a pain reliever. And it inhibits certain chemical reactions in that pathway in your body. And<br/>biology majors, I'm sure you know way more about this than I do.

So the S version works in the R version has no biological activity, at least in terms of the target of this drug, which is to treat pain and inflammation. But it's given as a racemic mixture because the second enantiomer does not have any negative effects that we know about. And your body can actually convert between the two. So if you're given all of the S form, which they have done to people and that actually has more of a benefit to them, but it'll convert some of it into the R form which is useless anyway.

So if we go back to our molecule carvone, we can look at the two types of carvone. And these are the two isomers, so they have the same molecular weight, same boiling point but different physical property in how they smell and how they taste.

So carvone is what we care about, but essential oils are not pure substances. So if we give you the spearmint oil and we give you the carraway oil, then they'll have more in it than just the carvone that we care about. So the major impurity that you guys are going to see in both of these essential oils is called limonene. And that is the structure of limonene. It is also chiral. So it has a chiral center down here at the bottom. And-- oh, my notes didn't show up. Bummer.

And so one of the isomers of this, I believe it's the R smells like oranges and the S smells like lemons. So it's found in high quantities in citrus plants. It's a monoterpene. It has two of these isoprene units. And in our case, it's going to be an impurity. So we are going to try and separate the carvone in our essential oil from the limonene in our essential oil.

So if we look at these two compounds side by side, how are we going to separate them? Alec.

STUDENT: Carvone has an oxygen so we're going to pull it in limonene?

SARAH Yeah. So one way you could think about separating these is by they are very similar in structure, but the only
major difference here is this oxygen, which makes the carvone slightly more polar. So you could think about using some sort of chromatographic technique, maybe, like we did before to separate them based on polarity. So that's one way we could do it. What else is different about them?

**STUDENT:** Boiling point.

SARAH HEWETT: The boiling point. And so using the polarity is a little bit more complicated. But if we want to use straightforward basic gen chem principles, then we can separate them by our boiling point using distillation which is a technique that you all probably heard of. And I have the distillation apparatus set up here. So this distillation apparatus is a short path simple distillation. So the way distillation works is if you have either a mixture of two liquids that have different boiling points or if you have a liquid that has some nonvolatile impurities in it, you can put your mixture in here, heat it up, and then re condense the vapor and then you can collect, hopefully, your purified substance at the end of it.

So if we have this mixture of two liquids, then we need to talk about different laws that govern how two liquids and how to vapors interact with each other. So the first thing that we want to talk about is Raoult's law. And what Raoult's law says is that the partial pressure of a substance is equal to the mole fraction of that substance, which is the x, times the vapor pressure of the pure substance.

So if we have a mixture of two things, and we want to figure out what the vapor pressure of one of them is above the mixture, and we look at the mole fraction within the mixture. And the mole fraction is just the moles of the component that we care about over the total moles in your mixture. So if you have two things, you add to the total number of moles, divide that by the number of moles of the thing that you care about.

And then you can look up the vapor pressure of what the pure substance would be, and then you can get the vapor pressure of that substance above a mixture. And then Dalton's law of partial pressure says that the total pressure of a gas is going to be equal to the sum of the partial pressures. So if you can figure out your partial pressures using Raoult's law, then if you have a mixture of pentane and hexane-- and this was taken from your textbook if you want to look this up. If you read this, it's in figure 12.3. Chapter 12 is all about distillation. So that's a good resource for this lab in particular.

But if you have pure pentane, then your total pressure is going to be just the pressure of pentane. And this is your pressure of pentane. And then you can see that if you add up these two lines, then you'll get the total equaling your blue line, which is your total pressure at various different mole fractions of each of these substances. So the way that it plays out for us, if we're going to be heating up a mixture and at-- hello-- and a different mole fractions, then we'll have different amounts of each vapor in our total vapor pressure, then we need to think about that when we are doing our distillation.

And so you can make a temperature composition diagram. And this is a little bit confusing to look at it first, or at least it was for me. So we can go through how this plays out in real life. So if you have a mixture of hexane and pentane-- this is just a theoretical situation. And the boiling points of hexane are 68 degrees and pentane boils at 36 degrees. And we wanted to get pure pentane which is our lower boiling point thing.

So if we start out with a mixture that's about 75% hexane and we vaporize it, then the boiling point of that liquid will be closer to the boiling point of hexane because there's more hexane in it. If we cool that vapor down so it's at the same temperature, so heat it up to 65, cool it to a vapor at 65, and then re-condense it to a liquid phase, now our mole fraction of hexane is about half. So we've purified our vapor.

If we take a starting mixture that has about half and half hexane and pentane and we heat that up to its boiling point, which is now going to be lower because it has a higher fraction of pentane in it, if we cool it down at the same temperature and then re-condense it to the liquid phase, now our mixture has about a little less than 0.4 of our more fraction of hexane. And then if we heat that back up and vaporize it, cool it back down, now we have even less hexane. Heat it up, cool it down, now we are getting very close to pure pentane with no hexane in it. So we get very close to a mole fraction of zero for a hexane, and we get closer to the actual boiling point of pure pentane.

So if you look at this diagram, then how many of those heating and cooling and re-vaporization cycles did we have to go through to get down to a vapor that is pure in pentane? So in this case, it's about five cycles of that. And depending on the difference in boiling point of your two liquids that you're trying to separate, it may take even more cycles to get to your pure compound that you care about. And if the boiling points are greater in difference, then it'll take fewer cycles to get to your pure liquid phase.

So if we have these two liquids that we were trying to separate and we want to get down to a pure fractions of limonene in carvone and we want to separate them and make them pure, how are we going to do that? A lot of distillations. Or we can do what's called a fractional distillation. So I told you this before. This one is a simple distillation, and you can see that there is not a lot of room in here for the vapor to vaporize and then re-condense before you collect it in your collection vessels over here.

If you provide more surface area, then the vapor has more chances to come into contact with the cooler glass. It'l re-condense into the liquid phase. And then if you're heating this, it'll drip down, it'll be reheated by the hot vapor coming up, and so then you can have multiple of these heating and cooling cycles before you start collecting your final product. And so this is a fractionating column or a Vigreux column. And you can see, hopefully maybe, that it is not just straight glass, that it has all of these little divots in it and it has a lot of kind of spikes going into the middle that provide a lot of surface area for this vapor to hit to cool and to reheat.

So this provides a much more efficient separation than just the simple distillation when you can only go through a couple of heating and cooling cycles before you collect it. So this will help us to get a more pure product. And it is very helpful for when the two things that you're trying to separate have boiling points that are relatively close to each other, being like less than 50 or 60 degrees apart.

So there are some things to consider if you're going to use distillation as a technique anyway. And so one of them is the boiling point of liquids that you're trying to separate. So how hot does this thing need to get? Is it a feasible temperature for you to reach in the lab? One of them is how pure your fractions need to be. So if you don't really care about the purity of your fractions, then you can do a quick simple distillation, collect your product, call it a day.

And then do your compounds decompose if you heat them too high? And in our case, yes, they do. So if we heat our compounds too high, so they have a pretty high boiling point if you remember back or if you look back in your slides. So we try to hit them aggressively to that boiling point, then our products may decompose before we can collect them. So we have to figure out a way to make things boil at a lower temperature. So if we think about our definition of boiling, it is the temperature at which the vapor pressure of a liquid equals the pressure above the liquid. So there are two ways that if we need to lower our boiling points that we don't have to heat it up to over 200 degrees, then how do we lower the boiling point? We can either increase the vapor pressure of the liquid. So usually, we increase the pressure of the liquid by just heating it. You heat the molecules up, they become into the gas phase, and you increase the pressure within the liquid itself. Or you can reduce the pressure above the solution.

So if we reduce the pressure above the solution, then we don't have to heat it as much in order for those pressures to be equal. So that is what we're going to do in class. We're going to use both of these. We're going to have to heat it, and we're going to reduce the vapor pressure in order to make the vapor pressure a reasonable temperature for us to get to in the lab.

And the way that we're going to be doing this is with a vacuum distillation. So this distillation setup is identical to the one that you will be setting up in the lab. And this is kind of nice because the glassware is all sealed together. So your column, your condenser, and your distilling head over here have all been made as one piece you have fewer joints where you could lose your vacuum.

So when you get this, you're going to get it as a kit from the stockroom, and it will have this one big piece of glassware in it, so be very careful when you are opening and taking things out of this kit because this is a large piece of glass where it should be wrapped in bubble wrap in most cases, but we don't want to break it because it is expensive and a specialty piece of equipment.

So if we go through this and talk about different pieces, they're all very important. So this is where your mixture will go. And if you look at the picture of an actual set up in lab, you'll have your stirring plate. So whenever you heat something you want to stir it so that it helps the bubbles to form because you don't want your solution to get superheated and then all start boiling all at one time because it'll shoot up into your column. And then if you flood your column with your mixture, then there aren't any surfaces for the vaporization cycles to happen, and so you will get very inefficient separation of your two liquids.

So you want to make sure that you have stirring and you don't heat it too quickly so that you don't flood your column with the bubbles and that you give it a chance to just have the vapor go up your fractionating column. So you'll have your heating mantle, your stirring plate, and then you'll have your mixture in here. This is your fractionating column. And then you'll have your thermometer.

And our thermometers are all set up to have a ground glass joint, so they'll just sit right in the top here. And you want to make sure that the thermometer bulb is right in the path of where your vapor is traveling so that you can monitor the temperature of the vapor that you're collecting so you know what the temperature is. And you can use it to help identify which fraction is which. So if you know the temperature of the vapor that you're collecting, then you can compare that to the boiling point you know what you're collecting.

Then you'll have your condenser, and this is going to be filled with cold water. So we have cold water supplies from the hood. You will attach the water in at the bottom and out at the top. Your TAs will go over this, too, but this is very important because you want this whole thing to be full of water. So if you put the water in the top, then gravity will kind of do this thing, you can see it's on an angle. So the gravity will come, it'll just go straight out the bottom, and then this whole top part will be full of air. That's not helpful. So we can use gravity to help us here. If you put the water in the bottom, it'll fill up the whole condenser and then go out the top. And this could be, possibly, the most important thing I will tell you today. This little metal piece is very important to the success of your lab. Does anyone know what this does? So this piece goes when you attach your tubing from your waterlines, you want to have one of these little metal guys. And it'll wrap around your tubing and your glassware, and then it'll clip together to secure your glassware to your tubing.

And this is important because once you turn the water on, the water is at a pretty decently high pressure. And if you are not paying attention, the tubing does not stay on these little adapters very well. Your tubing will pop off, and then you have a faucet of water coming through a rubber hose. I don't know if you've ever seen those like wild inflatable tube bend, yeah, like that's what's going to happen in your hood. And so your lab partner will be upset with you. You'll be upset put yourself and everything will get very wet. And there are no drains in the hoods, so it's a big mess to clean up.

So before you turn your water on, make sure that all of your tubing is clamped. It's my public service announcement for the lab. And your TAs, I'm sure, will also be checking this very carefully. They know what happens. So then, we have our condenser, and then we will have this piece here. And you can see that there's another spout out here. And this connects to your vacuum line. So you know those manifolds that you used with the nitrogen lines in the ferrozine lab? Yeah, so one end is connected to the nitrogen lines and the other end is connected to a vacuum pump.

So you're going to use the vacuum side of those this time. And so there's a pressure gauge on there that will tell you what the pressure is once you turn the vacuum on in the system. It's a digital one, so you can just read it. It gives you the output value in torr. And we're going to get this down to close to one torr or below for this particular experiment.

So you will make sure that your vacuum line is connected here. You'll turn your vacuum on. And then if we're going to make sure this whole system is under vacuum and you want to be able to collect different fractions so your things will boil at different temperatures, so you can't be swapping your collection vessel in and out if your whole system is under vacuum. So we use what's called a cow adapter. So this is a cow thing because it looks like some cow udders. And you can attach four different flasks to the bottom of it. And the ones that you'll see in lab will have a little spout here.

And so you can see which flask your spout is pointing towards when you are using it. So if you want, you can rotate this so that you collect your different fractions in different files or in different flasks. And you want to make sure that when you do all of this that all of your joints are nice and tightly clamped. So you want to put Keck clips, these little yellow guys, on all of the joints that you attach to each other. And then you're going to use vacuum grease, and your TAs will show you how to do that. You just need a little bit of grease, but you want to make sure that the grease is there to provide a good seal and so that you can undo your glassware after you've put it under vacuum.

So that is the case. And then the last thing that you need to know is the ice bath. So once you have heated up your mixture, you've re-condensed your vapor, and you're collecting hopefully another liquid over here, we have this under a reduced pressure, which means that the boiling point has now been lowered. So you don't want what you've already collected as pure product to re-vaporize and go back into your system. And you don't want these to kind of evaporate and then re-condense into each other and contaminate the fractions that you've worked so hard to collect.

So you're going to have to kind of engineer your ice bath to only cool one flask at a time, especially the limonene fraction. That one tends to be very volatile. So your TAs, again, will tell you about this when you get to lab, but you want to make sure that you are cooling your fractions but not cooling too many of them so that you don't get spillage into different vessels.

And so that will be how you collect your limonene and your carvone. And hopefully, you should be able to separate both of those components from your essential oil mixture that you're given. So some of you guys will start with the carraway oil, some of you will start with the spearmint oil, and then you will both end up with limonene and carvone. It will just depend on which isomer of the carvone that you have.

So when we were doing this, if we know the boiling point of the limonene and the carvone at room temperature, then we need to be able to figure out what the boiling point is going to be under a vacuum. So you can use what's called a nomograph. And there's one of these in your lab manual, and I've reprinted it here just to give an example of how you use one of these.

And so the way that you can use this to calculate your corrected boiling point at lower pressure is to figure out what the pressure is that you are doing your experiment at. So you can read, like I said, the pressure off of the pressure gauges in your vacuum lines. And let's say, for example, that you have gotten your experiment down to about one torr. So you put it out there. And then that this is your boiling point at 760 torr, which is atmospheric pressure. You know that the boiling point of whatever you're starting with is a little bit over 300. Then you put a point there.

And then you draw a straight line through those points that intersects the observed boiling point line. Ta-da! And then you can find out that your boiling point at reduced pressure would be somewhere around 120 degrees. So you can see that by reducing the pressure down by significantly, that we can also significantly reduce the boiling point so we don't have to heat it up as much. And we can reasonably get to these temperatures in the lab. Any questions about how to use that? It's pretty straightforward.

So you can use that to predict the temperatures that you will collect your limonene and your carvone fractions so you know when to be looking on your thermometer to switch your fractions and when you should be collecting which ones. And it'll help you identify what you've collected. So that is all of day one of the lab. If we made it through day one of the four day lab. And so then how will we know if it worked? Smell it.

So yeah, so part of the way that you can tell is you can smell it. Waft it gently, don't ever stick your nose-- so whenever you're trying to smell these, even though that these are chemicals that people eat, be gentle. You guys know and your TAs can show you if you're unsure, but don't ever stick your nose straight into a vial of any chemical. So yeah, we can check by smell. And there are a whole host of other ways that we are going to tell whether you successfully separated your two components of your mixture. And I will leave you with that, and we will talk about it on Thursday all of the different ways you can determine your success.

Are there any questions about anything from essential oils today? Anything else? Yeah.

## **STUDENT:** Do we make the essential oil in the labs?

No. We have already gotten it from the plant, so you don't have to do the steam distillation part. We will hand you the oil, and then you will just separate it into its components. All right. See you on Thursday.