[SQUEAKING] [RUSTLING] [CLICKING]

SARAH HEWETT: All right, good afternoon. We should get started, because we have a lot to talk about today. So today is the second in a series of three lectures about the essential oil lab. You'll get a third lecture and a little bit about X-ray crystallography. But today, we're going to finish talking about the main synthetic parts that you'll be doing in the essential oil lab.

So today, we're going to talk about how if your separation worked. And before I get too far into that, there was a question in the Tuesday lecture about the naming of terpenes. And so if you remember, the terpenes come from the isoprene structure, which was five carbons and eight hydrogens. But then there are some terpene derivatives, like menthol, that don't necessarily have that five carbon, eight hydrogen structure. So this obviously has an O-H group and more hydrogens.

And so what I found in the literature is that there are terpenes, which follow the strict pattern. And then there are terpenoids, which are derivatives of terpenes. They are synthesized from the same backbone. But then they get extra hydrogenation or oxidation done to them.

So, like, your carvone has the oxygen double bond in it. So that's technically a terpenoid. They seem to be referred to pretty interchangeably in the literature, which I thought was interesting. But that is probably the more accurate way to name these types of compounds. So hopefully, that clears up a little bit of that confusion.

So jumping in to the rest of the essential oil lab, on Tuesday, we talked about what you're going to do on day one of the lab, which is you'll get your essential oil, which will either be spearmint oil or caraway oil, and you will separate it into its two major components, which are your carvone, there on the left, and your limonene. So essentially, you'll take an oil that looks like that, and you'll separate it into two other oils that will also look really similar to that first one, except hopefully, they're pure compounds.

So if you do your vacuum distillation really well, and you separate those, and you get two pure compounds, we need to figure out how to characterize your separation, and figure out how well you were able to separate your carvone from your limonene. And we're going to do that in a whole number of ways. You'll get a lot of experience with different analytical techniques.

And these are the major methods that we're going to be using to characterize the success of your separation. So we'll be doing refractometry, gas chromatography, infrared spectroscopy, polarimetry, and X-ray crystallography. And so today, we're going to talk about the first four of those. And then, like I said, in a week or two, Peter Muller from the X-ray crystallography lab will come and do a much more detailed explanation of X-ray crystallography and what information you can get from that.

So to start with, refractometry-- refractometry is a way to measure the refractive index of a compound. And that is a characteristic property of different compounds. And it is-- it comes from the ratio of the velocity of light and air to the velocity of light in a liquid. So you have your light traveling through the air. Then it hits the liquid and it slows down, and it changes the angle. So you'll notice, if you ever look through some water, you'll know that the image is slightly distorted. And that is because of water having a different refractive index, or water having-- the light slows down as it travels through the water.

So all of our organic liquids-- so like I said, you're going to be separating your oil into two other oils. So we'll have liquids, which means that they will have refractive indices as well. And most organic liquids will have a refractive index somewhere from 1.3 to 1.7. And the way that you calculate that is it's represented as n. And you can either do the ratio of the velocity of the light in the air to the velocity of the light in the liquid.

But that is hard to measure. So in the lab, we can measure the angles at which the light travels. So if you have light that is coming through the air-- so if this part is the air, and you have your light, it'll hit your liquid interface at a certain angle, which is theta from straight up and down. And then, it'll get refracted at a different angle. We call that theta prime. So you can measure that in the lab and calculate your refractive index.

The refractive index is dependent on the wavelength of light. So you might imagine that if the refractive index and how much this angle-- and how much the speed of the light changes is dependent on how the light interacts with your liquid, then it'll depend on the properties of your light-- so the wavelength-- and the properties of your liquid. And one that we care about is the density.

So the way that we account for this is that we have the refractive index. And we call it refractive index D20. And so D represents that you use light from the sodium D line. So if you remember from, maybe, gen chem or physics, if you heat up an element very hot so it emits light, you can-- each element has a characteristic set of wavelengths of light that it emits. And sodium's happens to be around-- its brightest emission happens to be around 586 nanometers.

So if you use a sodium lamp, and you can select for these 586 nanometers, then you get a characteristic one wavelength of light that you can pass through your sample. So you know what the wavelength is, and you can know that you're just getting that one wavelength of light.

And then, you want to make sure that all of your samples-- or your measurements are taken at 20 degrees Celsius so that the density of your liquid. So if you change the temperature, you change the density. So we'd make all of our measurements at 20 degrees Celsius.

And here are the approximate refractive indices of limonene and carvone. And you can find different values for them in the literature, because the compounds that they used to measure these in the literature are of different purities. So you can by limonene and carvone at 95%, or 96%, 98% purity. And depending on what purity you use, you'll get a slightly different value. But they should be within that range. And when we measure them in the lab, we go out to four decimal places.

The way that we'll do this in the lab is with the refractometer. And this is a picture of the refractometer in the lab. And this part over here is the prism or the crystal. And so you'll put your liquid sample-- you just put a few drops right on the crystal. And then, you close the lid and the refractometer instrument shines light through your sample. It can measure-- it knows what angle the light is hitting the sample at. And then it can measure how much light it gets back out.

So it'll change the angle of the incident light until it gets total internal reflectance until it reaches the critical angle. You remember from physics? And so once it figures out what the critical angle is, then it can figure out the refractive index of your sample. And so it'll tell you what the refractive index is right here. You don't even really have to do any math.

And it'll also tell you what the temperature is. So you want to make sure that it's stabilized at 20 degrees before you actually take your measurement. And that's about it. So it's a pretty easy measurement to take, but it will help you to identify the purity of your compounds, because the refractive index is-- we can think of it as a linear quantity made up of the refractive indices of the two substances that we have in our mixture, multiplied by their molar fraction.

So your refractive index of a mixture is the sum of the refractive index of pure limonene times your mole fraction of your limonene, plus your refractive index of your carvone times your molar fraction of your carvone. And so that you don't have to sell for a million variables, if you remember the mole fraction, mole fractions have to add up to 1. So your mole fraction of limonene is 1 minus your mole fraction of carvone. So you can plug that in up there and, using the measurements that you take in the lab, you can solve for the mole fractions of each of these components in both of your fractions that you collect from your distillation. And you can determine their purity that way.

So another way to determine the purity of your-- or fractions that you've collected is through gas chromatography. And this is a picture of the GC instrument in the lab. And it's right next to the ICPMS, so you may have seen it if you were in lab yesterday. And if not, you can take a peek and see it today.

So this is the instrument itself. This is the autosampler. So you guys will make your sample vials, put them in up here, and then it'll pick them up, put them into the instrument, and then it'll automatically inject the sample for you, which is kind of nice. And then this thing over here, it generates hydrogen and air, which are used in the detector of the instrument.

So the way that gas chromatography works, if you wanted to take a peek inside the instrument in a very, very simplified fashion, you have a carrier gas, which in our case is helium. And the carrier gas, if you think about-- so this is a type of chromatography. And you guys did chromatography in the ferrocene lab with your thin layer chromatography and your column chromatography. So in your TLC and in your column chromatography, what was your stationary phase?

AUDIENCE: Alumina?

SARAH HEWETT: Alumina, yes. So you had your alumina, and it was coated on the TLC plate. Or you packed your column with the alumina, and that's your stationery phase. And then, what was your mobile phase?

AUDIENCE: Hexanes and ethyl acetate?

SARAH HEWETT: Hexanes and ethyl acetate. And you guys made different mixtures of those to determine the best separation of your compound. So in thin layer chromatography and in column chromatography, which of those phases had a bigger determination on the separation of your two components?

AUDIENCE: The mobile phase?

SARAH HEWETT: The mobile phase, yeah. So you could change the polarity and it would change the separation. In gas chromatography, it's kind of the opposite. So you have your carry your gas. And that's going to be your mobile phase. So that'll help push your compounds through the column. And then the column is your stationary phase. And you can change the properties of your stationary phase in order to separate-- get different types of separation.

So there are two major types of compounds that they use in column chromatography. And the first one, this is-- anyone know what this is? Polyethylene glycol, which is something you may have heard of before. So these are your ethyl groups, and then the glycol, because it's got alcohol groups. And so this notation is the notation for a polymer. So the way that this is written is you could have any number of this repeating unit until the end.

And this is a polar stationary phase. So if you're trying to separate compounds that have differences-- large differences in polarity, a lot of times you'll use the polar compound. Because it has these oxygens that have their lone pairs. So it can interact with polar compounds, it can form hydrogen bonds, all kinds of neat stuff.

But the one that we're going to use in the lab is this compound here. And it is called a polysiloxane. So polysiloxane-- and it has these silicon groups that have to methyl groups attached. And another name for this particular-- so polysiloxane is the overarching name for this class of compounds. This one has two methyl groups attached. So one of the other names for it is dimethicone. I don't know if you've heard of that before.

It's in a lot of things. They use it extensively in lotions, shampoos, and sometimes in food. It prevents foaming and it makes things feel nice and slippery. So that's why they use it in a lot of cosmetics, and lotions, and everything. So the next time you're in the bathroom, you can take a look at some of your products. And you may see dimethicone. And now you know what it looks like. And we're going to use it in the lab to help separate your limonene and your carvone to see how pure your separation was.

And the GC column looks like this. So it's represented in this diagram as a curled up circle. And so this is actually what it looks like. So this is an old column taken out of the GC in the lab. And you can see that it's very, very thin. So this is a capillary column. So this is actually a hollow tube. And your sample will travel through this tube around, around, around. Does anyone have a guess about how long this is?

AUDIENCE: Long.

SARAH HEWETT: Long. [LAUGHS] Yeah, so this is 30 meters of capillary tubing all wrapped up here. And on the inside of the capillary tubing, it is coated with this polysiloxane mixture which will interact with your sample. And it'll help cause the separation.

So your sample gets inserted into the injector port. This is an oven, so it gets heated up. And you can change the difference in the temperature. And so the difference in the temperature will change the amount of separation that you get. So if you do it hotter, then your compounds will travel through faster, and you don't maybe get as good of a separation. If you cool it down a little bit, then you tend to get better separation, but sometimes it can broaden your peaks. So there's a trade-off there.

We have already made a program for you that optimizes the separation of the two compounds that you guys are looking for. So you don't have to worry about that. You'll just make up your sample, inject it, it'll go through the column, and then it reaches the detector.

And we use a flame ionization detector. So that hydrogen in the air, they get lit on fire. The stuff that comes out of the column goes into the fire. It gets lit on fire, it produces ions, and then the ions are detected by the detector, changes the electrical current. And that is what gives you your signal.

This is a very sensitive technique. So you do not need to prepare very concentrated amounts of samples. So we're not going to inject your oil straight into the instrument. You are going to do what's called a double dilution.

So you will take 50 microliters of your sample in one of those micropipettes that you guys have been using. You'll dissolve it in 1 milliliter of pentane, so you're already making a very dilute solution. And then you'll take 50 microliters of that solution, and you will dissolve it in another milliliter of pentane. And that's what you will inject into the GC instrument. Your TAs will help you with that. But that's just to show you that it is a very, very sensitive technique. So you do not need a lot of volume.

This is what the output of the GC looks like. And you will get your GC chromatograph. And the units on this are in abundance and time in minutes. And so you can see that this is a GC chromatograph of the oil before it's been separated. So it has two major components and a couple of smaller impurities. And hopefully, if you guys do your distillation correctly, then you'll get-- your fractions will look like there's not very much of one and a lot of the other, and then vice versa for your limonene and your carvone.

And the way that you can tell which peak is which is that-- so this column is pretty non non-polar, like I said. And the interactions with the column are what separates your mixture. But the way that it is separated is mostly through boiling point. So when you heat this compound up-- and we'll talk about it a little bit more in the next slide-- but it'll travel through the column.

So things that are more volatile will travel through the column faster, so things with lower boiling point. And then, things with a higher boiling point stay on the column for longer. So you can use boiling point to sort of identify your compounds.

And you also get this information at the bottom, which tells you the retention times of your peaks in the minutes. And then, it'll also tell you the area of your peak. And you can use the percent area to calculate your percent purity. So in this case, we have 41% of one and 57% of the other. So it is not a very pure sample. And hopefully, yours will get on the order of-- you can get well above 90% separation. So that'll give you an idea of how well your distillation went.

So some things about gas chromatography that are important to note is that you can't really identify an unknown without a standard. So if you just-- if you don't know what your compound is already and you inject it into the GC, all you're going to know is that retention time. It doesn't give you any information about its structure or anything else.

So you can compare retention times. Those are consistent as long as you use the same method, and the same column, and the same carrier gas. But you need to have a standard that you can compare it to. Or if you know that there's two components in your mixture, if you know what's in them, then you can tell which is which, again, by their boiling point. So it is very useful for determining the purity or the percent composition of a sample, which is what we're going to use it for.

And like I said, separation is determined by your stationary phase. And the stationary phase is a liquid coating on there. So when you're using GC chromatography, you want to make sure that you pick a stationary phase that matches the type of compound that you're separating. So we're going to use this non-polar one because our compounds are relatively non-polar. But you'll see it used in extensively in other applications. So if you're doing a lot of polar compounds with a lot of oxygen groups or nitrogen groups, then you may want to use a more polar compound.

You can also get chiral stationary phases that will help separate enantiomers. There's all kinds. If you go on any of the Agilent website or anything, you can get a whole list of different types of stationary phases with different types of polarity. You can substitute these methyl groups out for other things depending on what application you're trying to use it for.

And so the way that we can characterize the efficiency of the separation is by theoretical plates. And we mentioned this really, really briefly when we were talking about the distillation. So if you remember, there was the grow column versus the simple distillation. So you get better separation when you have more and more of those cycles where the vapor can vaporize and recondense. Does that sound vaguely familiar? Good.

So essentially, that's what's happening in this GC column. So you have a liquid coating on the inside of this tube. And your compounds are traveling through it. And they can be heated up so they'll repeatedly dissolve and then revaporize in the liquid. And the more times they can do that as they travel through this 30 meters of column, the more efficient your separation is going to be.

And we can calculate that using a measure called theoretical plates. And it is based on the idea that one plate is one equilibrium between the liquid in the vapor phase, or one of those vaporization recondensation cycles. So you can refer to the temperature composition diagrams from the last lecture if you want to get a refresher on what I mean by the vaporization and recondensation.

And then, you can calculate this from your chromatogram data. So when you have that chromatograph data that has the peaks on it, you can use this equation. So n is the number of theoretical plates. This is just a standard constant. And then your tr is the retention time. So you'll use the retention time of your peak in some unit. And then you want to take the width of the peak at 1/2 of the height of the peak.

So I can't go backwards. Oh, there it is. So for this peak, you would say your retention time is 1.434. And then, you would want to go halfway up the peak, and then take the width of that peak, which is going to be a very small number. And the best way to do this is to probably use a ruler, and actually measure, physically, the height of the peak, and then measure the width at the halfway point.

So if you do that, you need to make sure that when you are using these measurements, that they are in the same units. So you can either do it in the units of time, which is what it's given to you on the GC. But it's probably a little easier if you do it in millimeters or centimeters, however you want to measure it. Just make sure that you also-- if you're going to measure your width in centimeters or millimeters that you also measure your retention time in centimeters or millimeters. And the retention time is just the time from the beginning to where your peak is.

So that's GC spectroscopy. And now, we can talk a little bit about IR spectroscopy. And if we take a moment and think about our electromagnetic spectrum, we can think about the different types of energy that you have available in the electromagnetic spectrum. So if this is our wavelength in meters, what is our thing with the smallest wavelength, highest energy? Anyone? Gamma rays. Then?

AUDIENCE: X-rays?

SARAH HEWETT: Then? UV. The little tiny one?

AUDIENCE: Visible.

SARAH HEWETT: Visible.

AUDIENCE: And then infrared.

SARAH HEWETT: Infrared.

AUDIENCE: Microwave.

SARAH HEWETT: And then, yeah, microwave and radio. All right, so from highest energy to lowest energy, this is our electromagnetic spectrum. And so IR spectroscopy uses IR radiation to give us information about a molecule. And the way that this happens is that it's very useful for identifying functional groups in a molecule. And you pass this IR radiation through the molecule. And all of the bonds in your molecule have some sort of vibrational energy associated with them. They're vibrating at a certain frequency which corresponds to energy in the IR region of the spectrum.

So if you pass IR radiation through your sample, then some of the wavelengths will match up with the frequencies of the vibrations of your bonds. And it will get absorbed. And then, you can measure how much light comes back out and how much light gets absorbed. And you can get an IR spectrum of your molecule, which is representative of all the different bonds and the energy that they absorb.

The frequency of light or energy that a bond absorbs is dependent on the mass of the two atoms that are attached in the bond, the bond strength, and the chemical environment. And this is related by Hooke's law, if you're familiar with physics, that talks about-- you can think about it as two masses on opposite ends of the string-- or a spring. And so how much force you need, or what the frequency is that that spring will oscillate on depends on all of these factors.

And so if you have something that has a high bond order, like a double bond or a triple bond, then those-- that's a really tight spring. So it's going to vibrate at a very high frequency, and it's going to take a lot more energy to get that vibration to happen. Whereas if there are two light atoms that are attached by a single bond, then they will vibrate at a much lower frequency-- less energy. Make sense, sort of?

So vibrations are only IR active if they change the dipole moment of a bond. So you can't see all the bonds in your molecule using IR spectroscopy. You'll be able to see the ones-- we can see most of them, since most molecules are not perfectly symmetric. But like carbon-carbon bonds, that stretch does not really change the dipole much. So you don't see those very strongly, if at all, in most of your IR spectra.

So there are a bunch of different ways that a bond or a molecule can vibrate. And the number of possible vibrational modes in a molecule is determined from the degrees of freedom. So if you think about a molecule, it can move in the three dimensions of space translationally. And so all of the atoms can also move in the three dimensions of space. So you get-- you start with 3N degrees of freedom, so N being the number of atoms in your molecule.

And then in a linear molecule, you have-- you can also have rotationally modes. But one of the rotationally modes does not quite work as well, because it's along the axis of the bond. So you're not changing anything if you rotate it that way. So we end up with 3N minus 5 vibrational stretching or bending modes.

And then, in a nonlinear molecule, you get all three translational and all three rotational modes. So you do 3N minus 6. And so that gets rid of the translational and rotational motion. So what's left is the vibrational stretching or bending modes in your molecule.

And so the possible vibrations that we can have are as follows-- the symmetric stretch, the asymmetric stretch, the bend, the wag, the twist, and rocking. So I need everybody to stand up. If you've done this before, we're doing it again. This is a rite of passage in a chemistry lab. We are going to-- I will wait. We are going to pretend that we are molecules, and we are going to act out all of these different vibrations that can happen in your molecule so that you have an idea of what you are looking for when you see your IR spectrum.

So if your body is like a carbon, and then the rest of, like, your legs are another carbon chain, then say you have two hydrogen atoms in your hands. Great. We are excellent methylene groups. So a symmetric stretch-- any ideas? Yeah, when they go at the same time. Excellent. The asymmetric stretch? Yeah, opposite, excellent.

The bend or scissor? Excellent. So you're all getting a workout today. This is great. The wag? The wag is back and forth, like-- yeah, there you go. Excellent. Twisting? One goes back, one goes forward. There we go. And then last one, rocking? That's what you had before. They're going back and forth at the same time. Excellent! Well done. Give yourselves a hand.

It's a good way to get everybody up and moving. So those are our IR vibrational modes, and those are all the ways that you can make your molecules vibrate. And so now, how do we measure this? And this is the ATR spectrometer. It's an infrared spectrometer. This is the one that we have in the lab. And it's in the very back corner by the door in A prime 4. So some of you guys may have seen it. You may have seen some of the other lab groups coming in to use it.

And the way that this works is, it's a pretty simple instrument in terms of what you need to do to use it. There is a crystal right here. And the crystals are usually made of zinc selenide, or I believe, in our case, it's a diamond. And it's a pretty tiny crystal. It's just that very, very small little dot in the center.

And you can take your compound and put it straight on the crystal. And if it's a solid, then you can use-- this is a little pressure thing. So you can lower that, and it'll press your solid right up against the crystal so you get good contact. Or you can just put a couple of drops of your liquid right on the crystal, and then it has good contact anyway because it's a liquid.

And-- oh, so the ATR stands for attenuated total reflectance. And how it works is you have a source of your IR energy. And these are some mirrors. And so you have your energy. It comes in here. It hits the mirror, and then it gets directed up through this crystal. And like I said, you're going to put your sample on top of the crystal. And you want to get really good contact, because the IR energy will travel through the crystal, and it'll interact with the very bottom layer of your sample there.

And then, it'll get reflected back down. And in some cases, there's only one reflection, and then it goes to the detector. And in other cases, depending on the size of your crystal, you can get multiple reflections. And when the light interacts with your sample, like I said, it'll absorb some of the wavelengths of the IR light.

So some of this will get absorbed. And then, that's the attenuation part is that some wavelengths will be decreased in intensity because they'll be absorbed by your compound. And then, the reflectance part is the light gets reflected down through this crystal into the detector. And then the detector can determine which wavelengths of light were absorbed and how much.

And that is IR in a very simplified fashion. There's more descriptions of it in your Mohrig book. So if you guys have this book or if you want to borrow it, there's an excellent description of how different IR spectrometers work. There are a couple of different ways to get IR samples.

So like ATR is great for liquids and solids. You can also have IR spectrometers that just-- you set your sample up on some sort of support that's, like, vertical. And then you pass the beam straight through it, and you measure what comes out on the other side, kind of like the UV-Vis that you did.

So there's a couple different ways to do IR. But this is the way that we're going to be using in the lab. And what you get out of it is an IR spectrum that looks something like this. So I took these straight from the lab manual. And these are IR spectra of carvone. And that's the carvone from the caraway seed oil, and that's the carvone from your peppermint oil. And do we see any differences?

Not really. So what do we know is different about the carvone from the caraway seed and the peppermint oil? The stereochemistry. So we know that one is the R form of the molecule, and the other is the S. So this is the S and this is the R. So is IR spectroscopy good at differentiating between isomers? Nope, not at all. It just tells you what functional groups are there. It doesn't really tell you in what order they are in. So you'll need to do a different spectroscopic technique if you want to figure that out. But we can get a lot of information about different bonds that are in our molecule, which is helpful for identifying different things.

What do we have next? So, yeah, we can talk about interpreting an IR spectrum. So in your book or on the internet there are many, many charts that have lists and lists of the IR-- oh, my bookmark fell out. Oh here it is. So there is a chart in the Mohrig book that has a list of all of the different stretching frequencies for different functional groups. And it has the stretching, the bending, and anything that you are typically able to see in an IR spectrum. And it tells you where you should look for it in the spectrum in terms of wave numbers.

So if you look at the axes of these things, the left axis here is percent transmittance, so how much of the light gets through. And then, the-- so the top is 100. So if nothing is absorbed, it'll be a baseline at the top. And then if things are absorbed strongly, then the transmittance goes down and you get a big peak.

And then the wave, the other axis along the bottom here, is-- well, on the bottom of this one, it's in micrometers. But in most cases in IR now, we use wave numbers because it's a linear measurement instead of non-linear. So wave numbers corresponds to the amount of energy in the molecule-- or in the photon. So we can get these charts of where different functional groups absorb in terms of wave numbers. So these are all in wave numbers, which is inverse centimeters.

So when you get your IR spectrum back and you take it in the lab, you're going to print it out. And then, you can use one of these charts to help you. And the best place to start looking at an IR spectrum is between 4,000 and 1,400 wave numbers, so, like, this half. Because you can see, on this side, there's a lot going on down here. And you might say, oh, that's got a ton of information in it. Not so much.

So this is called the fingerprint region. And it does have a lot of information in it. And before there were other spectroscopic techniques, people spent a lot of time trying to identify peaks down here. But they're less representative of actual specific bonds. A lot of it's, like, overtones and other resonance happening.

And these are unique to every chemical. So if you have a reference spectrum and then you have your spectrum, you can match them up and the fingerprint region should match really well. But it's kind of hard to interpret, and it's usually very messy. So we kind of ignore this at the beginning. And then, if you look over here, then there's a lot fewer peaks to deal with and they provide a lot of information.

So you'll see that the C-H stretches are typically around, like, 2,800 to 3,100 wave numbers. And it depends on whether it's a single bond like an alkane C-H, or alkene, or an aromatic where they come out. So these guys, you'll have different peaks, because we have some alkene C-H's and some alkane C-H's. So you'll have a whole variety of C-H stretching there. And then, what other functional groups do we have in the molecule? What's the other main one?

AUDIENCE: CO.

SARAH HEWETT: The CO. And the CO double bond is one of the most characteristic peaks that you can find in an IR spectrum. And it always comes out somewhere between 1600, 1700. So you can see, at 1700, there is this huge peak. It is the biggest peak in the spectrum. And that corresponds to that CO double bond there. So that's always a good place to start if you are looking to identify something that you think may have a carbonyl in it. Yes?

AUDIENCE: So can you tell how many C-c bonds there are based on the height of the peak?

SARAH HEWETT: No. So the height of the peak doesn't give you any specific information like how many there are. In amines, if you have a primary versus a secondary amine-- so if you have an amine with 1 H on it, and some other R group versus two H's, you'll see either two peaks and the amine or one peak. So that's when you can quantify atoms through this.

But no, the intensity does not always correspond to how many of the bond there are, since the intensity is mostly a function of how much the dipole moment changes. So you could have a lot of C-H bonds, but you won't see a whole-- like, a very intense peak. Yeah. Whoops! Go back.

So things that you should do is to look for what is there and look for what is not there. So if you know what's supposed to be there in your functional groups because you know the structure of your molecule, then you can try to identify the peaks that correspond to those functional groups. So if you are anticipating having an O-H stretch, like you've made an alcohol or a carboxylic acid, then you should see a giant, broad peak around 3,500 wave numbers.

And you should also, if you are making a carboxylic acid, you should see this O-H stretch, and you should see your carbonyl peak. So you need to be able to identify all the peaks that are associated with a certain functional group. So don't say that you have a carboxylic acid if you cannot find either-- if you cannot find both of these peaks. They will be there.

And then, less helpful for us maybe now, but in the ester lab, which we'll talk about later, you can also look for what is not there. So if you're trying to figure out if your synthesis was successful, and you start with an alcohol, and you're supposed to end with something that does not have an O-H peak, then you can look for the absence of an O-H stretch. Or if you're trying to get rid of a carbonyl, then you can say what's not there if you're trying to hydrogenate some sort of double bond. You can figure out what is there and what is not there.

So those are things you can talk about in your discussion. And your IR spectrum in your report should be attached as an appendix with key peaks labeled. So you can actually, on your spectrum, write what each peak represents. So if you see a giant peak at 1700, you can draw an arrow to it and say, C double bond O stretch.

And the way that this is typically reported in the literature-- and there's a bunch of different ways to do it. You can check out the ACS style guide for more information. But you'll say, IR spectroscopy. You use the type of IR spectroscopy, which in our case is going to be ATR. So there is, like, thin film, there's potassium bromide pellets, there's all kinds of different ways that you can take an IR spectrum. And the method that you use will also affect the peak intensity. So that's why it's not as helpful of a thing to look at.

And then, your units, wave numbers, and then your key peaks-- so if you have a peak that is really broad, you only have to report. And you'll see that most of the peaks in an IR spectrum are not super sharp. So this carbonyl peak, if you look at the base, it ranges through a good number of wave numbers. But when you report it, you only report the wave numbers of the highest intensity. And the instrument will print that out for you on your spectrum, so you'll know what the highest intensity is for your peak.

So you can report the highest intensity wave numbers for each of your peaks. And if you know what the peak is, in some cases, people will put what bond it represents. You don't have to. It was acceptable to do it both ways according to the ACS style guide. So that's kind of up to you. A little more helpful for the person reading it, but--

So if we want to take a look really quickly at a couple of IR spectra of molecules that-- well, this one you may know. So this is the IR spectrum of ethanol. And what is the major feature that we care about here?

So there's the C-- C-O, but the-- yeah, there's the O-H. So the biggest, strongest feature here is our O-H peak. And O-H peaks are typically quite broad. So they're not as sharp and defined as the other peaks in the spectrum. So that's one of the characteristic ways you can know if you have an alcohol.

And then, you can look. And we have some methyl C-H's and ethyl C-H's here. So you have alkane C-H's. And so those show up a little bit below 3,000 wave numbers. Great. And then, you may be able to find the C-O bond that typically shows up around 1,000, give or take. So maybe one of those peaks is your C-O stretch as well.

So sometimes you can go into that fingerprint region if you know that there is something there that you are looking for. So the C-O stretch is one that you typically can see, and it's typically pretty strong. So in this case, it is there.

But, yeah, and then another example is this compound, which is 3, 7 dimethyl octonal. And what do we have here that's really strong and sticking out? D double bond O, again, right here around 1700 wave numbers, that's always a dead giveaway that there is some sort of carbonyl peak. And if you have an aldehyde peak, then-- I didn't write it up here-- but you'll have a characteristic stretch for this aldehyde hydrogen that is also up here.

And then you have a couple of alkene C-H stretches, and a bunch of alkane C-H stretches. So in this case, there is a lot going on in our stretching region. And those are the major features of that spectrum.

So we'll go over this a little bit more also, again, when we talk about the ester lab. Because you're going to be using IR for that lab as well. We can talk about some of the different functional groups that you can see in that case, because we're going to be dealing with, obviously, esters, and some alcohols, and some ketones, and some carboxylic acids. So that is a brief overview of IR.

So we have one more technique left to talk about, and it is the polarimetry. And to talk about that, first, we're going to do another round of synthesis with your products that you've separated in your initial distillation. So you have your limonene fraction, your carvone fraction, and we're going to take the carvone fraction and synthesize a semi-carbazone, which will look something like this.

And you will make this molecule. It'll still have your stereocenter. We're not touching that. So it'll keep your RS configuration. And it'll be a solid, though. So you're going to start with an oil. You will go through this synthesis. And then you'll end up with white needle-like crystals. So you're going to recrystallize your product very, very slowly.

And your TAs will show you how to do that. There's a good procedure in your lab manual, but you're going to do the reaction, and then you're going to let it sit in your lab bench until the next lab period, so for a couple of days. And you want the crystals to grow really, really slowly. So you will not see them when you first make them, but you'll see them, hopefully, when you come to lab the next time.

And the slower the crystals grow, the more pure they are. And then, we are going to need very pure crystals, because these are what you're going to analyze by X-ray crystallography. And like I said, Peter Muller will come and give you more information on that. So stay tuned.

But what we can do with these crystals is use them to do some polarimetry. So one of the things that you'll know about your-- so I said you're going to make some white crystals. So one of the ways that we've been analyzing our solids is by melting point. And you can get two possible diastereomers out of this synthesis. So you can either have-- since there's not a lot of rotation around the C-N double bond, you either have that extra nitrogen group pointing up towards this methyl group or away from it.

And these two compounds have different melting points. So you will characterize your crystals by melting point to figure out which of these isomers you have, the alpha or the beta. Most people will make the beta, because it has less steric hindrance, so it's a little bit easier for that to happen, just synthetically. And so that's one of the first ways that you will characterize these compounds.

And the second way is by polarimetry. And so if we talk about polarized light really fast, so what does it mean for light to be polarized? It's all going in the same direction. And so all-- so you have a light source, and it emits light. And all of the waves are traveling in all different directions. But you can put it through a polarizing filter, and then you only filter out the light that is-- that has, like, the molecules are all arranged in slits so that it only selects for light traveling in one plane. So you can put that there.

And if you-- if I have two pieces of polarizing paper, and I put them on top of each other in the same orientation, then the light pretty much gets through. These are a little bit colored, so not all of it. But then, if I rotate so that the bottom one is selecting for light going this way, and the top one is selecting for light going the other direction, then no light gets through, because it can't pass through the filter. So that's what we're going to-- this is the essential principle behind polarimetry.

So if you remember from either your organic chemistry lectures or experience before, different molecules that are chiral will rotate plane polarized light. And so what that means is if you put a polarizing filter on your light, select for light all going in one direction, and then you shine that light through a chiral sample, it'll get rotated to a certain degree based on a number of features, but essentially, how the light interacts with your chiral sample. And so if you have two polarizing filters, and you have your sample in between them, you can rotate one of them, and eventually, it'll match up again, and you'll see all the light come through.

And so that's how you can tell how much your compound rotates the light. So you put a polarizing filter on each side, and then kind of rotate them until you get all of your light back. And then, you can measure the polarimetry. So that's kind of the way that they did it in the old days, but we have an instrument that'll do it for you. So you don't have to worry about that too much. I'll turn this back on really quick.

But the rotation, like I said, we can measure it. And it's characteristic of the molecules. So the R and the S forms will rotate in opposite directions. So in our case, the R form rotates light in the negative direction, or counterclockwise, and then the S form will rotate light in the positive direction. And the R and the S are not really related to the plus and the minus. So for different compounds, the R might be the plus isomer and the S might be the minus. It's something that you have to measure. You can't just know off the top of your head.

And from this, we can calculate the specific rotation of our light, which is how much the light has rotated. And it depends on the length of the sample, the concentration, and the wavelengths of light that is used, and then, again, the temperature, because that impacts the density of your solution or of your compound. So the way that you're going to do this in the lab is you're going to take a sample of your crystals, and then you're going to weigh them out, and you're going to dissolve them in ethanol. So you're going to make a solution that you know the concentration of so that we can plug that in for our concentration.

And you will put it in these tubes. And then you will insert the tube into the polar emitter, and it will shine the light through. And then it will calculate what the angle is that the light gets rotated by. And the polarimeter actually will calculate the specific rotation for you, which is kind of nice. But you can also calculate it. It gives you all the information to calculate it yourself as well.

So this is notated very similarly to our refractive index, in that it is taken at 20 degrees for the density. And we use the same wavelength of light from that sodium D-line, the 586 nanometers, in order to have a consistent representation. So you may see these constants in the literature. And if the temperature is different, it'll say something like 23 or 25, or whatever the temperature they took it at. And you can also change this to whatever nanometer wavelength of light that you used.

But we're going to be using these conditions. And then this is the rotation of the light in degrees, the length the travels in decimeters-- so you're going to measure the tube that you use in decimeters-- and then your concentration in grams per milliliter. And you can calculate the specific rotation of the molecules. So hopefully, the people who had different isomers will rotate-- the light will get rotated exactly in the same number of degrees, but in opposite directions if we've done everything correctly. So you'll see what you can get from there.

And I think that is all for today. Do you guys have any questions about anything that we are about to do in the essential oil lab? Autumn?

AUDIENCE: Why is-- in this case, why is gas [INAUDIBLE]?

SARAH HEWETT: In this case, to be perfectly honest, I am not super familiar with HPLC. I'm sure you could also use it to separate these compounds. GC is-- I mean, part of it is the techniques that we have available to us. So there's that. But this also gets separated really nicely based on boiling point. So it's kind of fast and easy for us to do. But I can also-- yeah, we can talk about it more. Yeah, no, it's a good question.