PHARMACOGNOSY AND PHYTOCHEMISTRY

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Week 10

Lecture49

Week 10: Lecture 49: Physical Methods of Quality Control of Herbal Drugs (Part 2)

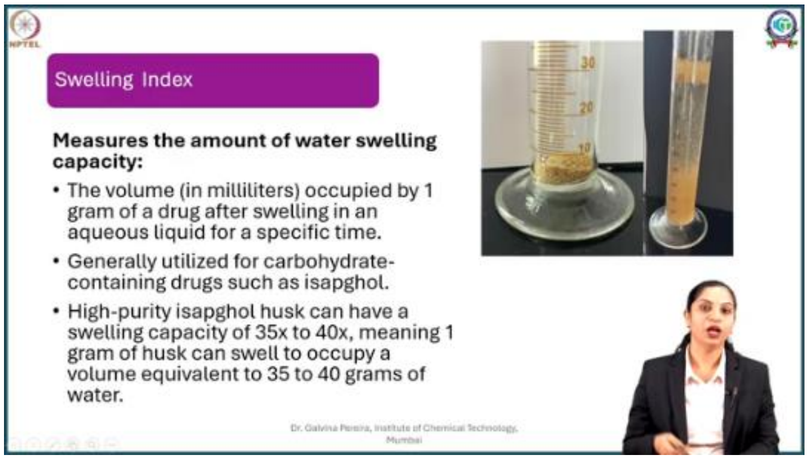
Hello everyone, and welcome to the NPTEL course in Pharmacognosy and Phytochemistry. This week, we are studying quality control methods, and so far, we have gone deep into understanding the physical methods of evaluation of herbal drugs. So far, we have covered organoleptic, microscopic, macroscopic, as well as some of the physical methods of evaluation of herbal drugs. When it comes to physical methods, you may recall from the previous session that we covered loss on drying, extractive value, ash value, and foreign organic matter. We are going to explore a few more values used for the physical methods of evaluation of herbal drugs.

These include the swelling index, foaming index, refractive index, optical activity, solubility, density, specific gravity, viscosity, and values such as melting, boiling, and congealing points. We will also see how measurements using these methods can help us ensure the quality of the drug. So, let's start exploring them one by one. We'll start with the swelling index.

So, what is the swelling index? You might have seen this when drinking falooda or sharbat, which contains chia seeds or sabja seeds. Or sometimes, you may observe certain drugs like agar. When you soak them in water, they gradually start swelling. The amount of water they absorb and the final volume they occupy differ from the initial volume.

And that is what we are seeing when you are seeing a swelling index. Swelling index is basically the capacity of one gram of drug to swell in a suitable solvent. Most of the cases the suitable solvent is water when it is soaked in that particular solvent for a specified amount of time. Now this is used for simple carbohydrate containing drugs like I said acacia. You could see it for your isabgol as well.

Now isabgol is used as bulk laxative. So I have isabgol in my image here. I weighed about 1 gram of it and can you see it just there at the bottom of my measuring cylinder as a dry powder. But then I add my water to it and what happens is in isabgol husk there is mucilage which starts imbibing water and swelling. Now it will go on swelling to a particular value.



Say for example the Indian pharmacopoeia says my isabgol when I keep it for a period of 24 hours should swell to about 40 ml. 1 gram should swell at least more than 40 ml. So I am looking for a value which is more than 40 ml that my drug is going to swell. So I keep it in 100 ml of water and I keep it intermittently. So I let all the air bubbles go in, shake it properly in a stoppered measuring cylinder and then I keep it for 24 hours.

After 24 hours, what happens is you can see this isabgol has swollen and reached a value of 40 here, slightly more than that, like 41 or 42. So let's say it's 42 here, and again, you will have another 10 here. So, some part of it has started floating. Floating is mainly due to air bubbles. So, you might have 10 here.

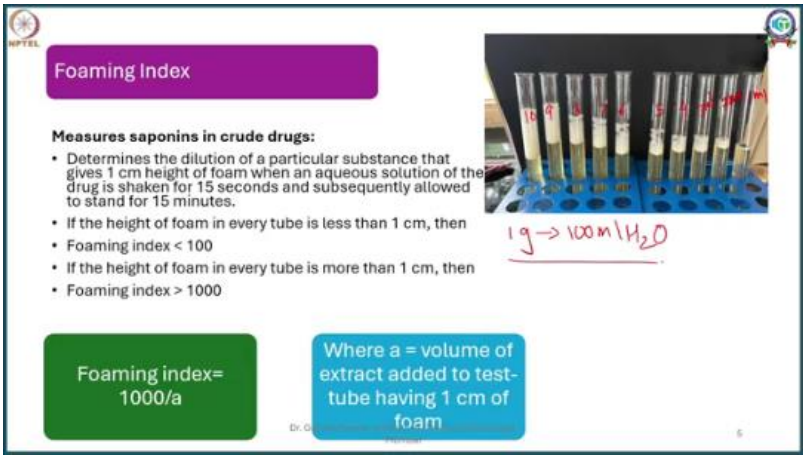
So, 42 plus 10, you get about 52 ml. My isabgol has now swollen to a value of 52 ml when I keep it for 24 hours. Now, that is what I call the swelling index. So, the swelling index is the volume that one gram of drug occupies when soaked in a suitable solvent for a period of about 24 hours. Now, swelling capacity, like we've seen, is about 30 to 40x. Now, imagine what happens if my drug doesn't swell at all or it swells just 20 ml. What does that indicate? That indicates my isabgol husk is mixed with other husks, like rice husk or wheat husk or any other husk, which do not have mucilage. Because it doesn't have mucilage, it's not going to swell, and as a result, my effective value lowers. So, I say my isabgol husk is not of good quality, or it is again adulterated or substituted partly with rice husk or any other husk.

Now, therapeutically, what is going to happen is initially one gram of isabgol was enough for me to give or deliver laxative effects. But now, what is going to happen because it's mixed with another husk is it's not going to swell, and it's not going to give you complete efficacy. So, generally, for mucilage-containing drugs, this is a good quality indicator. Now, moving on to the next value, the next value is referred to as the foaming index. Now, the foaming index is just the ability of a drug to produce foam.

Now, why would a drug foam? Imagine samples or drugs like your reetha or shikakai, which are used in herbal shampoos. We often see them containing compounds such as saponins. And saponins, owing to their surfactant-like properties, have a good ability to foam. So, what we are going to do here is check the foaming index.

You just take about 1 gram of the drug. And this 1 gram of the drug, you boil it in 100 ml of water. Generally, water is preferred for the extraction. Now, this 100 ml of water, you are going to add it to a series of tubes. So, the first tube I am going to add is like 1 ml.

In the second tube, I am going to add 2 ml and 3 ml. I will just go on adding 5, 6, 7, 8, 9, and 10. So, this extract I have added in increasing quantities. And then made up the volume to 10 ml with water. So, 1 ml extract plus 9 ml H2O.



2 ml extract plus 8 ml Water. Similarly, 3 ml extract plus 7 ml H2O. So, similarly, for the last one, there will be no water. For 9, I will have plus 1 ml H2O. I'll do all; I'll make all the tubes up to 10 ml.

Then what I do is I'll just shake every tube at the same time for 15 seconds, and thereafter, I'll allow it to rest for 15 minutes. Now, there's a reason for it: if you shake, even if it doesn't contain a surfactant, if you shake something vigorously, there is bound to be foam present. But if this foam is not stabilized by the surfactant, it breaks very fast. So, within 5 minutes, if there is no surfactant or saponin present in the drug, the foam will start subsiding. But if you check after 15 minutes and still if the foam persists, that means your drug contains saponin.

Now, in order to quantify how much saponin there is, you are going to do a foaming index. So now, what you are going to do is see the tube where the foam height is 1 cm. Now, you are going to take a scale. This is more than 1 cm, say, for example, in this case. My 3 ml tube is what is giving me a foam height of 1 centimeter. So, the foaming index is given by the formula 1000 upon A, where A is the volume, like 3 ml, that I am adding.

Volume of extract added to the test tube gives you about a centimeter of foam. So, in this case, the foaming index is 1000 upon 3. So, it is about 1000 divided by 3, which comes to around 333.33, something like that. So, your foaming index for this particular sample is 333.

Now, there are certain drugs that are very rich in saponins. So, in that case, what is going to happen is, even in the first tube, Okay. You might get a foam height higher than one centimeter. So, if it is very rich in saponin, imagine your first tube has this foam height.

Okay. So what does that indicate? That indicates that even if I take the last reading, what is the last reading? 1000. Okay, upon 1, 1 is my last reading that is possible is equal to 1000.

So if even if that is having a more than 1 centimeter height, that means my foaming index is higher than 1000. So that means a lot of saponins are there. In some cases, you can even do a further dilution and then multiply it by dilution factor. Similarly, reverse case can happen. That means my drug even in 10 centimeter tube doesn't produce a 1 centimeter foam.

Say for example, it just produces 0.5 centimeter foam. So in that case, what do I do? So in that case, just imagine what is the worst case scenario is 1000. The least value I can have with this test tubes is divided by 10.

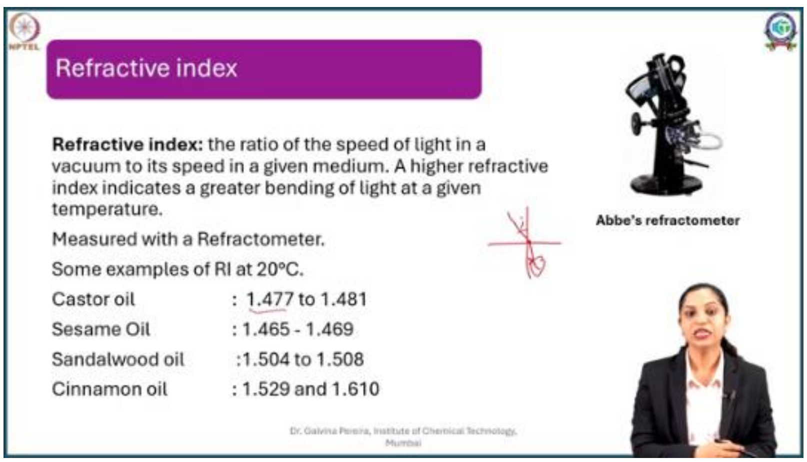
So it is 100 and even 100 is not there because it is just 0.5 centimeter. In that case I say my foaming index is less than 100. So you can get into the extremes. If they are at the extremes you can say that if it has a very less saponin content the foaming index is less than 100. If it has a very high saponin content you can say the foaming index is higher than 1000.

But yes you can do certain dilution and check it for this. But concentration is not generally recommended for drugs having lower saponin values. Now, moving on to the next value, the next physical value that we assess is the refractive index. Now, refractive index is generally carried for oils, especially of fixed oils, as well as volatile oils. Now, if you see a refractive index, you remember it is basically the ratio of sine i to

Is to sine r. So how much. Your. ray of light gets diffracted when it is moving from one media to another or more correctly I would say that refractive index is actually the ratio of speed of light in vacuum is to the speed of light in the given medium but this becomes little difficult to quantify so we quantify it by a very simple formula that is sine i upon sine r whereas it is the incident that is angle of incidence upon angle of refraction. Now, the good part of it, the most of the refractometers that we get in market nowadays are well calibrated to this value.

So, already sin i's to sin r values are kind of located and what they use are a set of prisms. So, they use a set of prisms to kind of actually see the ray or detect the ray and based on that you get a reading. Now this reading is say for example water. For water it is 1.33. For oils it goes higher than water.

The refractive index is again temperature specific. The reason being again higher temperature will change the density and because if there is a change in density its ability to refract will also change. So refractive index is again done at a constant temperature. So here are few refractive index values. If you carefully see your castor oil the refractive index is somewhere between 1.477 to 1.481.



Sesame oil, the refractive index is 1.465 to 1.469. Now, if you see or if you observe, this both are fixed oils. Now come to your essential oils or volatile oil. Say for example your sandalwood oil. Do you see a change in value?

It is generally said that your essential oils are much much more refractive as compared to your fixed oils. So your sandalwood oil somewhere has an RI or refractive index between 1.504 to 1.508 and your cinnamon that is your dalchini oil has a refractive index 1.529 is to 1 or you know between to 1.529 to 1.610 so this is for your volatile oil Now what happens is now you know that sandalwood oil is really expensive. Say you know the market rates may go as high as 15 lakh rupees a litre.

In that case there is a good chance that people mix fixed oils into sandalwood oil. Now, what is going to happen because less refractive substance is mixed with a more refractive one, the refractive index of your sandalwood will fall down. If the RI readings of the sandalwood oil is coming down, that means it has been adulterated with the fixed oil. That is how you use your refractive index to check the presence of other oils, especially the fixed oils. Moving on to the next value, the next value is optical activity.

Now, optical activity also we see in terms of molecules. Some molecules we call it a dextrorotatory, some molecules we call it as are levorotatory and what is exactly this optical activity? Now, if you take a, you know, like a source or a light source, especially if you see, you know, a light source which has a wavelength corresponding if you see the sodium spectrum the d line of sodium has a very specific wavelength and the specific wavelength is around 589 nanometers now this 589 nanometers is i mean plus minus 0.3 nanometers is what we use now this wavelength or this wavelength light or a plane of this wavelength light when it passes through a substance, the substance has the ability to rotate it to the left or rotate it to the right.

We call it rotating clockwise or rotating anticlockwise. So, the ability of a substance to rotate the plane of light is called optical activity, and this depending upon how this substance rotates, you can call them dextrorotatory—the ones which rotate the light clockwise—and levorotatory—the ones that rotate it counterclockwise. Okay, so how is this used, and where is this used? This is quantified or measured in a polarimeter because, in order for the light to be rotated, it should pass through at least what is called a 10-centimeter column of a substance of a specific dilution.

You can carry out a particular dilution. In that case, you can call it a polarimeter. SOR or specific optical rotation. You can do a dilution and then check. For example, with glucose, you can create a 1% solution of glucose and then check the optical activity.

It will be called specific optical rotation. Now, these values are used to detect adulteration in certain substances. So, if you come across peppermint oil, peppermint oil has a broad spectrum. The reason for this is, if you see, peppermint comes from Mentha species. We discussed this in the volatile oil section, and there are numerous Mentha species with a lot of variation in terms of compounds.

So, you get a range from minus 32, which is levorotatory. To a minus 18 range. Now, when it comes to honey. There are two substances which are principally present in honey. Those are fructose, glucose, and other sugars.

And you know, sugars are optically active. Glucose is something which is, you know, positive or what is called dextrorotatory, and fructose is something which is levorotatory. So, they both counteract each other, and eventually, the value of honey—because glucose is on the higher side—falls between plus 3 degrees to plus 15 degrees.

The optical rotation, because it's an angle, is expressed in degrees. Now, imagine if I'm adulterating the honey with syrup. Now, because the glucose and fructose are balanced, I'm getting this. Under glucose, which is slightly more, my plane is going or shifting more towards the positive side. So, this will change the direction or plane of light more towards what is called the dextrorotatory side—the right side. So, what is going to happen? The values of this are going to increase on the positive side. So, for adulterated honey, the values may go as high as 85 degrees. And what does that indicate?

That indicates that glucose has been added to this. So whenever you have your artificial syrup or glucose syrup being added to this you will see that the values of your honey will be always on the positive side. And that is an indicator of adulteration. Similarly, with your lemongrass oil, you might have it adulterated with other oil and the range might change. When you see molecules, in case of molecules also, when you are doing specific optical rotation, you will see naturally if you are dealing with mint, the mint oil contains L-menthol, the levomenthol.

But if you use synthetic menthol, synthetic menthol because the reaction is non-specific that leads to racemization. That means in synthetic you have D plus L menthol. Now D plus L menthol will no longer be optically active and I can easily know if it is a natural mint oil or artificial menthol or synthetic menthol has been added to it. So that's how your optical activity helps. The next quality control parameter is solubility.

Now, solubility is a lot of descriptive terms are used in pharmacopoeia. I have taken a screenshot of it. Say, for example, when you take a drug or when you take a substance, the ability of that substance to dissolve in a solvent is referred to as solubility. Now, if you know like one gram of the substance dissolve in one ml. of my solute it is very soluble okay so these are the ranges which are given by pharmacopoeia and these are the descriptive terms to tell that how much the solubility of a drug is so you have freely soluble if you require for one gram about 10 ml soluble if you require between 10 to 30 ml but insoluble if you require for one gram more than 10 liters of solvent

Then you say that if 1 gram is not going into 10 liters, it is kind of insoluble or practically insoluble. Also, note that solubility is temperature-dependent. So these descriptive terms, when you are doing a solubility test, you do it at a temperature or at a constant temperature. Now, solubility values, depending upon different substances, are calculated with respect to different solvents. Say, for example, if I am testing the solubility of a

sucrose, I will do it in water. But if I am testing the solubility of my resins, resins are water-insoluble. So when I have to test resins, I will check their solubility in something like alcohol. If I have to test the solubility of oils, castor oil, or, say, for example, sesame oil, I will use organic solvents because they are going to be insoluble in water.

So sometimes, solubility is also a quality indicator. Let's discuss how. Say, for example, castor oil. Now, castor oil contains ricinoleic acid, and this ricinoleic acid is one of the ingredients responsible for imparting solubility. If you see most of your fixed oils, they are ethanol-insoluble.

But what is going to happen with castor oil? If you take an equal amount of castor oil as alcohol, it is going to be soluble or miscible in 95% ethanol. Now, when you're talking about resins, you can have your colophony, which is freely soluble. In alcohols, benzene, ether, glacial acetic acid, and carbon disulfide. So if it is freely soluble, that indicates it's genuine colophony.

What if it is not? If it is not, that means it has been adulterated with another substance. What if this castor oil is not dissolving in an equal part of alcohol? What does that mean? That means there has been another oil added which doesn't contain ricinoleic acid.

So there's a good chance of adulteration. Similarly, sometimes apart from solubility, insolubility is also taken into account. So in the IP monograph of acacia, You have what is called water-insoluble matter. So, for example, you are taking 5 grams of acacia and dissolving it in 100 ml of water.

When you filter it, the residue on the filter paper should not exceed 50 mg. If it exceeds 50 mg, that means some other powder or chalk has been added, which is making it water-insoluble. So solubility is also a key indicator of the nature of substances that have been added and helps us in the assessment of quality. Now, moving on to the next one. The next one is density and specific gravity.

Now, both of these terms are used synonymously, but there is a slight difference between them. When I say density, density is given by what is called a simple formula: mass by volume or often called weight per ml. So, grams per ml. So, the density of a substance is how much mass and how much volume it occupies. If you divide it, you get how dense it is.

Whereas when you say specific gravity, specific gravity is actually the ratio of densities. So, specific gravity is the relative density of the substance when compared to water. For example, I am measuring the specific gravity of my castor oil. So in that case, I will measure the density of castor oil and divide it by the density of water. Comparing the density of castor oil to that of water is what is called specific gravity.

Now since both of these are... ratio, what happens is the units get canceled, and it becomes a dimensionless quantity. Now generally, when you do or when you see herbal drugs, we are more interested in specific gravity for the reason we check whether it's going to be lighter than water or denser than water. That helps us evaluate it during the extraction steps. Specific gravity, let's say for example, castor oil is 0.957. What does that mean? When I am pressing the seeds to obtain the castor oil, the oil is going to float, and the water or the juices of those castor seeds are going to settle down.

But take an example of clove. where it is more or slightly denser than water. What is going to happen when I extract clove oil? Water is going to be on top, and my clove oil is going to sink to the bottom. So that gives me an idea whether the top layer or the bottom layer is actually what you're looking for.

Beeswax is also one thing you will see when you boil the honeycomb. Because of its specific gravity, which is less than water, from the honeycomb, your beeswax oozes out and starts floating. So it is easier for me to collect and compare, and as a result, specific gravity is often a term used in herbal quality control. The next one is viscosity. Now this is something which is often given or used for liquid substances.

Now, this is a good indicator of flowability. When you say something is viscous, what does that mean? It is resisting flow. Say, for example, your honey—your honey is very viscous. Your shampoos—they are very viscous.

Or compare them with water; water is just pourable. So, viscosity is basically the fluid's resistance to flow. How much it resists flow when I kind of tilt that bottle. Now, this resistance to flow may be due to what is called, you know, internal forces, or the solid content, or even, for that matter, the ability—or, you know, the forces that bind the solute molecules into that solvent. So, whatever has been dissolved,

And because it is dissolved, it forms an association. Say, for example, your acacia. When you disperse it in water, Because it is forming an association in water. Because it is withdrawing water.

Now, no water is available to move. And that's the reason you create a good resistance to flow. The water is unable to flow. Because the acacia polymer has kind of blocked it. That's what is giving it, or that's what is making it more viscous.

It is important for us to know because, in processing, you require the liquid to flow through certain pipes. The viscosity will tell us how much energy is required to push through it. Now, this viscosity is referred to as dynamic viscosity and expressed as Pascal seconds—how much force is required, or often referred to as centipoise.

The reason why we use centipoise and not poise is that often 1 Pascal second is equal to 10 poise. So, in order to make the ratio 1:1, they use the term centipoise. So now, 1 Pascal second is equated to 1 centipoise. Now, in addition to dynamic viscosity, sometimes in certain cases, kinematic viscosity is also measured. Now, this is nothing but the same as your viscosity, but once you divide it by density, that gives you an idea of, you know,

Another factor is how or how density affects the flowability. In that case, what you get is called kinematic viscosity. Now, in order to measure viscosity, we use apparatus such as viscometers like Brookfield viscometers or certain rheometers with spindles. These spindles spin with a certain force. If the liquid resists that spin,

some force is exerted on the spindle due to this resistance, and that is accurately measured by the machine. For example, the viscosity of water is about 1 millipascal second. This is equal to 1 centipoise, but oils are slightly more viscous. Sesame oil, for instance, is about 65 millipascal seconds at 20 degrees Celsius. Now compare that to honey. Honey is 1000 to 2000. Again, depending on the source, raw honey is much more viscous than processed honey.

So, if you compare it with sesame oil, honey is almost 1000 to 10,000 millipascal seconds when measured at 25 degrees Celsius. Again, temperature is important here. The reason is that the moment you change the temperature, the liquids become less dense and more fluid. Moving on to the next point, the next parameters are physical constants of a particular oil or chemical, which include melting, boiling, and congealing points. These are often used as quality control parameters because they are fixed values, known as transition indicators or phase transition indicators. For example, melting is a transition from solid to liquid, boiling is a transition from liquid to gas, and congealing is the conversion of liquid back to solid.

So this phase transitions for a particular substances occur at a particular temperature. And if that is what we know, any impurities which are added in the substances can change the physical constants. which includes your melting, boiling as well as congealing point. So when you have, say for example, impurity, the melting point of the substance decreases. Because the crystal structure is no longer left intact and that is how you know that it's going to change and that change has been brought out by impurities.

So say for example melting point of coca butter is at the body temperature say 34 to 38 degree Celsius and that is the reason it is there or used in suppositories it is used in creams because at body temperature from its waxy consistency it becomes oils. So this is something what we do require. But imagine if it is adulterated and its melting point decreases to 20 degrees Celsius. That means you will have not a smooth lotion, you will have a pourable lotion. And then in order to increase the viscosity, you will have to add viscosatives.

Similarly, coconut oil. The boiling point of coconut oil again depending upon different different grades ranges somewhere between 204 to 232 degree celcius. Now this is also used for something which is called as or obtaining fractionated coconut oil. So you can cool, you can heat, you can congeal and get different fractions of coconut oil. Now similarly if you see congealing point the you can see here this is a clear one this is a waxy one that means it has kind of become solidified.

This point at which your liquid coconut oil transits into a solid phase is referred to as congealing point. So for coconut oil it is about 24 to 23 26 degrees Celsius. So what happens if your temperature during winter season dips more than a Say, for example, dips more than 24 degrees Celsius, slowly, slowly what is going to happen is this your coconut oil is going to slowly congeal and solidify. So what happens your coconut at an elevated temperature is liquid and at a lower temperature starts congealing.

So my consistency of preparations if I am preparing a formulation for cold countries versus if I am preparing a formulation for my temperate countries. The consistency of that will vary if I am using coconut oil as a ingredient. But at the same time, I also know that if I mix my paraffin or any other substance with coconut oil, its congealing point is going to change. So that also serves as my quality control indicator. So here are a few references that you can go across to check this physical methods of evaluation.

And thank you everyone for your patient listening. Thank you.