Natural Dyes Prof.

Padma Vankar

Department of Chemistry

Indian Institute of Technology, Kanpur

Lecture No. # 09

Since the last few lectures, we have been looking at the various aspects of dyes and their toxicity; their degradation product. And therefore, we have come to conclusion that slowly and steadily the use of natural dyes is much safer and it is also that it is now made available, many new sources have come up and therefore, there should be some very good methods of extraction.

So that, most of the dye content can be extracted from the plant material, because this was one of the draw backs in the earlier stages of extraction, that many a times the plant material still retain the colorant, and the colorant natural dyes were not fully extracted.

So, to overcome all those you know factors, that, how to extract most efficiently in cheap manner, and most effectively retaining the color property is a big challenge, and research has really progressed in this area as you will see, as we go along this lecture. That the extraction process has really taken up very advanced level. And so, we now try to look at the various methods of extraction of natural dyes.

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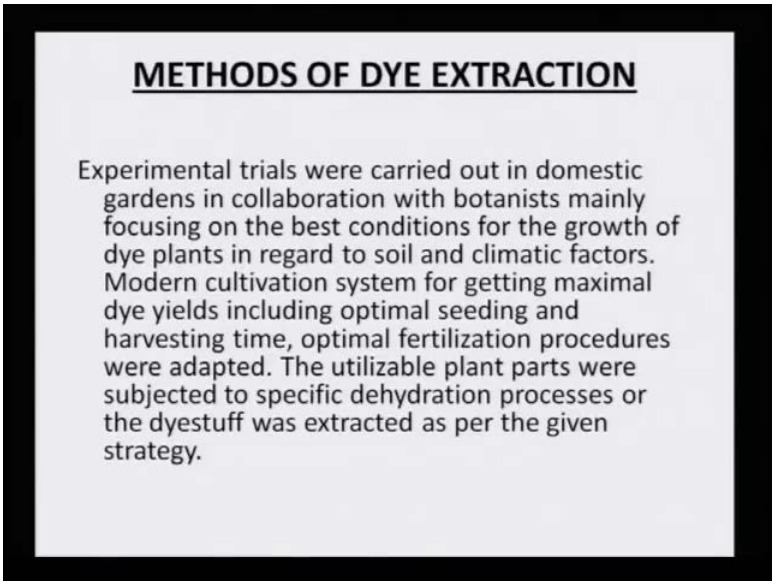

Methods of dye extraction: Experimental trials were carried out in domestic gardens in collaboration with botanists, mainly focusing on the best conditions for the growth of dye plants in regard to soil and climatic factors. Modern cultivation system for getting maximum dye yields including optimal seeding and harvesting time, optimal fertilization procedures were adapted. The utilization or utilizable plant parts were subjected to specific dehydration process or the dyestuff was extracted as per the given strategy.

So, how it happen? It all started with domestic garden growing the plant and with in collaboration with botanies, how to grow a plant more healthily and all that was taken care into the consideration, and these factors help to grow a very healthy plant. Now, if the plant is healthy; obviously, the flora of the plant also will be or the flower will be very healthy, the fruits will be very healthy the leaves and stem and root and another parts of the plant will be very healthy. So, it was expected that by proper utilization of fertilization and by taking into account the modern cultivation system. Plant organized forming of such dye yielding plants can be carried out very efficiently.

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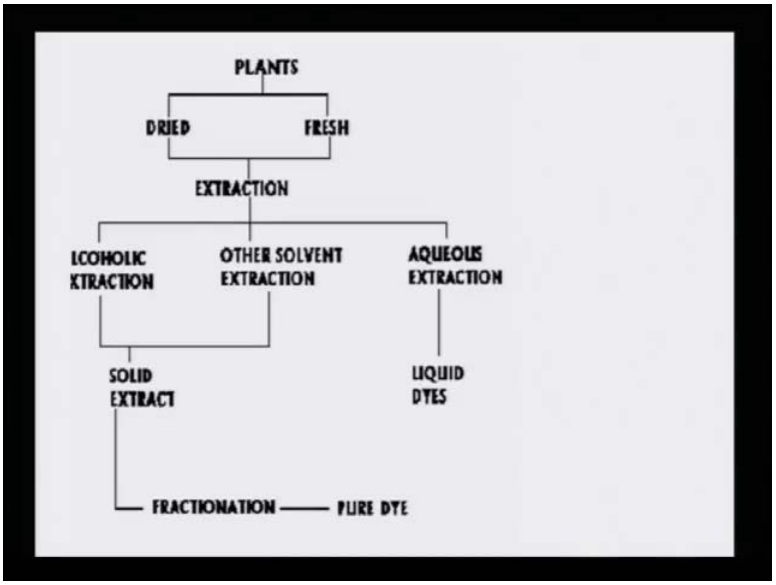

Now once that is been carried out, then by the means of this following strategy the plant can be extracted; that means, the plant can either be used as a dried plant; that means, the plant part will be first dried in oven, and then the colorant will be extracted or the plant can be used fresh both can be subjected to extraction in of course, separate systems.

Then during the extraction there can be an alcoholic extraction, any other solvent extraction or water extrication, which is also known as aqueous extraction. So, three types of extractions are possible. One is the methanol extraction, the second one is that any other solvent like hexane or acetone or ethyl acetate can be used. And the third one is the water extraction, which is the conventional method of extraction.

Now from these extra tents of the alcoholic type and any other solvent type, this solvent can be evaporated on rotary evaporator. And solid extract or semisolid extract or pasty extract can be obtained. Similarly from the aqueous extract liquid dyes can be extracted by removal of water. So, by gradual removal of water it can be concentrated. And then this solid extract can be further fractionated and pass through column chromatography as what we learnt yesterday, that column chromatography not only helps in identification of the compound, but it also help in the separation of various dyes that may be present together.

As I mentioned yesterday also. I want to repeat once more that natural dyes always occur in two, three or four types of similar structurally matching similar compounds. So, therefore,

they need to be separated, and the best method for separation of such similar compounds is by column chromatography. So, thus pure dye can be obtained.

So, both from the dye dried plant be it flower, be it fruit, be it leaves, be its stem, be it bark or roots all these parts of natural plants can be used for extracting dye, if they have any special colorant. Second thing is both dried plant part or fresh plant part either can be used. And three types of extractions are possible one is with methanol, because methanol is considered to be a very good solvent for all types of polar, non polar dye content of the plant part. But all the solvents also can play a very good role. For example, colors which are non polar can be extracted in hexane, colors which are polar can extracted in ethyl acetate or acetone.

So, you see that depending on the polarity of the compound, and the polarity is decided by the fact that how many O H groups, or how many such you know polar groups are attached as auxochromes to the molecule.

Now aqueous extract of course, has been one of the most traditional, conventional methods and from time and again it has been a full proof method. But the only problem with the aqueous method that is requires very long hours of extraction, and some of the dyes which are heat sensitive may get decolorized or discolored, there are two different words decolorized means it may lose its color completely, or discolored means from a bright nice color it may get converted to very dull bad looking color. So, this is the basic strategy that is followed for the extraction of dyes.

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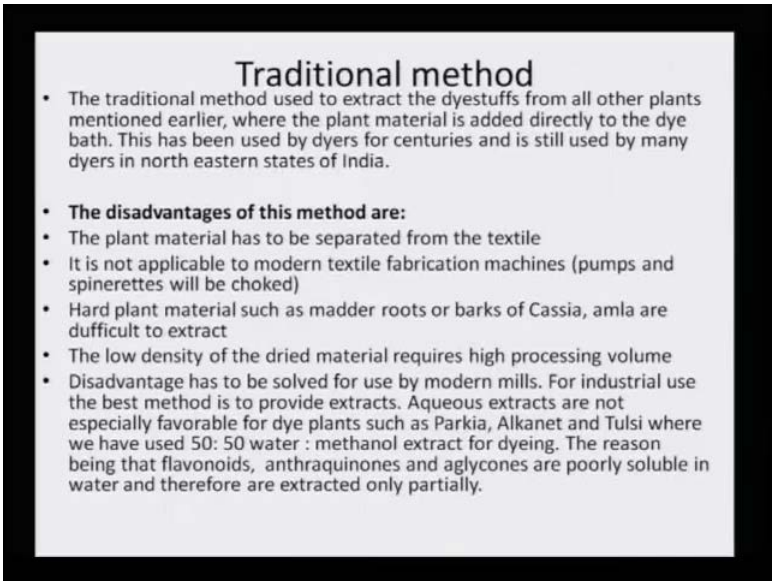

Traditional Method: The Traditional Method used to extract the dyestuff from all other plant mentioned earlier, were the plant material where the plant material is added directly to the dye bath. This has been used by dyers for centuries and is still used by the many dyers in north eastern states of India. So, this is the traditional method by just aqueous extraction.

However, this method had several disadvantages. Why I mentioned north eastern states of India, because still there are pockets of various groups where natural dyeing is being practice in Manipur, in Nagaland, in many other north eastern, Arunachal Pradesh and so, on.

The disadvantages of this method are that the plant material has to be separated from the textile. It is not possible to modern textile fabrication machines, because the pumps and spinerettes will be choked. Hard plant material such as madder roots or barks of cassia, amla are difficult to extract.

The low density of dried material requires high processing volume. Disadvantage has to be solved for use by modern mills. For industrial use the best method is to provide extracts. Aqueous extracts are not especially favorable for dye plants such as Parkia, Alkanet and Tulsi where we have to use 50 percent of 50:50 water: methanol extract for dyeing. The reason being that flavonoids, anthraquinones and aglycones are poorly soluble in water and therefore, are extracted only partially.

So, you see that when traditional method was that the fabric, the plant, and everything was put in one dye bath and it was just simply heated. Now some times when the dye is not very soluble in water there will be a problem that it you know it will not soubise in water, then in such a case like for example, parkia dye, dye from parkia plant, or dye from alkanet plant, or dye from Tulsi plant. Balsum Osmium is very you know in a state where it can only be dissolved in methanol, and such a plant can not show very good method of extraction by the traditional method and therefore, methanol has to be added 50 percent. Now all this plus if the plants parts are very hard, they will interfere with the modern machinery and therefore, there can be choking.

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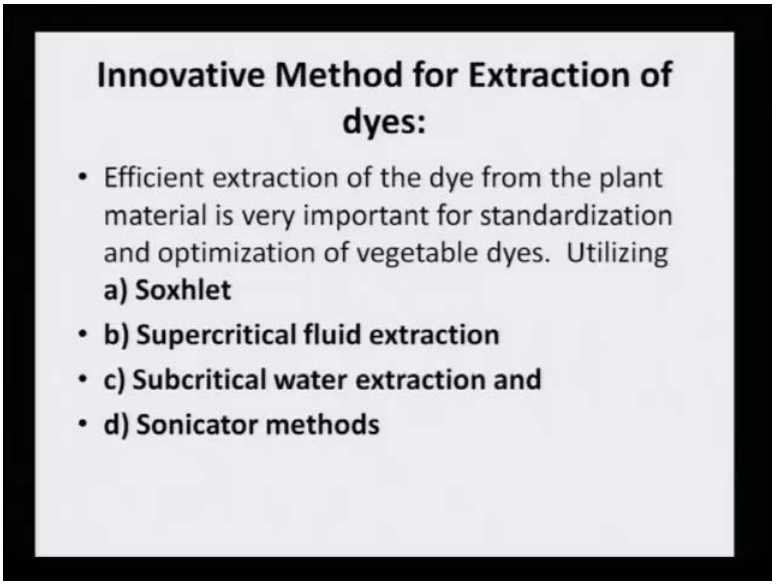

So, there that is the reason why traditional method is not very feasible any more with the modern technology. Innovative methods of Extraction of dyes: Efficient extraction of dye from the plant material is important for standardization and optimization of natural dyes or vegetable dyes. So, we come to a conclusion that the traditional method is not the best method.

And therefore, there are always possibilities of not being able to extract the entire amount of colorant, as it is the plants have only 2 to 10 percent dye, very rarely 10 percent it is between 2 to 5 percent mostly, and out of that also if the dye is not extracted properly it would mean that there is a loss of colorant in the waste material. And so efficient method of modern extraction methods that were device are Soxhlet, supercritical fluid extraction, subcritical water extraction and Sonicator methods.

So, these were the four methods which were popularly, or which have been popularly used in the extraction of natural dyes, of which Soxhlet is the most popular one why? Why, because early every laboratory can offer a Soxhlet machine. I will show you as we go along, how does the Soxhlet machine look like.

The second one is supercritical fluid extraction, which is also refered as SCFE now supercritical fluid extraction machine is very expensive, and because it is a one time investment. It is kind of very hard too you know procure this machine, but once it can be procured, it is one of the best techniques which can recover different types of plant chemicals,

such as are fit chemical which can be of different views to different industries. So, you can see that this machine can not only dye, but it can also essential as can give various types of other fragrance products and so on.

And so, forth subcritical water extraction is another new technology, as we go along we will try to learn more about it. It is a kind of super heated water, where water can be become a very good extractent. And the fourth method is the sonicator method, where ultra sound energy is being used for the extraction purpose.

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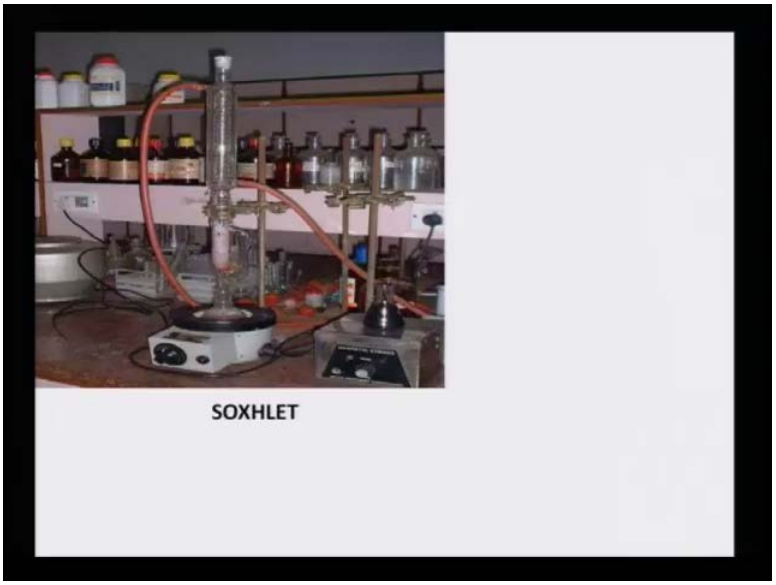

Now this is how the Soxhlet looks like, you see there is a round bottom flask, then there is a vessel where the plant material is wrapped up in filter paper, and there is a distillation head where the distillation takes place, and are almost eight hour this cycle goes on, till all the color actually collected in the round bottom flask, the round bottom flask is heated from beneath by a heating mantel. So, this is what this Soxhlet looks like.

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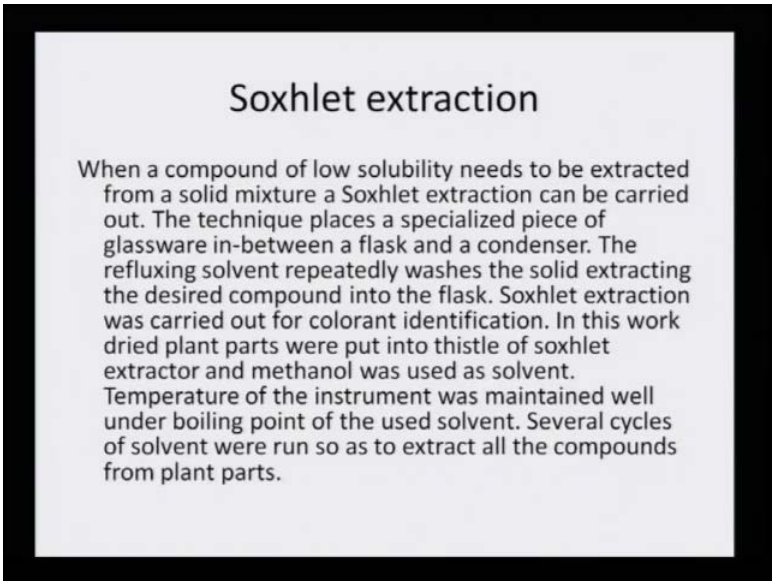

When a compound of low solubility needs to be extracted from a solid mixture a Soxhlet extraction can be carried out. So; that means, solid plant material cab be wrapped up, and with the help of appropriate solvent their solid extract ant can be or the dye can be extracted. The technique places a specialized piece of glassware in-between a flask and a condenser.

The refluxing solvent repeatedly washes the solid extracting the desired compound into the flask. Soxhlet extraction was carried out for colorant identification. In this work dried plant parts was used into thistle of the Soxhlet extractor and methanol was used as a solvent. Temperature of the instrument was maintained well under boiling point of the used solvent.

Several cycles of solvent were run so, as to extract all the compounds from the plant parts. So, you see that the choice of solvent in the case of Soxhlet is popularly methanol first thing, second thing is repeated cycle need to be done, because it is not possible to extract the entire amount of colorant in one cycle therefore, it requires almost four to eight hours, for the solvent to go on refluxing and then conducing and every time the solvent is condensing it is washing through that filter paper and back in to the round bottom flask.

So, that is how the process, but it is very efficient and its most popularly used in every laboratory where natural dye extraction is been carried out. Now another thing that, I would like to point out that it is very cheap and it is a affordable. So, therefore, one would find the Soxhlet exactor in every laboratory.

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Now SUPERCRITICAL EXTRACTOR: This is how it looks and there is this liquefied carbon dioxide, which is popularly used for the extraction of the colorant. Sometimes some entertainers are added as a solvent like ethyl acetate, to facilitate the solubility of the colorant. Now because of the rarefaction of carbon dioxide, and liquid fraction of carbon dioxide, it adds on property to the liquid carbon dioxide, to be a good solvent.

Now this is a new concept all together, why because you have always known carbon dioxide as a gas. Now how would you imagine that this can happen, you also can recall the gas lows that you have learnt in your tweleth standard that gases can be compressed, and at one particular point of temperature and pressure then gases can be converted into liquid. Now this is the critical temperature and the pressure at with gaseous material can be liquefied, now when it is rarefied again the release of pressure can bring them back to the gaseous state. So, thus the fundamental that adapted in super critical extractor.

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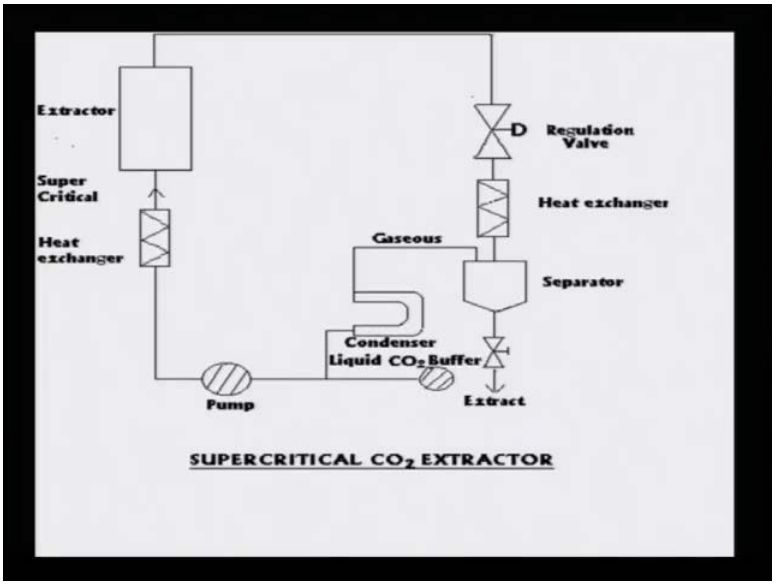

If, I show you the flow diagram, you will be able to understand the working. Now super critical extractor, here is a regulation valve there is a heat exchanger, there is separator, there is a gas exchange then there is the condenser which passes liquefied carbon dioxide, and through the extract ant, it then come to back the carbon dioxide into the system. So, you see that all the carbon dioxide is liquefied rarified, liquefied rarified, and it is recycled. There is very minimum loss of carbon dioxide, but nevertheless it acts as a solvent very efficiently.

What it does in the liquefied states? It takes away the colorant from the plant material, and then when it is rarified, it emits out the plant material extractant. So, therefore, the carbon dioxide in the gasses state is recycled, and that plant extractant is collected.

Now you will also understand that at different temperature and pressure, when the super critical extractor is set at a different temperature and pressure it can then be very be fitting for extracting steroids from the plants. Another parameter can be set up where only terpenoids can be extracted. So, you see from the same plant material which is fed into the supercritical extractor, many value addition products can be extracted by simply changing the parameters of the critical temperature and pressure and by using in different and trainers with the carbon dioxide gas.

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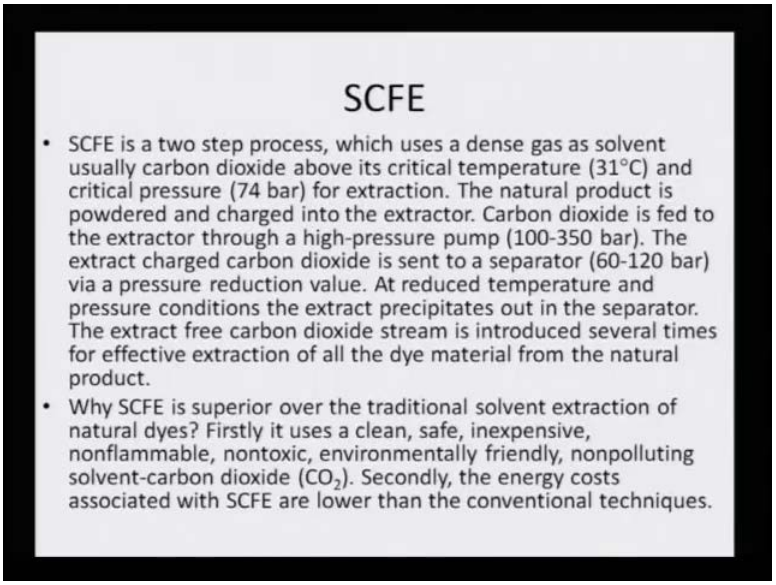

Supercritical fluid extraction or SCFE is a two step process, which uses a dense gas as a solvent usually carbon dioxide above its critical temperature that is 31 degree centigrade, and critical pressure which is 74 bar for extraction. The natural product is powdered and charged into the extractor. Carbon dioxide is fed to the extractor through a high-pressure pump that is working at 100-350 bars.

The extract charged carbon dioxide is sent to the separator which is at a pressure 60-120 bar via a pressure reduction value. At reduced temperature and pressure conditions the extract precipitates out in the separator. The extract free carbon dioxide stream is introduced several times for effective extraction of all the dye material from the natural product.

Why SCFE is superior over the traditional solvent extraction of natural dyes? Now, one would wonder that, why is it that there was a need for SCFE at all? Why, because it is one of the best method, and as I told you that nothing is wasted, from the same plant material various other important fito chemicals can also be extracted after the extraction of the dye. And at a particular parameter which is the critical temperatures and pressure, above the critical temperatures and pressures, when these temperature and pressures are altered, different materials will come out differently.

So, there is no necessity for even doing any column chromatography. As, what we saw in the Soxhlet extraction, we always get a crude mixture, but in the case of supercritical fluid

extraction the purity of the compound is assured. Firstly, it uses a clean safe in expensive, non in flammable, non toxic, environmentally friendly, non polluting solvent carbon dioxide.

Now you see all other solvents that are used in Soxhlet extraction be it methanol, be it acetone, be it ethyl acetate, or any other aromatic solvents are always considered to be a non eco friendly and therefore, this particular method has certainly many superiority points as compared to the Soxhlet method.

Secondly, the energy cause associated with SCFE are lower than the conventional technique, because of the quick extraction process, the energy required for pumping the carbon dioxide is much faster, one can complete this cycle of passing through the plant material, the liquefied carbon dioxide, and then rarifying it in a much faster manner and may be within a half an hour the job is done, where as I told you in Soxhlet extraction it is always like four to eight hours and that much amount of heating is required continuously.

So, therefore, it is also not only energy saving, time saving, but the machine has a onetime cause no doubt about it, because of its specialized pressure equipment.

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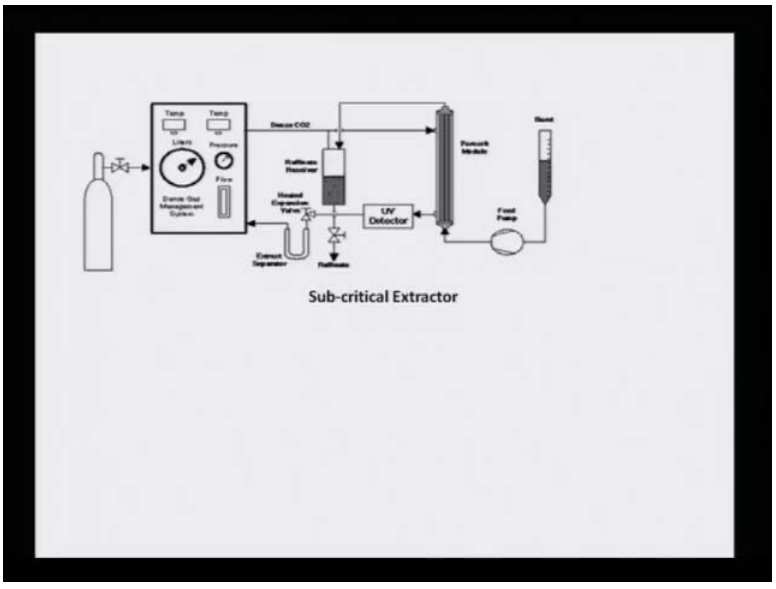

Now looking at the sub-critical extractor, Sub-critical Water extractor: This is how the layout looks like? It has a pump that is the controlling the temperature and then it also has an exist through which the extraction takes place, there is a feed pump then into the extractor there is

a very specialized kind of arrangement, where super heated water can be passed through the extractant. Normally the water have a temperature of 100 degrees at boiling.

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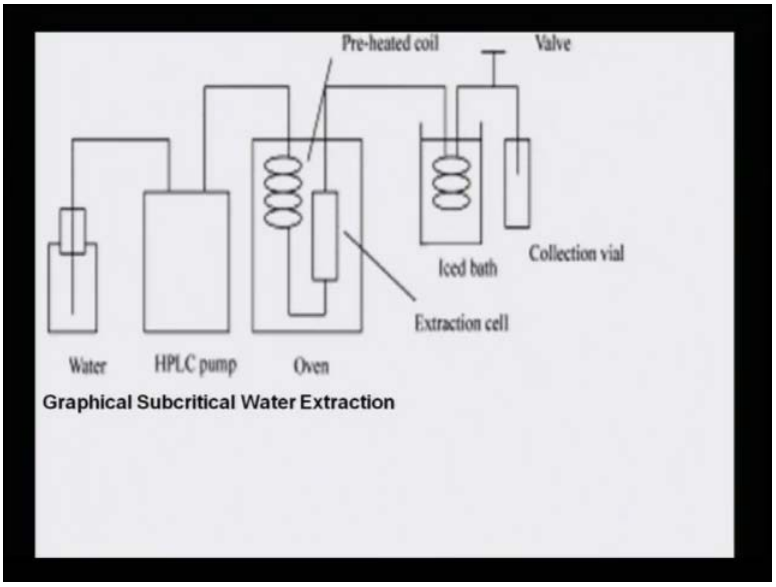

Now we are taking about temperature which are much above that. This is the graphical subcritical water extractant there is a pre-heating coil and extraction cell and therefore, it is you know water is through an HPLC pump is pumped into the Oven, and it is the heating is facilitated by the heating coil and therefore, the temperature of the pumped water is kept above 100 degree, and this gaseous water is a very good or I would save you know something which is in the in between the stage of gaseous and liquid water is a good extractant.

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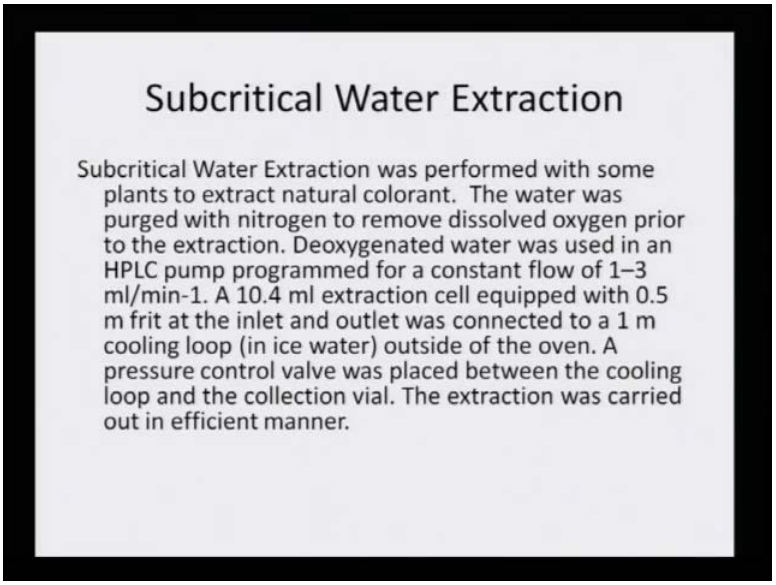

Subcritical Water Extraction: Subcritical Water Extraction was preformed with some plants to extract natural colorant. It is not one of the most popular methods, let me also tell you, but this was one of the methods that was practiced for a few plants. The water purged with nitrogen to remove dissolved oxygen prior to the extraction. Deoxygenated water was used in an HPLC pump programmed for a constant flow of 1-3 ml per minute.

A 10.4 ml extraction cell equipped with 0.5 meter frit at the inlet and outlet was connected to 1 meter cooling loop in ice water outside of the oven. A pressure control valve was placed between the cooling loop and the collection vial. The Extraction was carried out in efficient manner. So, this also actually was based on making liquefied water into a gaseous space and then cooling it again to get the liquid. So, that is how it was made used for extraction.

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Now comes the Sonicator, now this is the machine which uses the ultra sound energy, and the ultra sound energy of 20 kilo hertz frequency, at voltage of about 50 can creates agitation which can be very good for extracting the colorant from the plant material.

And this was for the first time used in our laboratory while we were working with some natural dyes, and how it started? I will give you a small story, because you know all new invention are accidental and this two was a not a very planned experiment, but we just wanted to see this, because we were facing with the extraction of super wool by a conventional water extraction, and what would happen?

The super wood shavings when they were heated for a prolong time in water wood instead of giving that bright magenta extract would get converted into a dull round extract. Therefore, we have understood that is getting discolored, because of prolonged heating. So, we first thought may be heat is the culprit so, we started trying to do with cooled water or not so, hot water, even then we faced the same difficulty.

So, it we understood that is not heat alone there is oxygen factor which is making this or the prolonged contact with oxygen is actually causing this discoloration. Therefore, we then tried to put the plant material into the Sonicator, and quickly we agitated for 5, 10 minutes and all the color came into the solvent, then we understood or into the water layer and we understood that the past method is the best method. So, we understood the use of sonicator for extraction, and this was used for the first time in our laboratory for this purpose.

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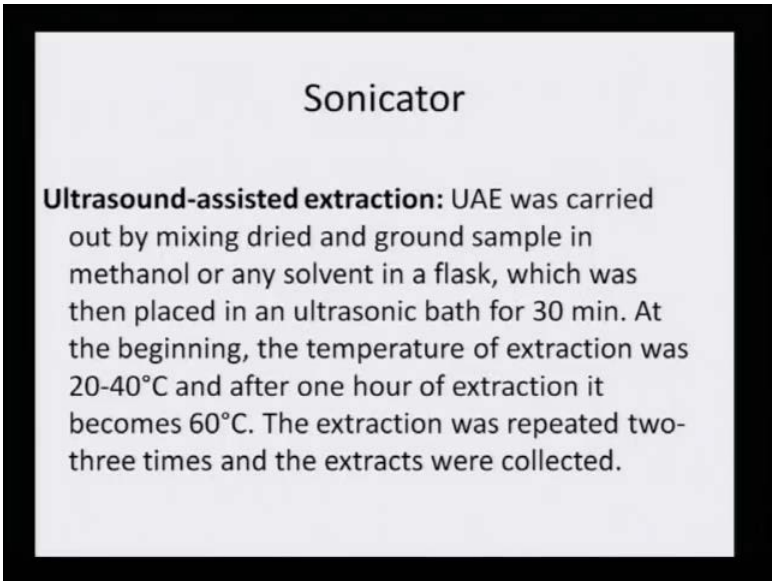

Ultrasound-assisted extraction was carried out by mixing dried and ground sample in methanol or any other solvent in a flak, which was then place in the ultrasonic bath for 30 minutes. Actually it did not required even 30 minutes it was less than 30 minutes. At the beginning the temperature of extraction was between 20- 40 degrees and after about 30 minutes that became, because there is a localized heat that is generated. The extraction was repeated two-three times and the extracts ware collected and evaporated on the rotary evaporator,

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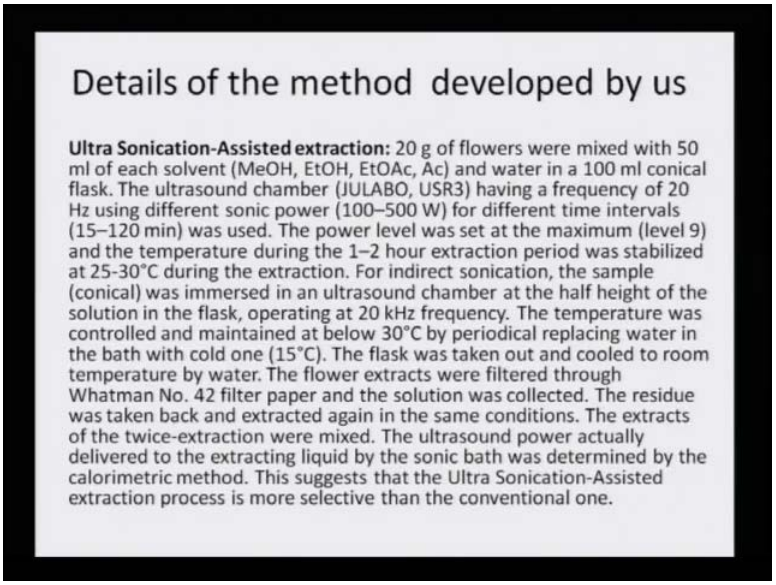

Details of this methods that was developed by us: I would like to share this, because this is one of the most innovative technology, Ultra sonication-Assisted extraction: Ideally 20 gram of flower were mixed with 50 ml of each solvent. Like one can use methanol, or ethanol, or ethyl acetate, or acetone and sometimes even water and in 100 ml conical flask it is just taken.

The ultra sound chamber that is the machine that I should use was the JULABO machine of the model USR3 having a frequency of 20 kilo hertz, using different sonic power between 100 to 500 watts, for different time intervals that is 15 minutes to even 2 hours was used. The power level was set at the maximum level 9, and the temperature during the 1-2 hour extraction period was stabilized, and 25-30 degrees during the extraction.

For indirect sanitation, the sample conical flask was immersed in an ultrasound chamber at half height of the solution of the flask, operating at 20 kilo hertz frequency, the temperature was controlled and maintained at below 30 degrees by periodically replace in water in the bath with cold one that is if cold water is added, then the temperature can be maintained, because it otherwise by continues using of the ultra sound energy the water gets little heated up. The flask was taken out and cooled to room temperature by water.

The flower extract were filtered through the watman number 42 filter paper and solution was collected. The residue was taken back and extracted again in the same conditions. The extracts of the twice extraction were mixed. The ultra sound power actually delivered to the extracting liquid by the sonic bath was determined by the colorimetric method; that means, from one extraction to the next extraction it was possible to see the color change and the intensifying of the color.

This suggested that Ultra Sanitation-Assisted Extraction process is most selective than the conventional one, and it is more effective also, it is also very good for temperature sensitive substrates. As I told you that accidental use for the Sonicator with the soften wood material. Let us to this important use of ultra sound, otherwise ultra sound was not used for this purpose earlier.

So, therefore, the energy consumption is much lower in the case of extraction, the efficiency of the extraction is much higher and not only that the time consumed is much less, and the cost of the machine is very optimal, it is not a very expensive machine like SCFE or for that matter subcritical water extractor. So, you see that this is an affordable machine, but it one

compares with the Soxhlet machine, this extracting machine is much efficient, it can be done directly into the bath or it can be done I mean putting it in a conical flask, and only utilizing the agitation that is created by water in the Sonicator bath. So, both ways one can do it one can have a choice for solvent, you can use any one of these solvent like methanol, ethanol, ethyl acetate, acetone, or even water.

So, you see there is a big flexibility just like Soxhlet where one can use any solvent, here also one can use any solvent here one can use it directly or indirectly. Then the third point is that it is very fast, it is very you know its saves a lot of energy, because it is fast and lastly it also fairly cheap.

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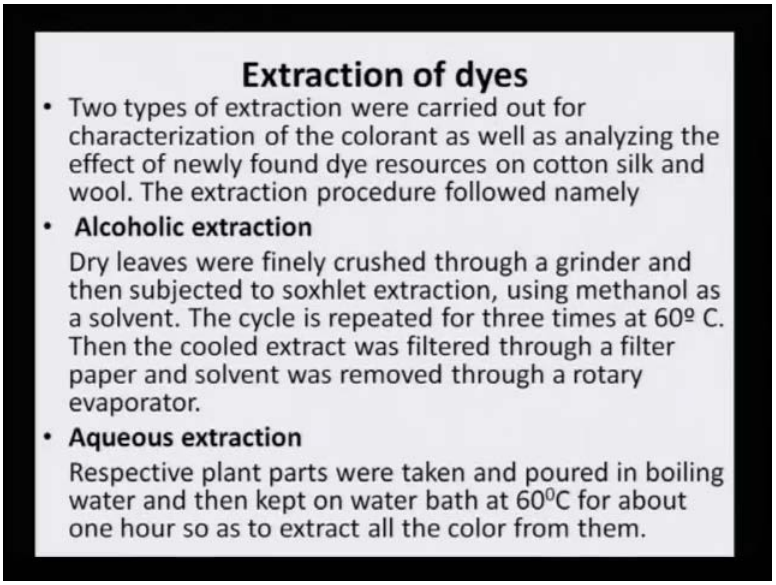

Therefore, extraction of dyes: Now can be summarize one can use Soxhlet method, one can use super critical method, one can use sub critical water, or one can use Sonicator method. But if I have to talk about extraction, then I will say that extraction of dyes are popularly done by two main solvents.

Two types of extractions were carried out for characterization of colorant as well as analyzing the effect of newly found dyes resources on cotton silk and wool. The Extraction produced followed namely Alcoholic extraction, where dry leave were finely crushed through a grinder and then subjected to Soxhlet extraction, using methanol as a solvent. The cycle is repeated for three times at 60 degree centigrade. Then the cooled extract was filtered through a filter paper and solvent was removed through a rotary evaporator.

Similarly Aqueous extraction, respective plant parts were taken and poured in boiling water and then kept on for Water bath for 60 degrees or about one hour, and the extract of all the color was then seen in the water layer.

Now this is also felted and then concentrated then the same procedure is carried out, but nevertheless these are the two main solvent methods or choice of solvent alcoholic method, or aqueous method for the extraction of dyes.

So, if I have to, now conclude I will say that for extraction one has to keep in mind that Soxhlet is one of the methods, but the better method is by the use of ultra sound energy using a Sonicator bath, and this was found to be not only cheap, but had lot of flexibility and any and every plant would be extracted on sonicator.