Natural Dyes

Prof. Padma Vankar

Department of Chemistry

Indian Institute of Technology, Kanpur

Lecture No. # 07

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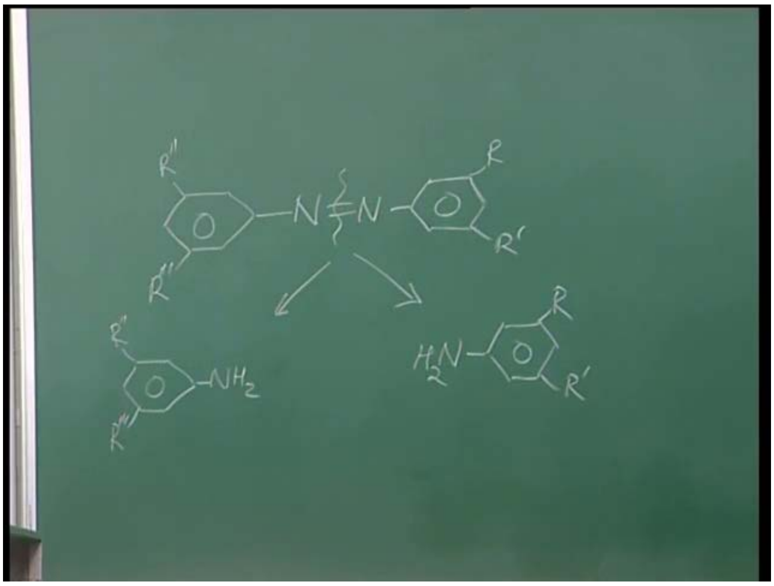

Yesterday we saw, how the azo dyes get reductively cleaved and different amino acids or amino compounds or amino naphthols are formed. Today, we will try to look at the oxidation of colors; you must have noticed that fading occurs, when light is observed some colors, when they are are color fabric, when they are put on the sun light for drying, their color seem to reduce over a couple of times. And this is due to certain oxidation of color that occurs due to the absorption of UV light; and therefore, we should have an understanding of what really happens during this oxidation of colors.

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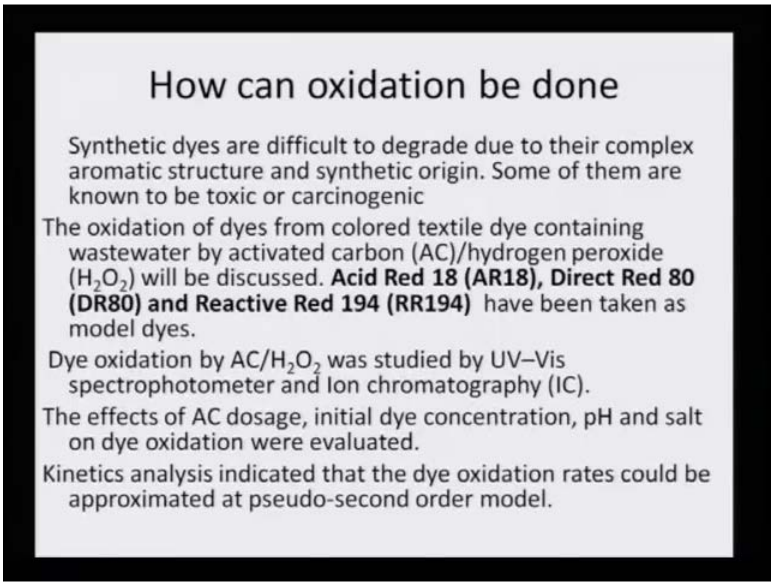

How can oxidation be done

Synthetic dyes are difficult to degrade due to their complex aromatic structure and synthetic origin. Some of them are known to be toxic or carcinogenic

The oxidation of dyes from colored textile dye containing wastewater by activated carbon (AC)/hydrogen peroxide (H_2O_2) will be discussed. **Acid Red 18 (AR18), Direct Red 80 (DR80) and Reactive Red 194 (RR194)** have been taken as model dyes.

Dye oxidation by AC/H_2O_2 was studied by UV–Vis spectrophotometer and Ion chromatography (IC).

The effects of AC dosage, initial dye concentration, pH and salt on dye oxidation were evaluated.

Kinetics analysis indicated that the dye oxidation rates could be approximated at pseudo-second order model.

How can oxidation be done? Synthetic dyes are difficult to degrade, because it is not only the process of fading, but also when dye effluent have to be degraded, before they are run into the reverse stream; that time also some kind of oxidation reaction has to be carried out in order to break down the dye molecule. Now, synthetic dyes are difficult to degrade, due to their complex aromatic structure and synthetic origin; some of them are known to be toxic or carcinogenic; we saw that when we were learning about toxicity, the oxidation of dyes from color textile dye containing waste water by activated carbon and hydrogen peroxide will be discussed.

So, the way the oxidation is carried out will be due to the two oxidative agents; one is the activated carbon, which will be the referred as AC and the other one is hydrogen peroxide, H 2 O 2; and the acid dyes that would be taken into consideration are Acid Red 18 or AR18, Direct Red 80 - DR 80 and Reactive Red 194 or RR194; these have been taken as a model dye to study or understand the oxidative degradation. By the dye oxidation by a activated carbon and hydrogen peroxide was studied by UV visible spectrophotometer and ion chromatography. So, these were the two methods for determining, whether the oxidation processes has taken place or not. The effect of activated carbon, dosage, initial dye concentration, pH and salt on dye oxidation were evaluated.

So, you see that you know, many parameters have to be taken into account that, how should be the process, how much of activated carbon should be used, what should be the initial dye concentration, we service the concentration of activated carbon, what should be the p H; and therefore, all these parameters need to be set up, and see which one gives the best result; kinetic analysis indicated that the dye oxidation rates could be approximated as pseudo-second order model. So, when kinetics was done, it was found that these oxidative processes are pseudo-second order in their kinetic reaction; you must have heard the Reaction; first order Reaction, second order Reaction, and this is a new type of kinetic order, which is called pseudo-second order model.

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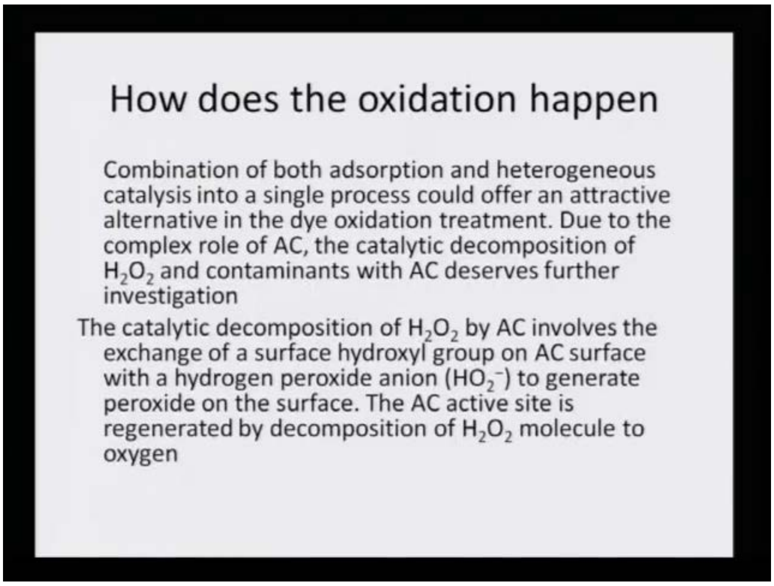

How does the oxidation happen

Combination of both adsorption and heterogeneous catalysis into a single process could offer an attractive alternative in the dye oxidation treatment. Due to the complex role of AC, the catalytic decomposition of H_2O_2 and contaminants with AC deserves further investigation

The catalytic decomposition of H_2O_2 by AC involves the exchange of a surface hydroxyl group on AC surface with a hydrogen peroxide anion (HO_2^-) to generate peroxide on the surface. The AC active site is regenerated by decomposition of H_2O_2 molecule to oxygen

How does the oxidation actually happen: Combination of both adsorption and heterogeneous catalysis into a single process could offer an attractive alternative in the dye oxidation treatment. Due to the complex role of Activated Carbon the catalytic decomposition of H 2 O 2 and contaminants with Activated Carbon deserves further investigation. So, what is done? It is taken in combination with hydrogen peroxide, activated charcoal and hydrogen peroxide; and there is a… So, on the surface of the activated carbon, the adsorption takes place; and then there is a heterogeneous catalysis, which occurs on the surface, and therefore the hydrogen peroxide then, is facilitated to react.

The catalytic decomposition of hydrogen peroxide by Activated Carbon involves the exchange of the surface hydroxyl group on Activated Carbon surface with the hydrogen peroxide anion that is HO 2 minus to generate peroxide on the surface. The Activated Carbon active site is regenerated by decomposition of hydrogen peroxide molecule to oxygen. So, this is how it releases oxygen, and this nascent oxygen is then very, very reactive; and this is facilitated only because it has adsorbed on the surface of Activated Carbon. So, Activated Carbon offers an adsorption surface, and there is heterogeneous catalysis that is occurring, and there is a decomposition of hydrogen peroxide to first hydro peroxide anion, and then subsequently to oxygen.

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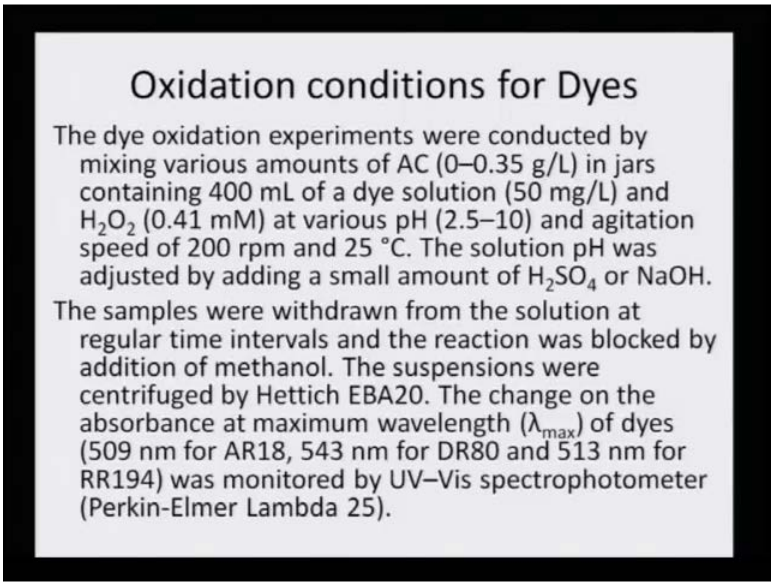

Oxidation conditions for Dyes

The dye oxidation experiments were conducted by mixing various amounts of AC (0–0.35 g/L) in jars containing 400 mL of a dye solution (50 mg/L) and H_2O_2 (0.41 mM) at various pH (2.5–10) and agitation speed of 200 rpm and 25 °C. The solution pH was adjusted by adding a small amount of H_2SO_4 or NaOH.

The samples were withdrawn from the solution at regular time intervals and the reaction was blocked by addition of methanol. The suspensions were centrifuged by Hettich EBA20. The change on the absorbance at maximum wavelength (λ_{max}) of dyes (509 nm for AR18, 543 nm for DR80 and 513 nm for RR194) was monitored by UV–Vis spectrophotometer (Perkin-Elmer Lambda 25).

Oxidation condition for dyes: The dye oxidation experiments were conducted by mixing various amount of Activated Carbon that is from 0 to 0.35 gram per liter in jars containing 400 mL of the dye solution, that is 50 milligram; the concentration of the dye solution was 50 milligram per litre; but the volume of this concentration of solution was 400 mL and hydrogen peroxide 0.41 millimole at various pH varying from 2.5 to 10 pH, and then agitation speed was 200 rpm and at 25 degrees, that is the ambient temperature or the room temperature was carried out

The solution p H was adjusted by adding a small amount of either sulphuric acid or if it is was required to make it alkaline, it was done by sodium hydroxide. The samples are withdrawn from the solution at regular time intervals and the reaction was blocked by addition of methanol. So, the way the reaction was carried out, to find out how, what is the kinetics of oxidation? The Reaction was quenched or stopped by the edition of methanol; the suspensions were centrifuged, and the change on the absorbance of maximum wave length of dyes that is for AR18 - it was 509; 543 nanometer for DR80 and 513 nanometer for RR194 were were all monitored by UV visible spectrophotometer.

So, you see that the procedure was so simple; it was just that pH, at different pH, this activated charcoal and hydrogen peroxide were added to the three different acid solutions that are rather the acidic dye solutions, that is AR18, DR80 and RR194. And then it was seen, how the reaction occurs; what is the best pH at which the oxidation occur; because it was monitored by UV visible spectrophotometer; therefore, the lambda max of these dyes were the main object to notice, whether it was lowering down, because that is the criteria of seeing any dye under this UV visible spectroscopy.

I will take a separate lesson on how dyes are analyzed, and there we will talk about the lambda max of a dye or a colored molecule, but right now, it is important for you to understand that the monitoring of the oxidative processes of these three dyes were done by their lambda maxes; and their lambda maxes were different 1 AR18 had a lambda max 509 nanometer; DR80 had lambda max of 543 nanometer; and the RR194 had a lambda max of 513 nanometers. So, you see that they were all different, so it was easy to monitor them.

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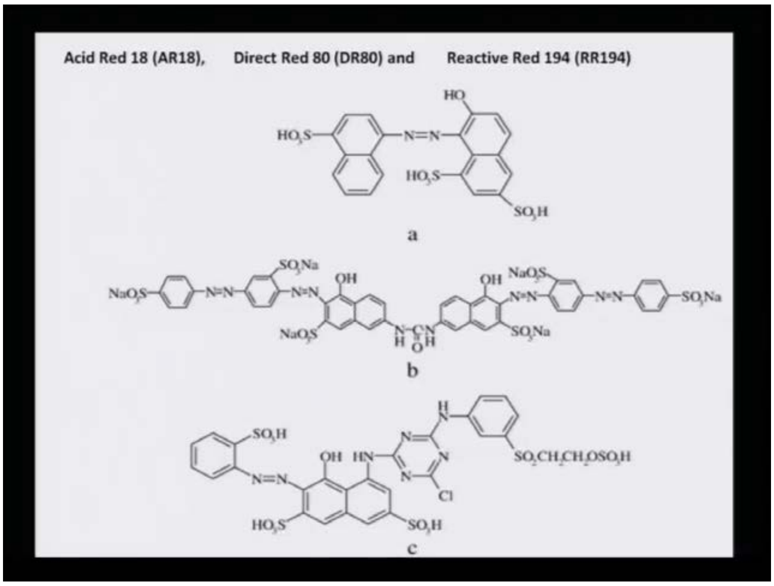

Acid Red 18 (AR18), Direct Red 80 (DR80) and Reactive Red 194 (RR194)

Now if one looks at the structure of the three dyes; a, b, c; you will see that the first acid Red 18 - AR18 has three sulphonic acid groups and one OH group; whereas, direct Red 80, DR80 has more conjugated system, it has azo linkages, and then it has sodium, it is a sodium salt of the sulphonic acid; and there are several, the already there are 4 azo linkages, and then there are 3 and 3, sulphonic - 6 sulphonic acid sodium salt of sulphonic acid. So, you see the direct red has a very conjugated long system, and the third one, the reactive red 194, is also having a fairly more complicated structure than the acid Red 80.

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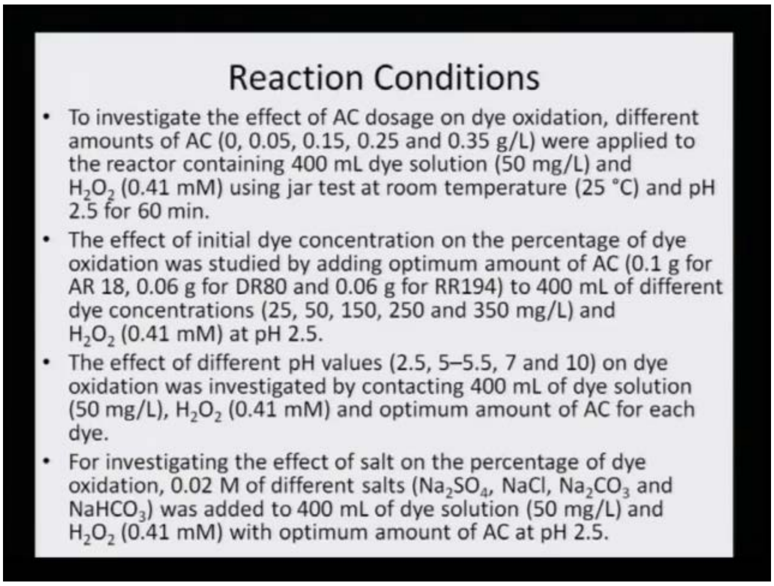

Reaction Conditions

- To investigate the effect of AC dosage on dye oxidation, different amounts of AC (0, 0.05, 0.15, 0.25 and 0.35 g/L) were applied to the reactor containing 400 mL dye solution (50 mg/L) and H_2O_2 (0.41 mM) using jar test at room temperature (25 °C) and pH 2.5 for 60 min.
- The effect of initial dye concentration on the percentage of dye oxidation was studied by adding optimum amount of AC (0.1 g for AR 18, 0.06 g for DR80 and 0.06 g for RR194) to 400 mL of different dye concentrations (25, 50, 150, 250 and 350 mg/L) and H_2O_2 (0.41 mM) at pH 2.5.
- The effect of different pH values (2.5, 5–5.5, 7 and 10) on dye oxidation was investigated by contacting 400 mL of dye solution (50 mg/L), H_2O_2 (0.41 mM) and optimum amount of AC for each dye.
- For investigating the effect of salt on the percentage of dye oxidation, 0.02 M of different salts (Na_2SO_4, NaCl, Na_2CO_3 and $NaHCO_3$) was added to 400 mL of dye solution (50 mg/L) and H_2O_2 (0.41 mM) with optimum amount of AC at pH 2.5.

Now Reaction Conditions: It is important when we are trying to understand the oxidative degradation of these synthetic dyes, what are the optimal parameters, under which they can be a degraded. You have learnt about the azo dye degradation in the intestinal micro bio term yesterday; today we will try to see that in the laboratory, what kind of conditions are set up for the oxidative process of degradation of these synthetic dyes. To investigate the effect of Activated Carbon dosage on dye oxidation; different amounts of activated charcoal or carbon that is 0.05, 0.15, 0. 25 and 0.35 gram per liter were applied to the reactor containing 400 mL of a dye solution of concentration 50 milligram per litre.

And then to that hydrogen peroxide 0.41 millimole was added; and all the jars were kept at 25 degree centigrade and at pH 2.5 per 60 minutes, that is for 1 hour. The effect of initial dye concentration on the percentage of dye oxidation was also studied, and that was done by adding optimum amount of activated carbon that is 0.1 gram for AR18, 0.6 gram for DR80 and 0.6 gram for RR194 to 400 mL of different dye concentrations that is 25, 50, 150, 250, 350 milligram per liter, and to that the same amount of hydrogen peroxide that is 0.41 millimole was added and the pH was maintained at 2.5.

So, you will see that the effective dose of the Activated Carbon or the effect of initial dye concentration; one has to set other parameters fixed that means, the concentration of hydrogen peroxide was not altered, the temperature was not altered, the pH was not altered only the dye concentrations were altered in this case; and in the earlier case, where Activated Carbon was, the dose was a varied and other parameters were kept fixed; the effect of different pH values were evaluated, and solutions at 2.5 to 5.57 and 10 on dye oxidation were set and investigated by contacting 400 mL of the dye solution having the same concentration 50 mL per liter hydrogen peroxide 0.41 milli mole and optimum amount of Activated Carbon for each dye.

For investigating, the effect of the salt on the percentage of the dye oxidation, 0.02 molar of the different salts such as sodium sulphate, sodium chloride, sodium carbonate and sodium bicarbonate were added to the same amount of 400 mL of dye solution of concentration concentration 50 milligram per liter, and the same amount of hydrogen peroxide, because it is also important to know, whether these salts play any role or not in the facilitation of oxidation or do they act as a retardant in the oxidation. And therefore, all other parameters like pH was kept at 2.5, and the study was carried out.

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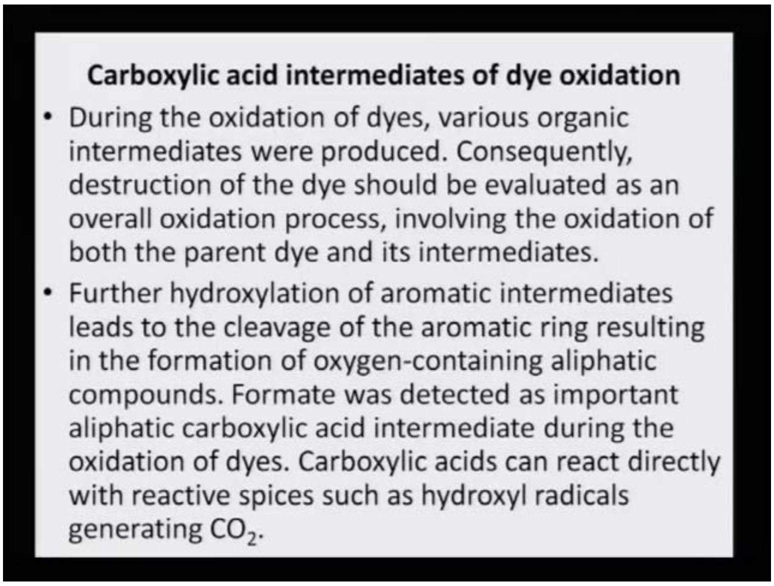

Carboxylic acid intermediates of dye oxidation

- During the oxidation of dyes, various organic intermediates were produced. Consequently, destruction of the dye should be evaluated as an overall oxidation process, involving the oxidation of both the parent dye and its intermediates.
- Further hydroxylation of aromatic intermediates leads to the cleavage of the aromatic ring resulting in the formation of oxygen-containing aliphatic compounds. Formate was detected as important aliphatic carboxylic acid intermediate during the oxidation of dyes. Carboxylic acids can react directly with reactive spices such as hydroxyl radicals generating CO_2.

Carboxylic Acid intermediates of dye oxidation: Normally what happens is that the there is a formation of COOH, during the oxidation of dyes various organic intermediates were produced; consequently destruction of the dye should be evaluated as an overall oxidation process involving the oxidation of both the parent dye and its intermediate. So, that the destruction of the dye, when we are looking at it, it is an overall process; and therefore, what happens to the parent dye what are its subsequent intermediates that are formed during the process of oxidation need to be understood and evaluated. Further hydroxylation of aromatic intermediates leads to the cleavage of aromatic ring, resulting in the formation of oxygen containing aliphatic compounds.

Formats were detected as important aliphatic carboxylic acid intermediate, during the oxidation of dyes; Carboxylic acids can react directly with reactive spices such as hydroxyl radicals generating carbon dioxide radical. So, you see that that is what is the procedure, when the oxidative process starts taking place; one radical generates another radical and the carboxylic acid that was popularly noticed to be formed as an oxidative degraded product was the formic acid or the format, and then that was… It is an aliphatic acids. So, you saw that most of the dyes were actually aromatic compounds, but these aromatic compounds did not have too much of aromaticity, still retained even after the degradation; therefore, it you have to understand that from the aromatic ring, the degradation product gives aliphatic compounds.

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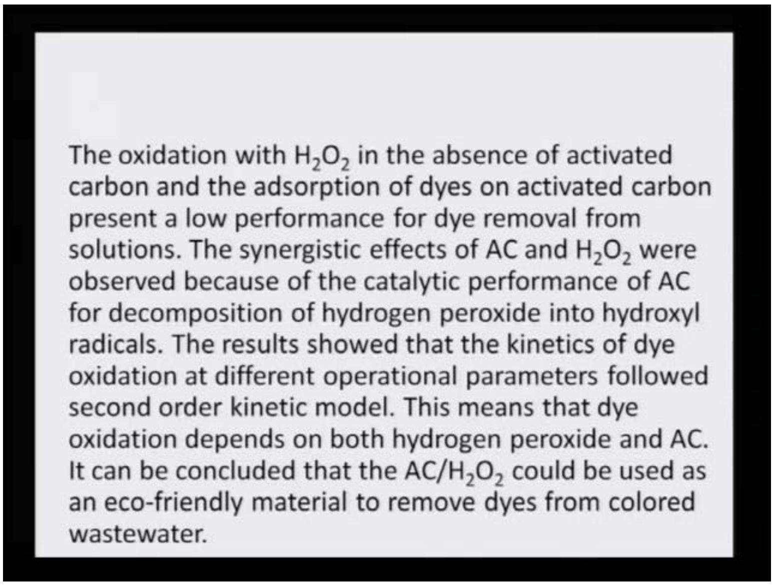

The oxidation with H_2O_2 in the absence of activated carbon and the adsorption of dyes on activated carbon present a low performance for dye removal from solutions. The synergistic effects of AC and H_2O_2 were observed because of the catalytic performance of AC for decomposition of hydrogen peroxide into hydroxyl radicals. The results showed that the kinetics of dye oxidation at different operational parameters followed second order kinetic model. This means that dye oxidation depends on both hydrogen peroxide and AC. It can be concluded that the AC/H_2O_2 could be used as an eco-friendly material to remove dyes from colored wastewater.

The oxidation with hydrogen peroxide in the absence of Activated Carbon and adsorption of dyes on Activated Carbon present a low performance for dye removal from solution. The synergistic effects of the activated carbon and hydrogen peroxide were observed, because of the catalytic performance of the activated carbon for decomposition of the hydrogen peroxide into hydroxyl radicals. The result showed that the kinetics of the dye oxidation, at different operational parameters followed second order kinetic model; this means that dye oxidation depends on both hydrogen peroxide and activated charcoal or carbon. It can be concluded that the activated carbon and hydrogen peroxide could be used as an eco-friendly material to remove dyes from colored wastewater.

So, this whole experiment actually, concludes one thing that, if we use only activated charcoal, it is not enough; if we use only hydrogen peroxide, it will not react very in a very fissile manner with the dye. So, the activated carbon provides and absorb surface, and then this hydrogen peroxide then gets reductively cleaved into hydroxyl radicals, and then the hydroxyl radicals then give out oxygen, which is nascent oxygen. And hence the nascent oxygen is very, very reactive species to degrade or fade or you know, remove the coloration of the dye.

So, that is the main crux, if we use either of them it is not a good solution; but when both are used in conjunction, the synergistic effects is very pronounced, and it can be safely used as a eco friendly method for removal of the color from the colored wastewater.

Then there are other materials also, I am just trying to give you overview, because it is important to understand that there is not just one single oxidative method, but there are other methods of oxidation of these colored dyes as well. Nanoceria is a nanoparticulate oxidizing agent in the oxidation of organic dyes. This is a very recent trend, and therefore I thought, I should introduce this Nanoceria to you.

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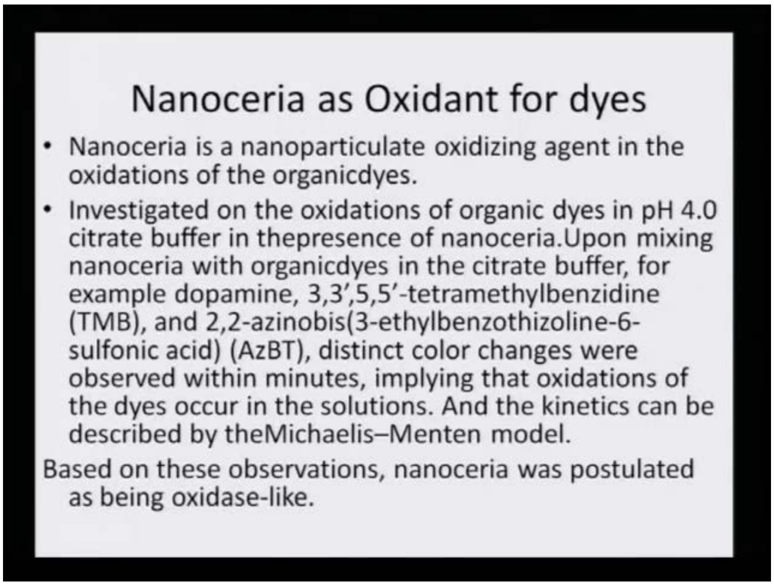

Nanoceria as Oxidant for dyes

- Nanoceria is a nanoparticulate oxidizing agent in the oxidations of the organicdyes.
- Investigated on the oxidations of organic dyes in pH 4.0 citrate buffer in thepresence of nanoceria.Upon mixing nanoceria with organicdyes in the citrate buffer, for example dopamine, 3,3′,5,5′-tetramethylbenzidine (TMB), and 2,2-azinobis(3-ethylbenzothizoline-6-sulfonic acid) (AzBT), distinct color changes were observed within minutes, implying that oxidations of the dyes occur in the solutions. And the kinetics can be described by theMichaelis–Menten model.

Based on these observations, nanoceria was postulated as being oxidase-like.

Investigated on the oxidation of organic dyes in pH 4 citrate buffer in the presence of Nanoceria; upon mixing Nanoceria with organic dyes in the citrate buffer for example, dopamine which is 3,3 pime, 5,5 prime tetramethylbenzidine TMB, and 2, 2 - dye azinobis 3-ethylbenzothizoline 6- sulfonic acid, that is Az BT distinct color changes were observed within minutes, implying that oxidations of the dyes occur in the solutions, and the kinetics can be described by the Michaelis-Menten model.

Based on these observations, Nanoceria was postulated as being oxidased like. So, it was so faster reaction that within minutes, the decolonization of the dye took place; whereas, you saw in the previous case, where activated carbon and hydrogen peroxide were used; it took more than an hour for decolonization. So, Nanoceria definitely has an edge over and it can be evaluated or compared as one of the methods, which is must faster and acts like the enzymes, and enzymes which are of the category of oxidizes.

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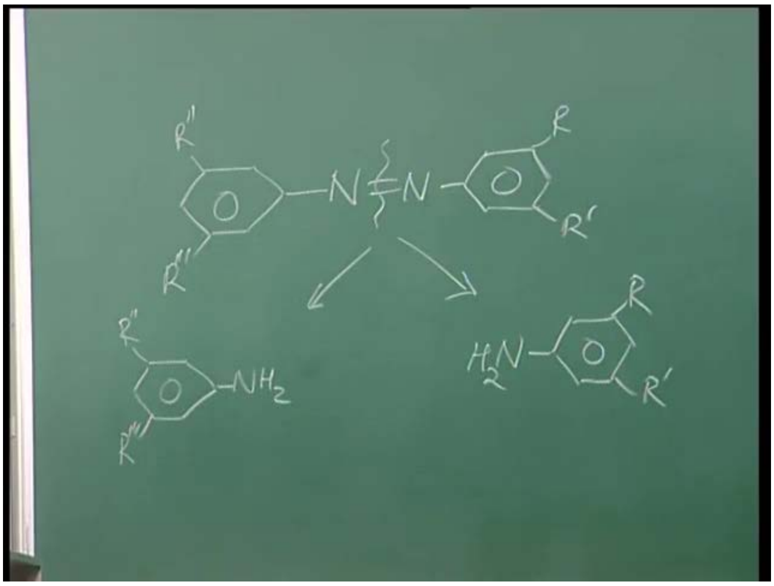

Yesterday, we saw that this molecule was actually broken down by the azo reductase, but today, we will learn something about comparing it with oxidizes. Now, definitely the series of enzymes, which are called azo reductase; and the series of enzymes, which come under the category of oxidizes are not the same. As the name suggest, they are two different categories; but nevertheless these enzymatic reactions are very, very fast as compared to the normal chemical reaction.

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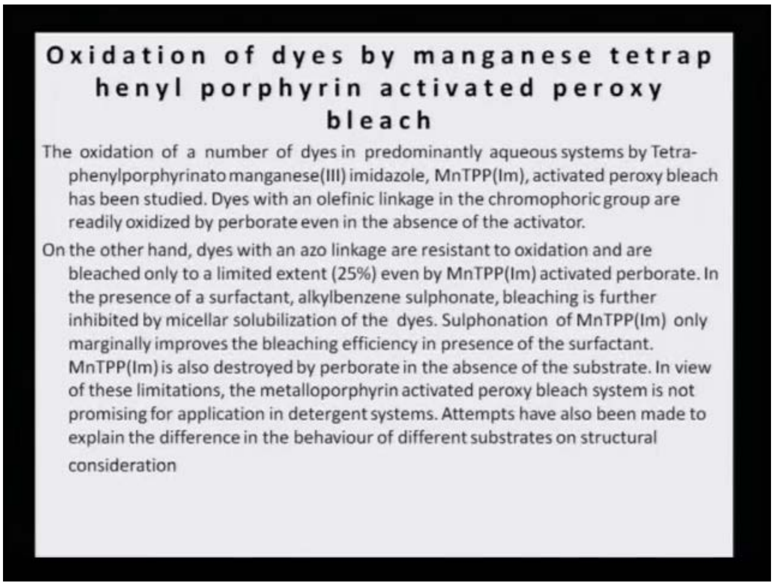

Oxidation of dyes by manganese tetraphenyl porphyrin activated peroxy bleach

The oxidation of a number of dyes in predominantly aqueous systems by Tetra-phenylporphyrinato manganese(III) imidazole, MnTPP(Im), activated peroxy bleach has been studied. Dyes with an olefinic linkage in the chromophoric group are readily oxidized by perborate even in the absence of the activator.

On the other hand, dyes with an azo linkage are resistant to oxidation and are bleached only to a limited extent (25%) even by MnTPP(Im) activated perborate. In the presence of a surfactant, alkylbenzene sulphonate, bleaching is further inhibited by micellar solubilization of the dyes. Sulphonation of MnTPP(Im) only marginally improves the bleaching efficiency in presence of the surfactant. MnTPP(Im) is also destroyed by perborate in the absence of the substrate. In view of these limitations, the metalloporphyrin activated peroxy bleach system is not promising for application in detergent systems. Attempts have also been made to explain the difference in the behaviour of different substrates on structural consideration

Oxidation of dyes by manganese tetraphenyl porphyrin activated peroxy bleach: Now there are many, many examples; I have just taken a few of them, to make you aware that how the oxidation of the colored dye molecules is carried out in order to degrade these synthetic dyes. The oxidation of number of dyes in predominantly aqua system, by tetraphenylporphyrinato, manganese III imidazole, that is MnTPP Im, activated peroxy bleach has been studied, dyes with an olefinic linkage in the chromophoric group are readily oxidized by perborates, even in the absence of the activator. On the other hand dyes with azo linkages are resistant to oxidation, and are bleached only by a limited extent, to a limited extent even by MnTPP Im activated perborate.

In the presence of the surfactant, Alkylbenzene sulfonate, bleaching is further inhibited by Micellar solubilization of the dyes; sulphonation on MnTPP Im only marginally improves the bleaching efficiency in the presence of surfactant. MnTPP im is also destroyed by perborate in the absence of the substrate; in view of these limitations, the metaloporphyrins activated peroxy bleach system is not a very promising application in detergent system. Attempts have been made to explain the difference in behavior of different substrates on structural consideration.

So, you see that there people came up, scientist came up with the new bleaching agent, which was manganese tetra phenyl porphyrin; and with the perforate linkage, it was found to be quite reactive towards the dye; but the hindrance came when there were surfactants like alkylebenzene sulfonate, it would not bleach as efficiently as what it would do to the dye itself; and the perforate activator actually if there was no dye, it would destroy the metaloporphyrin.

So, there were many, many hindrances, but nevertheless you know, this was one of the promising bleaching agent or degrading oxidative agent in the series of synthetic dye degradation products. So, with these we have now come to an understanding that these compounds are degraded, there are several such things available; but the importance of these are still not fully understood, and it is very hard to say, which one is the best? There are several chemical moieties, which can degrade synthetic dyes; but the most important thing is to see, which one works best for which dye? So, with this we have come to an end of the chapter called oxidation of colors; and now we go on to understand the fundamentals of evaluation of dyestuff by analytical technique.

It is quite understandable that if there is a dye, there has to be some analytical tool to identify, which kind of information can be obtained to be able to a certain the type of dye. And therefore, there was a necessity to find out, how can we actually make use of these analytical chemistry machines, which can help us, because after all that there are synthetic dyes or natural dyes, they all have one thing in common and that is that they are chemical substances; and therefore, all the chemical analytical tools must be able to give some kind of information about these dye moieties, which are nothing but organic molecules.

So, therefore, we will now start understanding the fundamentals of evaluation of dyestuff by analytical techniques, which is made a mention that in the previous lecture that the lambda max of the AR dye, the DR dye and the RR dye were different and therefore, they were evaluated; and when the degradation with the activated carbon and hydrogen peroxide was carried out for these three dyes the way to study whether the degradation actually proceeded or not or whether it is ended or not after one year, after one hour was a certain by the help of UV visible spectrophotometer. So, at least you know that there is one instrument which is already mentioned to you which is used in the dyestuff evaluate on.

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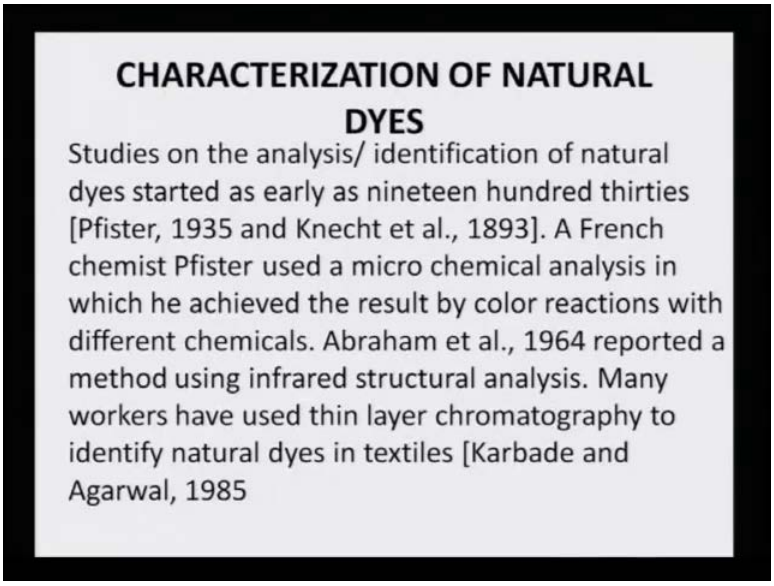

CHARACTERIZATION OF NATURAL DYES

Studies on the analysis/ identification of natural dyes started as early as nineteen hundred thirties [Pfister, 1935 and Knecht et al., 1893]. A French chemist Pfister used a micro chemical analysis in which he achieved the result by color reactions with different chemicals. Abraham et al., 1964 reported a method using infrared structural analysis. Many workers have used thin layer chromatography to identify natural dyes in textiles [Karbade and Agarwal, 1985

Characterization of any dyes, whether it is natural dye or synthetic dye, but we will take an example of natural dye, because all a long, now we will study more and more about natural dyes. Studies on the analysis identification of natural dyes started as early as 1930s that a French chemist Pfister used a micro chemical analysis, in which he achieved the result by color reactions with different chemicals.

Abraham et al in 1964 reported a method using infrared structural analysis, many workers have used thin layer chromatography to identify natural dyes in textiles. So, at least you do not know that there are methods such as you know, micro chemical analysis method or infrared method or thin layer method. So, at least these names have now come newly to you, but at least we will now, when we talk in detail about it, you will be able to appreciate these processes in a better manner.

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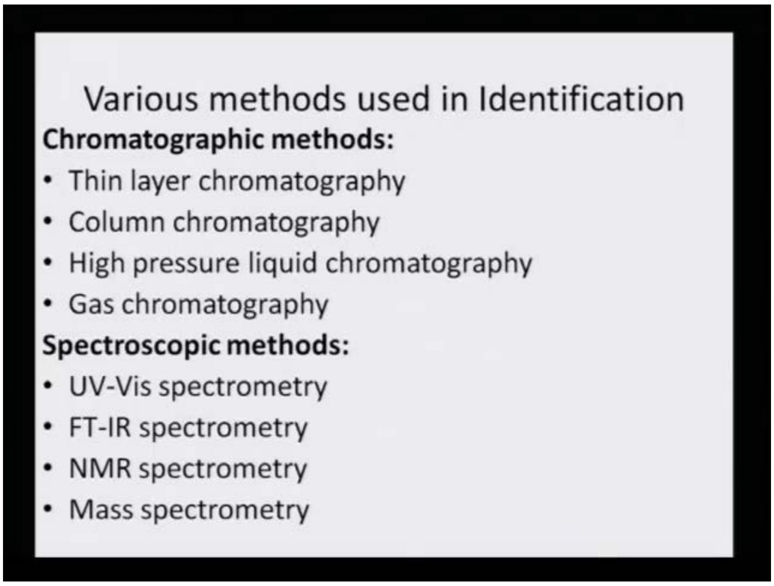

Various methods used in Identification

Chromatographic methods:

- Thin layer chromatography
- Column chromatography
- High pressure liquid chromatography
- Gas chromatography

Spectroscopic methods:

- UV-Vis spectrometry
- FT-IR spectrometry
- NMR spectrometry
- Mass spectrometry

The Various methods used in identification are under the category of Chromatographic methods, thin layer chromatography, column chromatography, high pressure liquid chromatography, gas chromatography. So, 4 types of chromatographic techniques have been used for the identification. Similarly, there are 4 spectroscopic methods popularly used; one is UV visible spectrometry, FT-IR spectrometry, NMR spectrometry and Mass spectrometry. So, these are the 4 spectroscopic methods of identification of dyes, be it natural dye or be its synthetic dye

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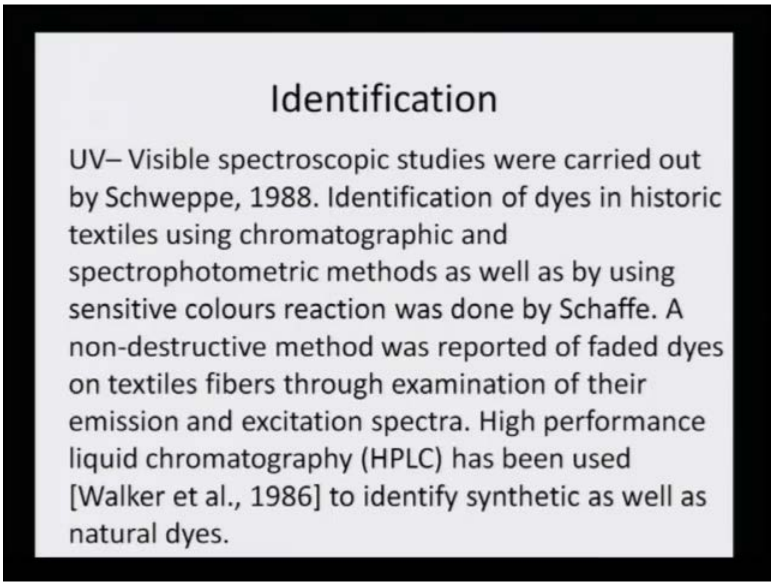

Identification

UV– Visible spectroscopic studies were carried out by Schweppe, 1988. Identification of dyes in historic textiles using chromatographic and spectrophotometric methods as well as by using sensitive colours reaction was done by Schaffe. A non-destructive method was reported of faded dyes on textiles fibers through examination of their emission and excitation spectra. High performance liquid chromatography (HPLC) has been used [Walker et al., 1986] to identify synthetic as well as natural dyes.

The way identification is done is UV visible spectroscopic studies were carried out first by Schweppes in 1988, identification of dyes in historic textiles using chromatographic and spectrometric methods. As well as by using sensitive colors reactions were done by Schaffer. A non-destructive method was reported of faded dyes on textile fibers through examination of their emission, and excitation spectra. High performance liquid chromatography that is HPLC has been used by walker et al in 18 in 1986 to identify synthetic as well as natural dyes. So, it is not just a method which is meant for synthetic dye alone, but it is also a method which is valid for natural dyes and vice a versa. Because both are chemical entities.

And if they are organic chemicals, they need to be either a identified by spectroscopic methods and or by the means of chromatographic methods. And the 4 chromatographic methods that are used are TLC column, chromatography, HPLC and GC and the 4 spectroscopic methods that are popularly used are UV vis, FT- IR, NM R and mass. Then, another studies that prompted to show whether a it was a dye was through the ability to study the solubility.

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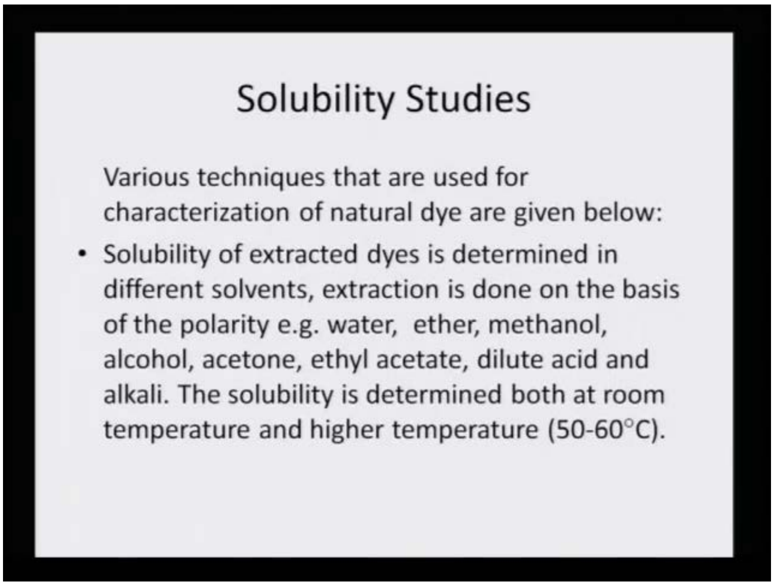

Solubility Studies

Various techniques that are used for characterization of natural dye are given below:

- Solubility of extracted dyes is determined in different solvents, extraction is done on the basis of the polarity e.g. water, ether, methanol, alcohol, acetone, ethyl acetate, dilute acid and alkali. The solubility is determined both at room temperature and higher temperature (50-60°C).

Various techniques that are used for characterization of natural dyes are mentioned below; one of them is Solubility of extracted dye is determined in different solvents. Extraction is done on the basis of the polarity that is an water, ether, methanol, alcohol, acetone, ethyl acetone, dilute Acid and alkali. The solubility is determined both at room temperature and higher temperature. So, that is one method of finding out, whether a dye is soluble, water soluble or solvent soluble, because some of the very very strict aromatic dyes which are colored compound may or may not have these oxochromic groups of OH, and NH to which does not make them a water soluble substance.

Therefore, it is important that one should know the solubility of these dyes and therefore, you know, a whole array of solvents like water, ether, methanol, alcohol that is ethyl alcohol, acetone, ethyl acetate, dilute acids, and alkalis all have been used subsequently, not in one group. A dye is taken, it is first dissolved in water; then kept aside. Then again another sample of the same dye is taken and ether is added. So, that gives an idea what is the kind of solubility of this colored moiety.

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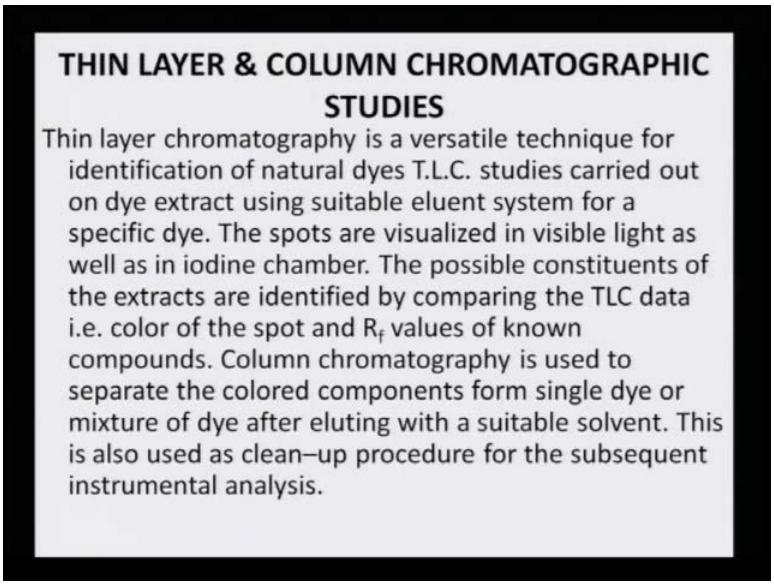

THIN LAYER & COLUMN CHROMATOGRAPHIC STUDIES

Thin layer chromatography is a versatile technique for identification of natural dyes T.L.C. studies carried out on dye extract using suitable eluent system for a specific dye. The spots are visualized in visible light as well as in iodine chamber. The possible constituents of the extracts are identified by comparing the TLC data i.e. color of the spot and R_f values of known compounds. Column chromatography is used to separate the colored components form single dye or mixture of dye after eluting with a suitable solvent. This is also used as clean–up procedure for the subsequent instrumental analysis.

Thin layer and column chromatographic studies; thin layer chromatography is a versatile technique for identification of natural dyes. It is popularly known as TLC. The TLC studies carried out on dye extract using suitable eluent system for a specific dye. The spots are visualized in visible light, as well as in iodine chamber. The possible constituents of the extracts are identified by comparing the TLC data that is the color of the spot, and R f value of known compounds.

Column chromatography is used to separate the colored compounds from single dye or mixture of dyes, after eluting with a suitable solvent. This is also used as clean-up procedure for subsequent instrumental analysis. So, the first and the primary analysis that one does after understanding the solubility, is to evaluate the thin layer chromatography. The TLC is carried out on small glass plates which are coated with silica mainly, sometimes with alumina; and these are very nicely you know coated. So that, the coating is very fine and thin on the glass plate; and then when the compound or the dye is spotted, and it is allowed to elute in a solvent system; thus spot start moving. And when the spot start moving, they because of the difference in polarity, this showed different spots on the plate.

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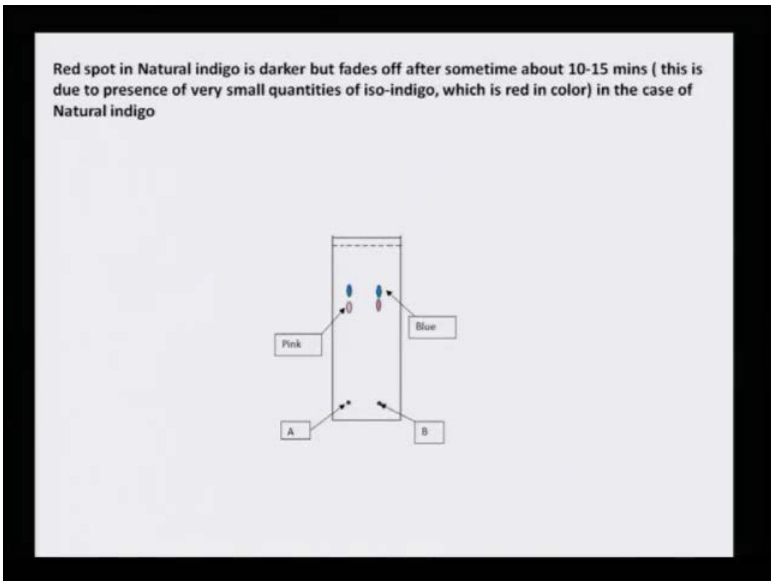

Red spot in Natural indigo is darker but fades off after sometime about 10-15 mins (this is due to presence of very small quantities of iso-indigo, which is red in color) in the case of Natural indigo

Similarly, the magnified version of a TLC is a column chromatography, we will come to that a little later. Now, if I have to show you, you know TLC, this is how it looks like; indigo red spot in the natural indigo is darker, but fades off after sometime about 10 to 15 minutes. This is due to the presence of very small quantities of is indigo which is red in color. And in the case of natural indigo only it will be present. So, suppose yesterday, we were talking about you know the the demand being met by this synthetic d indigo; and natural indigo is not sufficient to we catering to the textile industry.

So, how do we identify? It is very easy to identify, whether it is a sample of synthetic indigo or whether it is a sample of natural indigo; why because natural indigo will have a reddish spot below the blue spot, and that is only possible in the natural isomer, because iso indigo comes hand in hand with natural indigo, but when it is prepared in the laboratory, when we make samples of synthetic indigo, it does not form the red colored compound; but the tendency for the red color compound to retain on the slide is very for a short while, it is only there for 10 to 15 minutes, and then it fades off. So, one should be very careful in looking at the TLC, and then evaluating whether it is a sample of natural indigo or synthetic indigo. So, this is how TLC is used.