Natural dyes

Prof. Padma Vankar
Department of Chemistry
Indian Institute of Technology, Kanpur

Lecture No. # 02

The second lecture dealing with dyes and dyeing is related to light color and different dye stuffs. We will take an over view of what the light can do in order to produce color and what are the different types of dye stuff, both natural and synthetic structurally, because we will now try to get in to a bit of chemistry. We took an over view of the history of dye stuff and now we try to look at the chemistry.

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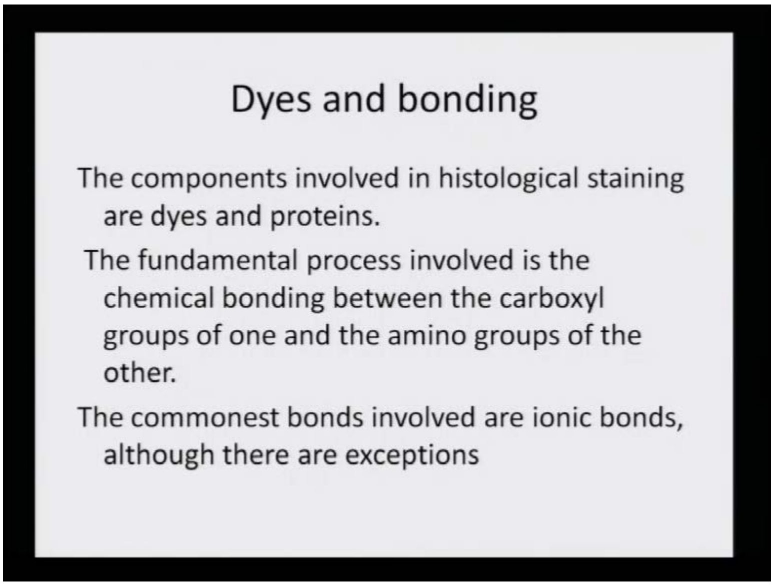

Dyes and bonding

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The commonest bonds involved are ionic bonds, although there are exceptions

Dyes and bonding: The components involved in histological staining are dyes and proteins. If we just take a simple case of you know doing blood test or some kind of a color difference there has to be some source of protein and some color. The fundamental process involved is the carbon is the chemical bonding between the carboxylic group of one and the amino groups of the other. The commonest bonds involved are ionic bonds, although there are exceptions. So, you see that dyes when they adhere to any material be it protein material that is wool or silk, be it cellulosic material which is cotton, it is always that there is some kind of linkage or bonding occurring between the dyes and the material, and it is through this bonding and the most common type of bonding that one comes across is the ionic types, but there are other types of bonding to between dyes and the material.

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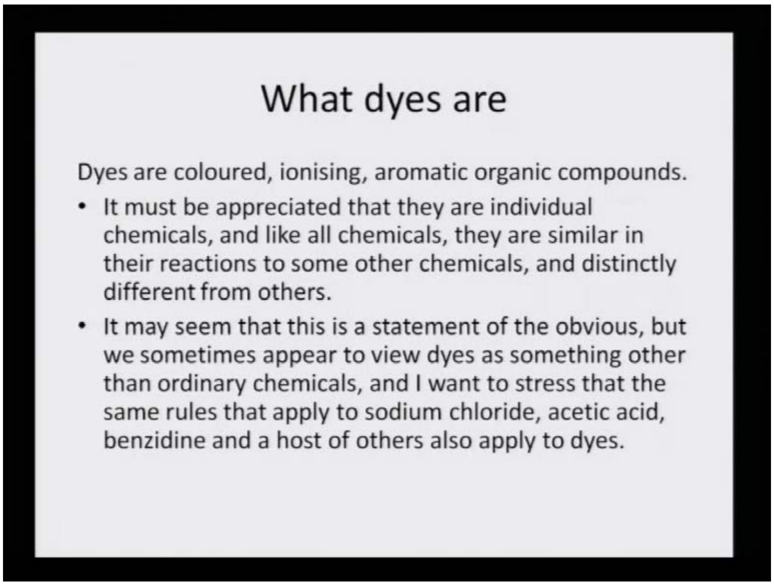

What dyes are

Dyes are coloured, ionising, aromatic organic compounds.

- It must be appreciated that they are individual chemicals, and like all chemicals, they are similar in their reactions to some other chemicals, and distinctly different from others.
- It may seem that this is a statement of the obvious, but we sometimes appear to view dyes as something other than ordinary chemicals, and I want to stress that the same rules that apply to sodium chloride, acetic acid, benzidine and a host of others also apply to dyes.

What are dyes then? Dyes are colored, ionizing, aromatic organic compounds. It must be appreciated that they are individual chemicals, and like all chemicals, they are similar in their reactions to some other chemicals, and distinctly different from others. So, dyes are nothing whether they are synthetic dyes or natural dyes, they have these salient features in common that these are colored compound, and they may or may not be completely aromatic, but they do have a lot of conjugation. That means they have in their structural detailing a lot of alpha-beta unsaturated carbon systems in the entire molecular structure, and that they are like any other chemicals.

So, basically a dye is a chemical, whether it is a natural dye or a synthetic dye it remains the same. It may seem that this statement of the obvious, but we sometimes appear to view dyes as something other than ordinary chemicals, and I want to stress that the same rules that apply to sodium chloride, acetic acid, benzidine and a host of others also applies to dyes. So, if we talk in terms of dyes as being chemicals, and I said dyes are all could be synthetic dye or chemical dye, they will all have a chemical structure. They can be aromatic, they can be non-aromatic, but all the rules that apply to any chemical will also apply to these dyes.

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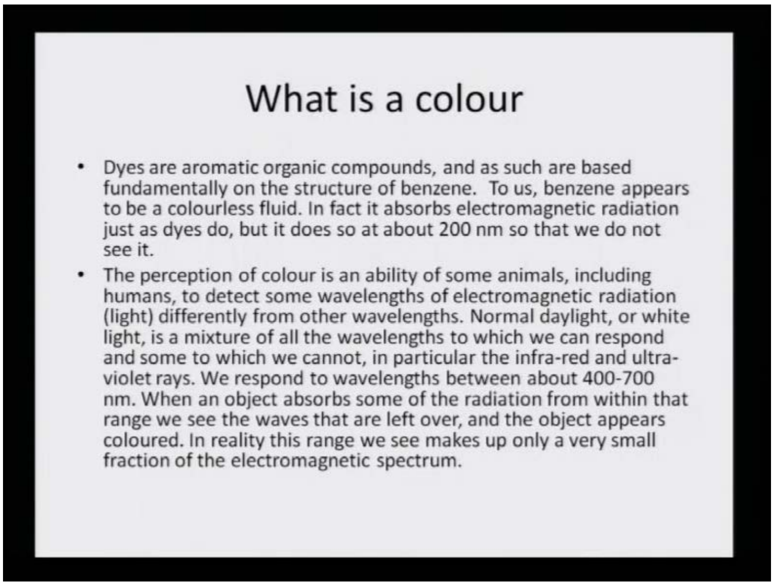

What is a colour

- Dyes are aromatic organic compounds, and as such are based fundamentally on the structure of benzene. To us, benzene appears to be a colourless fluid. In fact it absorbs electromagnetic radiation just as dyes do, but it does so at about 200 nm so that we do not see it.
- The perception of colour is an ability of some animals, including humans, to detect some wavelengths of electromagnetic radiation (light) differently from other wavelengths. Normal daylight, or white light, is a mixture of all the wavelengths to which we can respond and some to which we cannot, in particular the infra-red and ultra-violet rays. We respond to wavelengths between about 400-700 nm. When an object absorbs some of the radiation from within that range we see the waves that are left over, and the object appears coloured. In reality this range we see makes up only a very small fraction of the electromagnetic spectrum.

What is the color? When we try to understand that dyes impart color, we should also understand what is the physics of this color. Dyes are aromatic organic compounds, and as such are based fundamentally on the structure of benzene. To us, benzene appears to be colorless fluid. In fact it absorbs electromagnetic radiation just as dyes do, but it does so at about 200 nanometer, so that we do not see it. Now, let us try to understand between colorless and colored compound, what both are chemicals, both have some structure. Now, this structure what makes a compound colorless and a compound colored would depend on one very basic factor that any compound which on UV spectrometry shows our lambda max below 200 or rather below 400 would all be colorless. And a compound which shows a chemical compound which shows a lambda max in UV spectrometry up between 400 to 700 nanometers will be colored. So that means that they have to be structurally different to be able to absorb UV light in different regions.

The perception of color is an ability of some animals, including humans, to detect some wave lengths of electromagnetic radiation that is the light differently from other wavelengths. Normal daylight or white light is a mixture of all the wave lengths to which we can respond and some of which we cannot, in particular the infrared and the ultra-violet rays. We respond to wavelengths between about 400 to 700 nanometer. When an object absorbs some of the radiation from within that range we see the waves that are left over, and the object appears colored. In reality this range we see makes up only a very small fraction of electromagnetic spectrum. So, we come to a conclusion that colors can be observed only by the visible region of the electromagnetic radiation. We have to also understand that the electromagnetic radiation varies from ultra-violet to UV to IR and so on. And when and in between the UV and the IR we have a small visible region. Now, this visible region is what is generating the perception of color.

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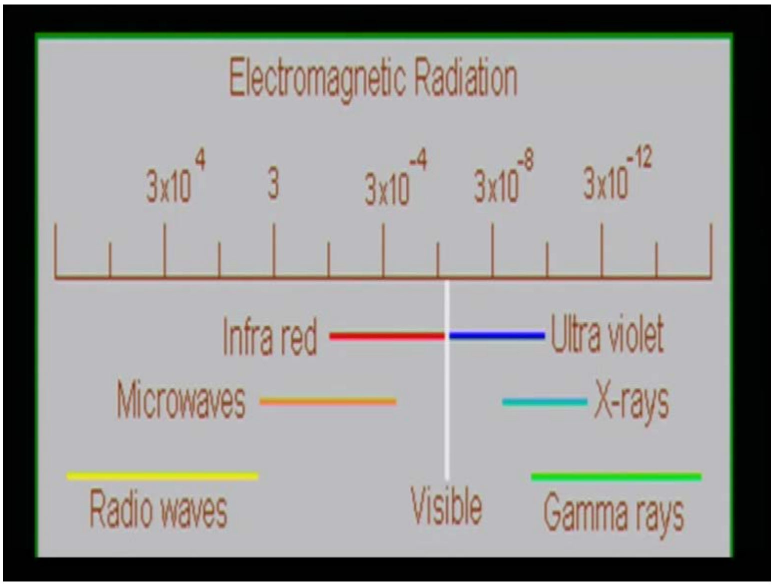

Electromagnetic Radiation

This is how I would like to depict my statement by this light where electromagnetic radiation and there you know frequencies have been mentioned how between the infra red and the ultra violet ray. A very small region belongs to visible region, beyond infra red is the microwaves, and beyond microwaves is the radio waves, and beyond ultra violet is the X-rays, and beyond X-rays is the gamma rays. So, this is the kind of entire range of electromagnetic spectrum. And in between that lies the visible region which causes color perception.

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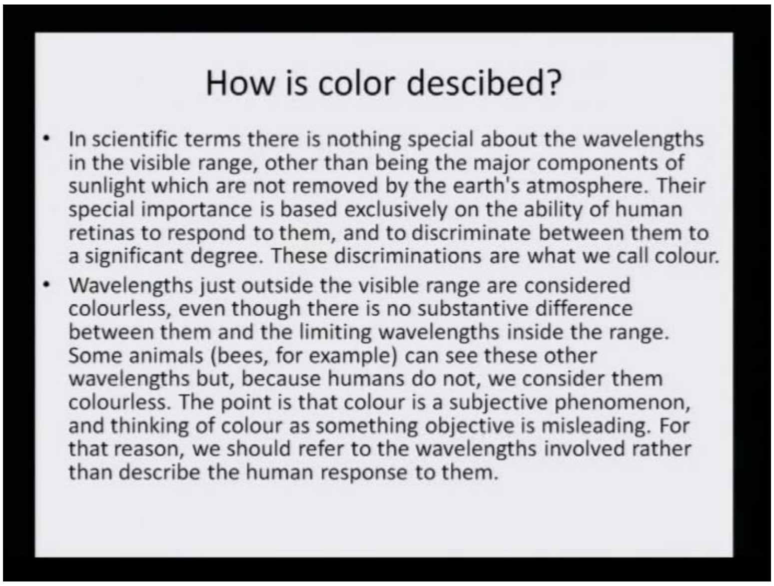

How is color descibed?

- In scientific terms there is nothing special about the wavelengths in the visible range, other than being the major components of sunlight which are not removed by the earth's atmosphere. Their special importance is based exclusively on the ability of human retinas to respond to them, and to discriminate between them to a significant degree. These discriminations are what we call colour.
- Wavelengths just outside the visible range are considered colourless, even though there is no substantive difference between them and the limiting wavelengths inside the range. Some animals (bees, for example) can see these other wavelengths but, because humans do not, we consider them colourless. The point is that colour is a subjective phenomenon, and thinking of colour as something objective is misleading. For that reason, we should refer to the wavelengths involved rather than describe the human response to them.

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Some animals can see these other wavelengths but, because humans do not, we consider them colorless. The point is that color is a subjective phenomenon, and thinking of color is something objective is misleading. For that reason, we should refer to the wavelengths involved rather than describe the human response to them. So, what it is a basic fundamental of observation of a particular wavelength. It has nothing to do with what we are thinking. We cannot think that this color is like that and so the color becomes like that. Actually, what is the wave length that is involved is what creates the color.

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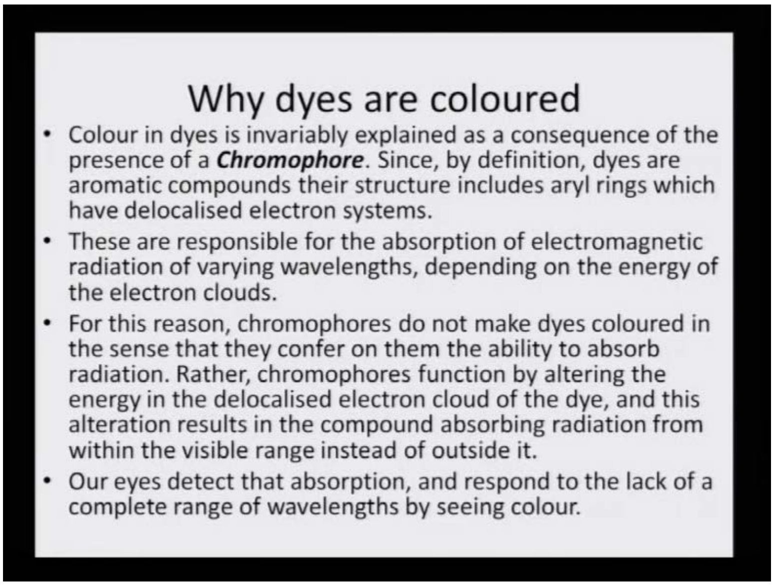

Why dyes are coloured

- Colour in dyes is invariably explained as a consequence of the presence of a ***Chromophore***. Since, by definition, dyes are aromatic compounds their structure includes aryl rings which have delocalised electron systems.
- These are responsible for the absorption of electromagnetic radiation of varying wavelengths, depending on the energy of the electron clouds.
- For this reason, chromophores do not make dyes coloured in the sense that they confer on them the ability to absorb radiation. Rather, chromophores function by altering the energy in the delocalised electron cloud of the dye, and this alteration results in the compound absorbing radiation from within the visible range instead of outside it.
- Our eyes detect that absorption, and respond to the lack of a complete range of wavelengths by seeing colour.

Why dyes are colored? Now, let us we have understood that there is a color perception by the retina and the range is 400 to 700 nanometers. But how does this color get related to dyes? Color in dyes is invariably explained as a consequence of the presence of chromophore. Just a while ago I told that dyes are chemicals which have a structure, and the structure must have a lot of conjugation or alpha-beta unsaturation and that alpha-beta unstauration or conjugation is what causes the absorption of wavelength in this visible region and that is what is called a chromophrore. So, it must be very, very clear that these terminologies should be clear to the students before we proceed. Since, by definition, dyes are aromatic compounds their structure includes aryl rings which have delocalized electron system. They are responsible for the absorption of electromagnetic radiation of varying wavelength depending on the energy of the electron clouds.

For this reason, chromophores do not make dyes colored in the sense that they confer to them the ability to absorb radiation. Rather, chromophore function by altering the energy in the delocalized electron cloud of the dye, and this alteration results in the compound absorbing radiation from within the visible region instead of outside it. So, what makes a chromophore absorb light is because of the delocalization of the electrons and that is what causes the it to fall within the visible region of the electromagnetic spectrum. Our eyes detect that absorption, and correspond to the lack of complete range of wavelengths seeing the color.

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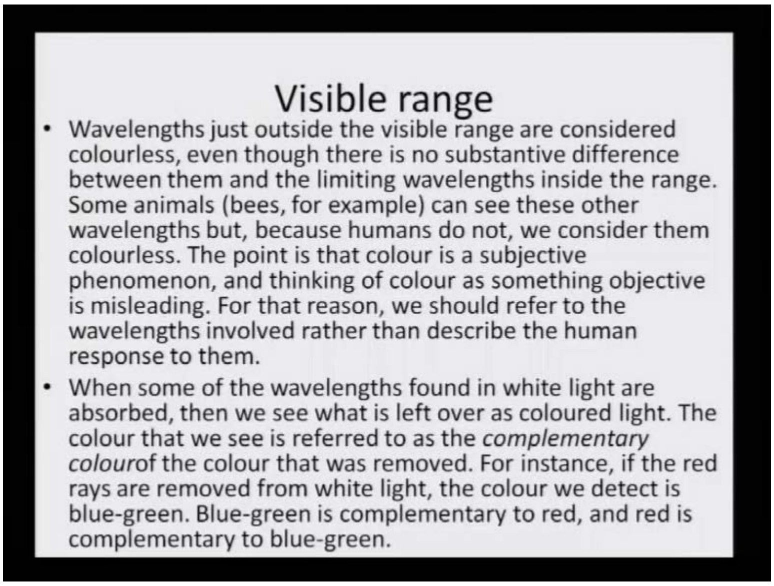

Visible range

- Wavelengths just outside the visible range are considered colourless, even though there is no substantive difference between them and the limiting wavelengths inside the range. Some animals (bees, for example) can see these other wavelengths but, because humans do not, we consider them colourless. The point is that colour is a subjective phenomenon, and thinking of colour as something objective is misleading. For that reason, we should refer to the wavelengths involved rather than describe the human response to them.
- When some of the wavelengths found in white light are absorbed, then we see what is left over as coloured light. The colour that we see is referred to as the *complementary colour*of the colour that was removed. For instance, if the red rays are removed from white light, the colour we detect is blue-green. Blue-green is complementary to red, and red is complementary to blue-green.

Visible range: Wavelengths just outside the visible range are considered colorless, even though there is no substantive difference between them and in limiting wavelengths inside the range. Some animals and bees can absorb them, but for us it is colorless. The point is that the color is a subjective phenomenon. When some of the wavelengths found in white light are absorbed, then we see what is left over as colored light. The color that we see is referred to as complimentary color of the color that was removed. So, let me tell you that what we observe is actually the complimentary color that means the color that was absorbed has been absorbed and whatever is transmitted is what we see. So, the transmitted color is the complimentary color.

For instance, if the red rays are removed from white light, the color we detect is blue-green. Blue-green is complimentary to red, and red is complimentary to blue-green. So, depending on what we are observing the opposite would have been absorbed.

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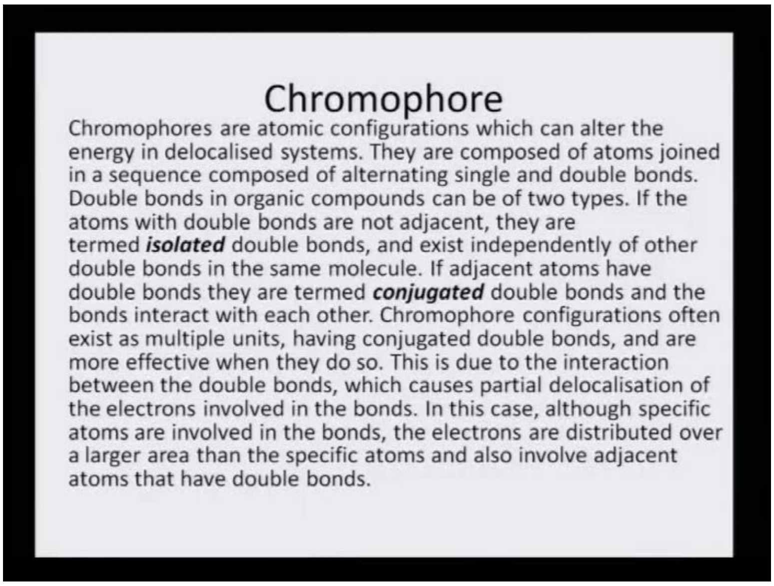

Chromophore

Chromophores are atomic configurations which can alter the energy in delocalised systems. They are composed of atoms joined in a sequence composed of alternating single and double bonds. Double bonds in organic compounds can be of two types. If the atoms with double bonds are not adjacent, they are termed ***isolated*** double bonds, and exist independently of other double bonds in the same molecule. If adjacent atoms have double bonds they are termed ***conjugated*** double bonds and the bonds interact with each other. Chromophore configurations often exist as multiple units, having conjugated double bonds, and are more effective when they do so. This is due to the interaction between the double bonds, which causes partial delocalisation of the electrons involved in the bonds. In this case, although specific atoms are involved in the bonds, the electrons are distributed over a larger area than the specific atoms and also involve adjacent atoms that have double bonds.

Now let us try to understand what a chromophore is. Chromophores are atomic configurations which can alter energy in delocalized system. They are composed of atoms joined in a sequence composed of alternating single and double bonds. Double bond in organic compounds can be of two types. If the atoms with two double bonds are not adjacent, they are termed as isolated double bonds and exist independently of double bonds in the same molecule. If the adjacent atoms have double bonds they are termed as conjugated double bonds and the bonds interact with each other. Chromophore configuration often exits as multiple unit, having conjugated double bonds, and are more effective when they do so. So, it is possible that the bonds can be for away double bonds or they could be simply altered by a single bond. So, in the earlier case we will say it is isolated double bonds into ends of the compound or if they are alternating with single bond double bond, single bond double bond, we will say it is a conjugated system.

So, now it is clear what is isolated and what is conjugated. It is the conjugated double bond system which creates a chromophore, and chromophore is what creates the excitation and absorbs the in the visible region. This is due to the interaction between the double bonds which cause partial delocalization of the electrons involved in the bond. In this case, although specific atoms are involved in the bonds, the electrons are distributed over a larger area than the specific atoms and also involved adjacent atoms that have double bond. So that is why there is a kind of a delocalization of the electrons.

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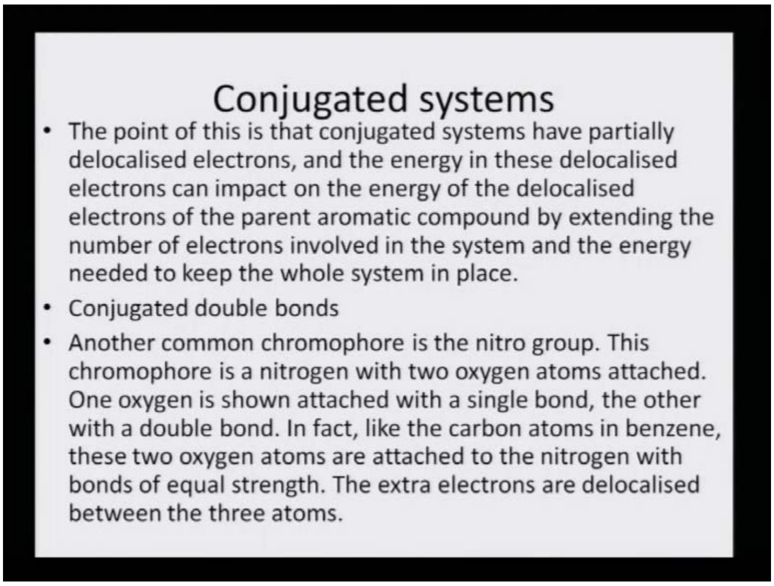

Conjugated systems

- The point of this is that conjugated systems have partially delocalised electrons, and the energy in these delocalised electrons can impact on the energy of the delocalised electrons of the parent aromatic compound by extending the number of electrons involved in the system and the energy needed to keep the whole system in place.
- Conjugated double bonds
- Another common chromophore is the nitro group. This chromophore is a nitrogen with two oxygen atoms attached. One oxygen is shown attached with a single bond, the other with a double bond. In fact, like the carbon atoms in benzene, these two oxygen atoms are attached to the nitrogen with bonds of equal strength. The extra electrons are delocalised between the three atoms.

Conjugated systems: The point of this is that conjugated systems have partially delocalized electrons, and the energy in these delocalized electrons can impact on the energy of the delocalized electrons of the parent aromatic compound by extending the number of electrons involved in the system, and the energy needed to keep the whole system in place. So, you see the more the conjugation the better it is and more deep would be the color. So, the if the conjugation is outside the aromatic ring it is extended into the aromatic ring. Conjugated double bonds extended double bonds with alternating with single bond double bond single bond create a good amount of conjugated system.

Another common chromophore is the nitro group. This chromophore is a nitrogen with two oxygen atoms attached. One oxygen is shown attached with the single bond and the other with the double bond. In fact, like the carbon atoms in the benzene, these two oxygen atoms are attached to the nitrogen with bonds of equal strength. The extra electrons are delocalized between the three atoms. So, the kind of nitro group or the kind of benzene ring delocalization are a few examples of conjugated chromophores.

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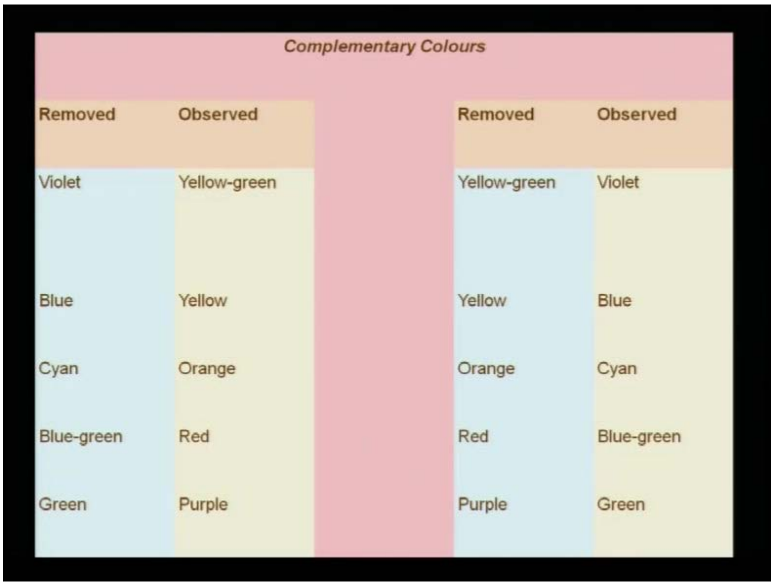

Complementary Colours

Removed	Observed	Removed	Observed
Violet	Yellow-green	Yellow-green	Violet
Blue	Yellow	Yellow	Blue
Cyan	Orange	Orange	Cyan
Blue-green	Red	Red	Blue-green
Green	Purple	Purple	Green

If we try to take a look as what is removed and what is absorbed. You will see that always if violet light is removed or absorbed. The color that would be complimentary that would be reflected would be yellow-green and similarly, if the yellow-green is removed or absorbed, the complimentary color that the eye will observe will be violet. And you can see the whole chart actually whatever is absorb just the opposite end would be the complementary color that would be observed by the eye.

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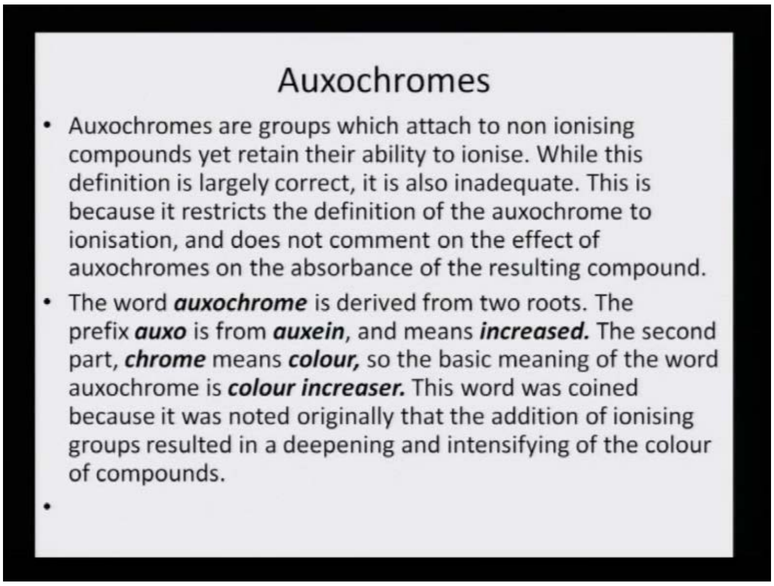

Auxochromes

- Auxochromes are groups which attach to non ionising compounds yet retain their ability to ionise. While this definition is largely correct, it is also inadequate. This is because it restricts the definition of the auxochrome to ionisation, and does not comment on the effect of auxochromes on the absorbance of the resulting compound.
- The word ***auxochrome*** is derived from two roots. The prefix ***auxo*** is from ***auxein***, and means ***increased.*** The second part, ***chrome*** means ***colour,*** so the basic meaning of the word auxochrome is ***colour increaser.*** This word was coined because it was noted originally that the addition of ionising groups resulted in a deepening and intensifying of the colour of compounds.
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Now let us try to understand that there are something called auxochromes also present in the molecule; the dye molecule or the color absorbing molecule. Chromophores are groups which attach to non-ionizing compound yet retain their ability to ionize. While this definition is largely correct, it is also inadequate. This is because it restricts the definition of the auxochrome to ionization, and does not comment on the effect of auxochromes on the absorbance of the resulting compound. It is considered that you know they can ionize, but does ionization really play a role in color production? No. So, this is a not a very appropriate definition for auxochrome. So, what would be the appropriate definition?

The word auxochrome is derived from two roots. The prefix auxo is from the auxein which means increased and the second part chrome which means color. So, the basic meaning of the word auxochrome is color increaser or enhancer. This word was coined because it was noted originally that addition of ionizing group resulting in deepening and intensifying of the color of the compound takes place if certain groups are also present. They themselves may not be chromophore that means they are not conjugated system, but they are helping the color to get deepen. So, they are known as auxochroms. So, it is very distinct now that chromophores absorb light, auxochrome help the chromophores.

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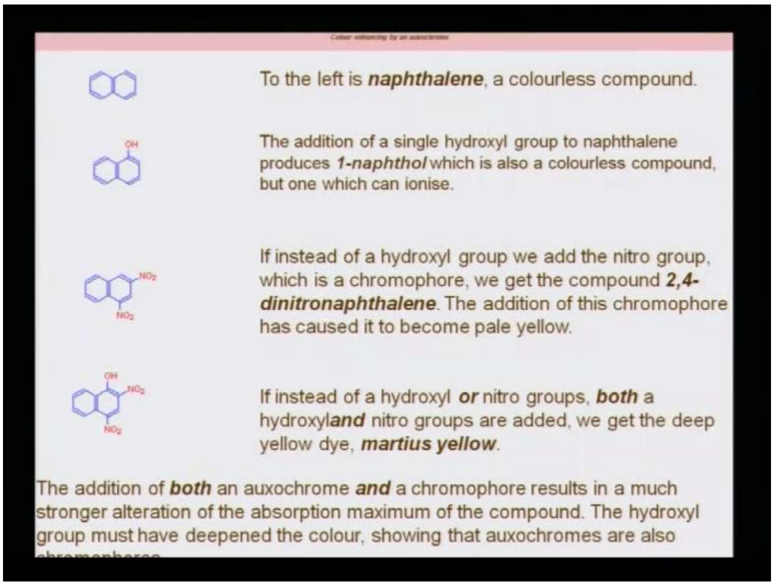

To the left is ***naphthalene***, a colourless compound.

The addition of a single hydroxyl group to naphthalene produces ***1-naphthol*** which is also a colourless compound, but one which can ionise.

If instead of a hydroxyl group we add the nitro group, which is a chromophore, we get the compound ***2,4-dinitronaphthalene***. The addition of this chromophore has caused it to become pale yellow.

If instead of a hydroxyl ***or*** nitro groups, ***both*** a hydroxyl***and*** nitro groups are added, we get the deep yellow dye, ***martius yellow***.

The addition of ***both*** an auxochrome ***and*** a chromophore results in a much stronger alteration of the absorption maximum of the compound. The hydroxyl group must have deepened the colour, showing that auxochromes are also

Now if we try to look at this example which is a naphthol naphthalene which is colorless compound by the near introduction of OH group. The addition of single hydroxyl group in naphthol naphthalene making it an 1-naphthol and the it is also colorless, but it start it can ionize. Similarly, instead of the hydroxyl group if we add nitro group as a chromophore, we get 2, 4-dinitronaphthalene, and the addition of this chromophore has caused the color to become pale yellow. And if we now have 1-hydroxyl and 2-nitro groups, the color becomes deep yellow or martius yellow.

Now, the addition of both an auxochrome and a chromophore results in a much stronger alteration of the absorption maximum of the compound. And the hydroxyl group must have deepened the color, showing that auxochromes are also enhancing the effect of the chromophores. So, you see taking this example was important to make you realize, how the color shifts, because of different functional groups.

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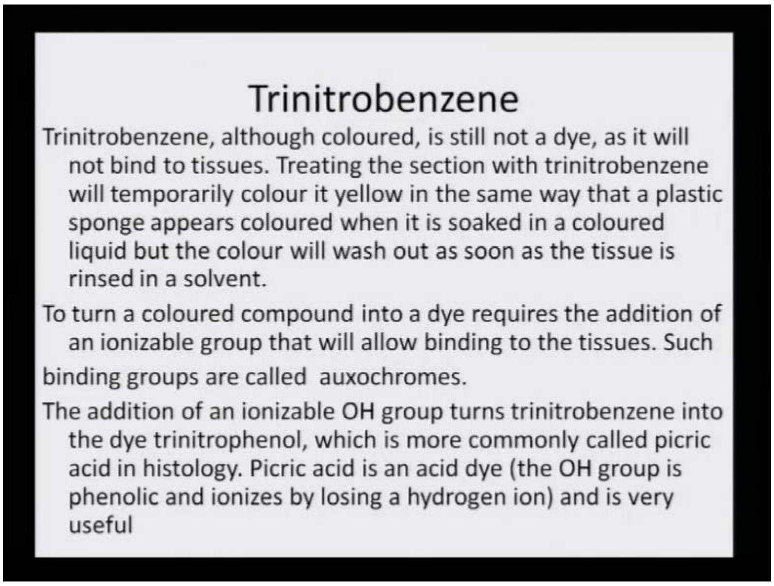

Trinitrobenzene

Trinitrobenzene, although coloured, is still not a dye, as it will not bind to tissues. Treating the section with trinitrobenzene will temporarily colour it yellow in the same way that a plastic sponge appears coloured when it is soaked in a coloured liquid but the colour will wash out as soon as the tissue is rinsed in a solvent.

To turn a coloured compound into a dye requires the addition of an ionizable group that will allow binding to the tissues. Such binding groups are called auxochromes.

The addition of an ionizable OH group turns trinitrobenzene into the dye trinitrophenol, which is more commonly called picric acid in histology. Picric acid is an acid dye (the OH group is phenolic and ionizes by losing a hydrogen ion) and is very useful

Trinitrobenzene, although colored, is still not a dye; it is it will not bind to tissues. Treating the section with trinitrobenzene will temporarily color it yellow in the same way that a plastic sponge appears colored when it is soaked in colored liquid, but the color will wash out as soon as the tissue is rinsed with solvent. To turn a colored compound into a (( )) dye, it requires to add additional ionizable group that will allow binding to the tissues or the material. Such binding roots are called auxochromes. So, you see what makes a chemical a dye. Now, you have understood that it the dye molecule should have a chromophore and an auxochrome. Even then when trinitrobenzene has the required chromophoric groups, it is still not a dye, why because it lacks in the binding property, and auxochrome addition of auxochrome enhances the binding property and makes it from a chemical to a dye. So that is the kind of transformation that one observed.

The addition of an ionizable OH group turns trinitrobenzene into the dye trinitrophenol, which is more commonly called picric acid and is used in histology. Picric acid is an acid dye, the OH group is phenolic and ionizes by losing hydrogen ion and is very useful. So, you see that if picric acid falls on the skin it stains the skin into dark yellow. Now that would not have happen with trinitrobenzene, because the trinitrobenzene did not have this ionizable group which is the ion auxochrome to make that trinitrobenzene chemical into dye, it needed this extra OH, and because of this extra OH the categorization to dye took place.

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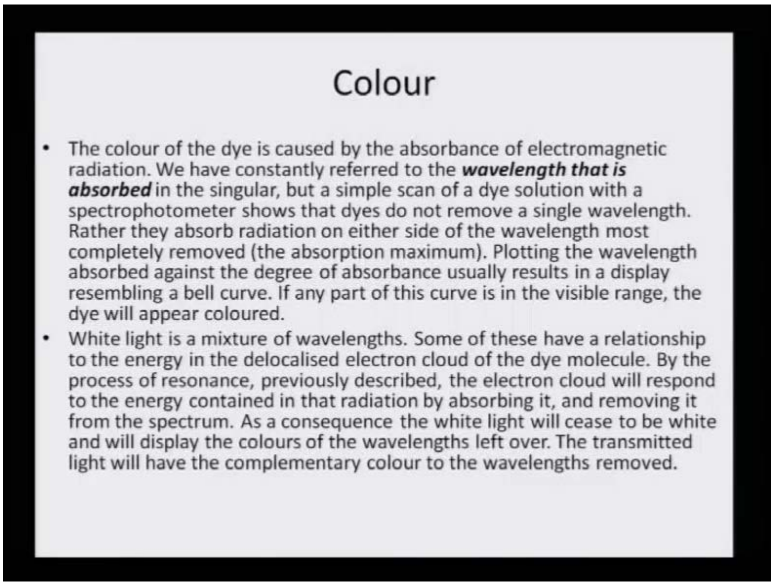

Colour

- The colour of the dye is caused by the absorbance of electromagnetic radiation. We have constantly referred to the ***wavelength that is absorbed*** in the singular, but a simple scan of a dye solution with a spectrophotometer shows that dyes do not remove a single wavelength. Rather they absorb radiation on either side of the wavelength most completely removed (the absorption maximum). Plotting the wavelength absorbed against the degree of absorbance usually results in a display resembling a bell curve. If any part of this curve is in the visible range, the dye will appear coloured.
- White light is a mixture of wavelengths. Some of these have a relationship to the energy in the delocalised electron cloud of the dye molecule. By the process of resonance, previously described, the electron cloud will respond to the energy contained in that radiation by absorbing it, and removing it from the spectrum. As a consequence the white light will cease to be white and will display the colours of the wavelengths left over. The transmitted light will have the complementary colour to the wavelengths removed.

Now, let us try to understand the physics of coloration. The dye or the color of the dye is caused by the absorbance of electromagnetic radiation. We have constantly referred to the wavelength that is absorbed in the singular, but a simple scan of a dye solution with a spectrometer shows that dye do not remove a single wavelength. Rather they absorb radiation on either sides of the wavelength most completely removed the absorption maximum. Plotting the wavelength absorbed against the degree of absorbance usually results in the display resembling a bell curve. It will in any part of that curve in the visible range, the dye will appear color. White light is a mixture of wavelengths. Some of these have a relationship to the energy in the delocalized electron cloud of the dye molecule.

By the processes of resonance, previously described, the electron cloud will respond to the energy contained in that radiation by absorbing it, and removing it from the spectrum. So the light that is absorbed is actually removed from the electromagnetic spectrum, and so the light that is transmitted is actually divide what has been absorbed. As a consequence the white light will cease to be white and will display colors of the wavelength that are left over or that are transmitted. The transmitted light will have the complimentary color to the wavelengths that are removed or absorbed. So, vice versa.

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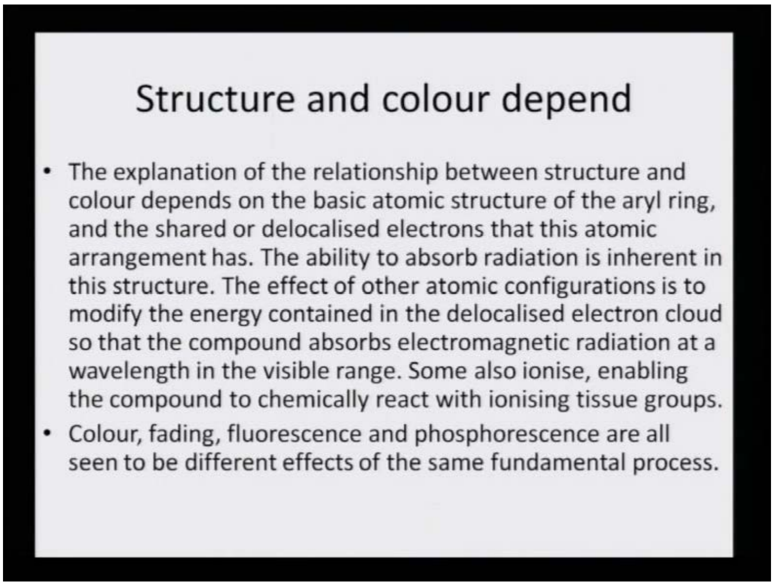

Structure and colour depend

- The explanation of the relationship between structure and colour depends on the basic atomic structure of the aryl ring, and the shared or delocalised electrons that this atomic arrangement has. The ability to absorb radiation is inherent in this structure. The effect of other atomic configurations is to modify the energy contained in the delocalised electron cloud so that the compound absorbs electromagnetic radiation at a wavelength in the visible range. Some also ionise, enabling the compound to chemically react with ionising tissue groups.
- Colour, fading, fluorescence and phosphorescence are all seen to be different effects of the same fundamental process.

Now, the structure and the color are actually related and dependent. The explanation of the relationship between the structure and color depends on the basic atomic structure of the aryl ring or the aromatic ring, and the shared or delocalized electrons that this atomic arrangement has. The ability to absorb radiation is inherent in this structure. The effect of other atomic configuration is to modify the energy contained in the delocalized electron cloud, so that the compound absorbs electromagnetic radiation at a wavelength in the visible region. So, the whole chemistry lies in the fact that there is a benzene ring, there is more delocalization of the electron due to auxochromes, and it is this auxochrome, chromophore put together that the light of a certain wavelength is absorbed, and absorption is from the electromagnetic radiation. Some also ionize, enabling the compound to chemically react with ionizing tissue groups or materials.

Color, fading, fluorescence, phosphorescence are all seen to be different effects of the same fundamental process. Now, once the light is you know absorbed, a color will be observed. But then this light may remain this color may remain there or may cause fading, there may be other effects like fluorescence and phosphorescence that may be occurring because of this color changing effect, because it is all a matter of electronic transformation. The electrons jump from the molecular orbital to anti bonding molecular orbital. So, when they are thrown back all these effects can be noted.

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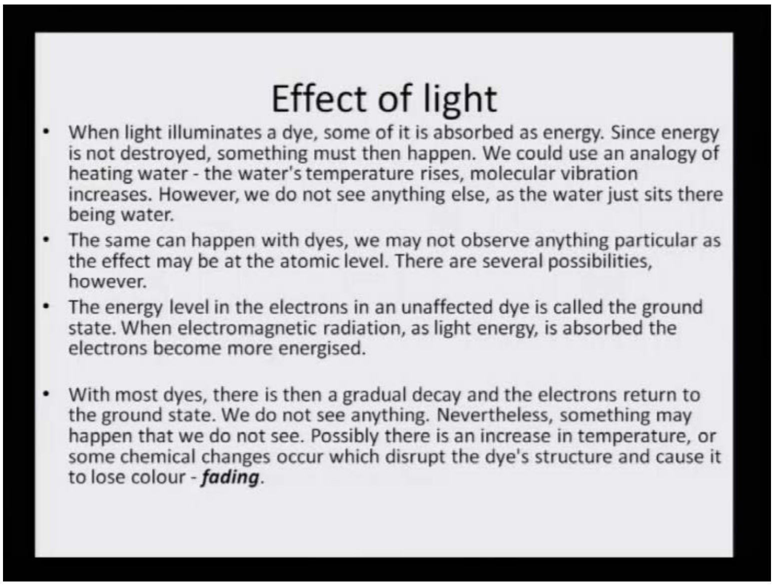

Effect of light

- When light illuminates a dye, some of it is absorbed as energy. Since energy is not destroyed, something must then happen. We could use an analogy of heating water - the water's temperature rises, molecular vibration increases. However, we do not see anything else, as the water just sits there being water.
- The same can happen with dyes, we may not observe anything particular as the effect may be at the atomic level. There are several possibilities, however.
- The energy level in the electrons in an unaffected dye is called the ground state. When electromagnetic radiation, as light energy, is absorbed the electrons become more energised.
- With most dyes, there is then a gradual decay and the electrons return to the ground state. We do not see anything. Nevertheless, something may happen that we do not see. Possibly there is an increase in temperature, or some chemical changes occur which disrupt the dye's structure and cause it to lose colour - ***fading***.

Effect of light: When light illuminates a dye, some of the absorbed some of it is absorbed as energy. Since energy is not destroyed, something must then happen. We could use an analogy of heating water - the water's temperature rises, molecular vibration increases. However, we do not see anything else, as the water just sits there being water. The same can happen with dyes, we may not observe anything particular as the effect may be at the atomic level. There are several possibilities, however. The energy level in the electron is an unaffected dye is called the ground state. When electromagnetic radiation, as light energy, is absorbed the electrons become energized.

With most dyes, there is then a gradual decay and the electrons return to the ground state. We do not see anything. Nevertheless, something may happen and that we do not see.

Possibly this is an increase in temperature or some chemical change is occur which disrupt the dye's structure and cause it to lose color or fading. So, now what, so many things can happen, when the electrons get excited to the anti bonding orbitals, they cannot reside there for too long. So, they have to come back and while they come back many changes can occur.

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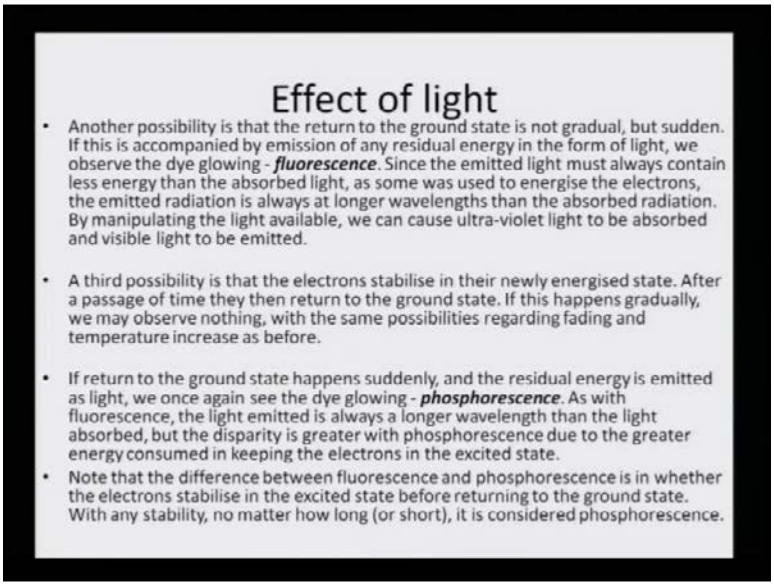

Effect of light

- Another possibility is that the return to the ground state is not gradual, but sudden. If this is accompanied by emission of any residual energy in the form of light, we observe the dye glowing - ***fluorescence***. Since the emitted light must always contain less energy than the absorbed light, as some was used to energise the electrons, the emitted radiation is always at longer wavelengths than the absorbed radiation. By manipulating the light available, we can cause ultra-violet light to be absorbed and visible light to be emitted.
- A third possibility is that the electrons stabilise in their newly energised state. After a passage of time they then return to the ground state. If this happens gradually, we may observe nothing, with the same possibilities regarding fading and temperature increase as before.
- If return to the ground state happens suddenly, and the residual energy is emitted as light, we once again see the dye glowing - ***phosphorescence***. As with fluorescence, the light emitted is always a longer wavelength than the light absorbed, but the disparity is greater with phosphorescence due to the greater energy consumed in keeping the electrons in the excited state.
- Note that the difference between fluorescence and phosphorescence is in whether the electrons stabilise in the excited state before returning to the ground state. With any stability, no matter how long (or short), it is considered phosphorescence.

Effect of light can also have another possibility is that the return of the ground state is not gradual, but sudden. So, it if it is accompanied by emission of any residual energy in the form of light, we observe the dye to glow and that causes fluorescence. Since the emitted lights must always contain less energy than the absorbed light, as some was used to energize the electrons, the emitted radiation is always at longer wavelength then the observed or absorbed radiation. By manipulating the light available, we can cause ultra-violet light to be absorbed and visible light to be emitted. So, these all possibilities are there because the electronic transition coming back to the ground level state is not gradual.

A third possibility can be that the electrons stabilize in their newly energized state. After a passage of time they then return to the ground state. If this happens gradually, we may observe nothing, but the same possibility regarding fading and temperature increase may come as what we learnt a little while ago. If return to the ground state happen suddenly, and the residual energy is emitted as light, we once again see the dye glowing and that is creating a phosphorescence effect. So, we had fluorescence effect. we So, whenever the dye glows it gives a different kind of an effect, because now the light that is now being transmitted is not the usual colored light but it has another aspect to it, and it is all because of the electronic interaction, it is not happening on its own. Electrons if they are jumping back in a particular manner will show a particular effect, if they are you know gradually coming down they will show fluorescence, if they are coming abruptly then they will glow and they will show phosphorescence.

As with fluorescence, the light emitted is always the longer wavelength than the light absorbed, but the disparity is greater with phosphorescence due to the greater energy consumed in keeping the electrons in the excited state. So, you see because they are more energized. Therefore, the qualities of fluorescence and phosphorescence or the effect of fluorescence and phosphorescence are ultimately very different. Note that the difference between fluorescence and phosphorescence is in whether the electrons stabilize in the excited state before returning to the ground state or not. With any stability, no matter how long or short, it is considered phosphorescence. So, you see that these vary because of their different abilities.

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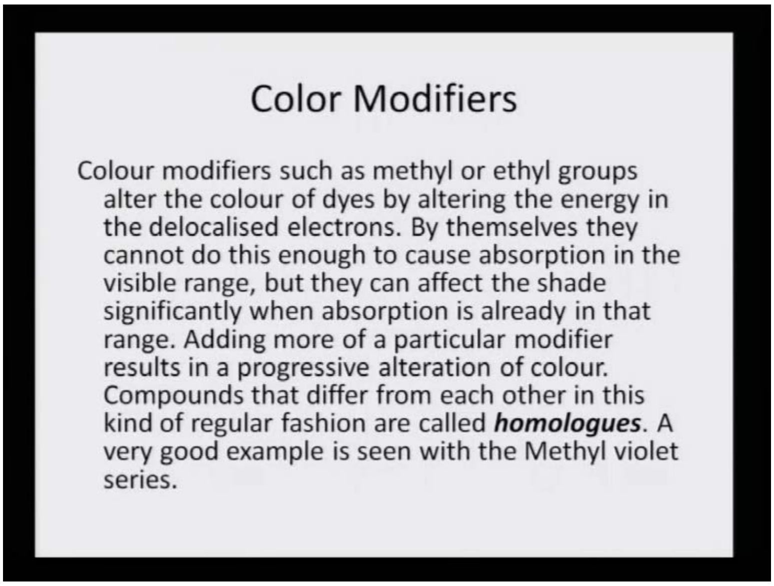

Color Modifiers

Colour modifiers such as methyl or ethyl groups alter the colour of dyes by altering the energy in the delocalised electrons. By themselves they cannot do this enough to cause absorption in the visible range, but they can affect the shade significantly when absorption is already in that range. Adding more of a particular modifier results in a progressive alteration of colour. Compounds that differ from each other in this kind of regular fashion are called ***homologues***. A very good example is seen with the Methyl violet series.

Then there are color modifiers; color modifiers such as methyl or ethyl groups alter the color of the dye by altering the energy in the delocalized electrons. That is by hyper-conjugation effect. If there is a methyl group it will try to push the electrons and increase the delocalization in the ring structure. By themselves they do not do they cannot do this enough to cause absorption in the visible range, but they can affect the shade significantly when absorption is already in that range. Adding more or a particular modifier results in progressive alteration of color. Compounds that differ from each other in this kind of regular fashion are called homologues. A very good example is seen with methyl violet series. So, when we try to look at different types of metal incorporation or ethyl incorporation, basically we are looking at the basic fundamentals of hyper conjugation, stabilization and that causes the color to get modify.

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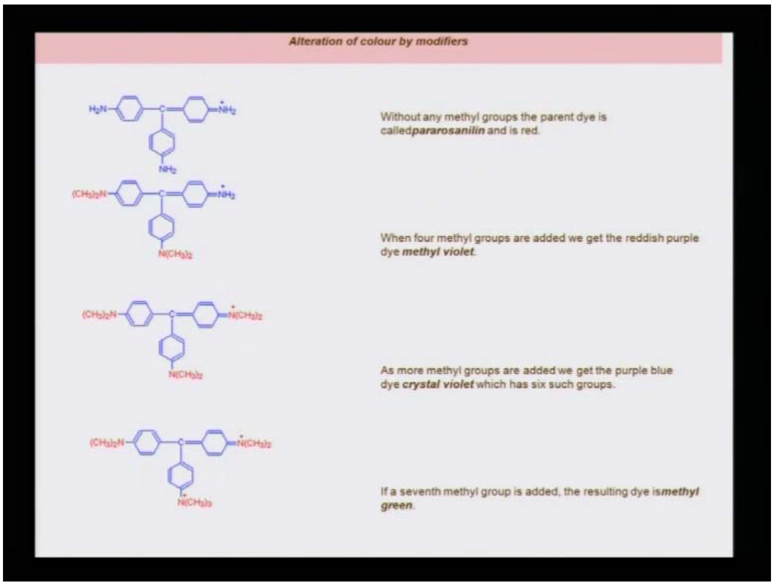

Alteration of colour by modifiers

Without any methyl groups the parent dye is called***pararosanilin*** and is red.

When four methyl groups are added we get the reddish purple dye ***methyl violet***.

As more methyl groups are added we get the purple blue dye ***crystal violet*** which has six such groups.

If a seventh methyl group is added, the resulting dye is***methyl green***.

If we now try to look at different types of modifiers; you look at the first structure without any methyl group the parent dye is called pararosanilin and it is red in color. With two nitrogen’s having 2-2-methyl’s, when four methyl groups are added to get the reddish purple color it becomes methyl violet. And when all the three nitrogen’s have two methyl groups as more methyl groups are added we get the purple blue which is crystal violet. And with charged two of them being charged, the nitrogen is now having a surplus methyl group in the last structure. A seventh methyl group is added and resulting in methyl green. So, you see that deepening of the color takes place and the methyl groups on the nitrogen are just creating a more hyper conjugation effect and deepening the colors.

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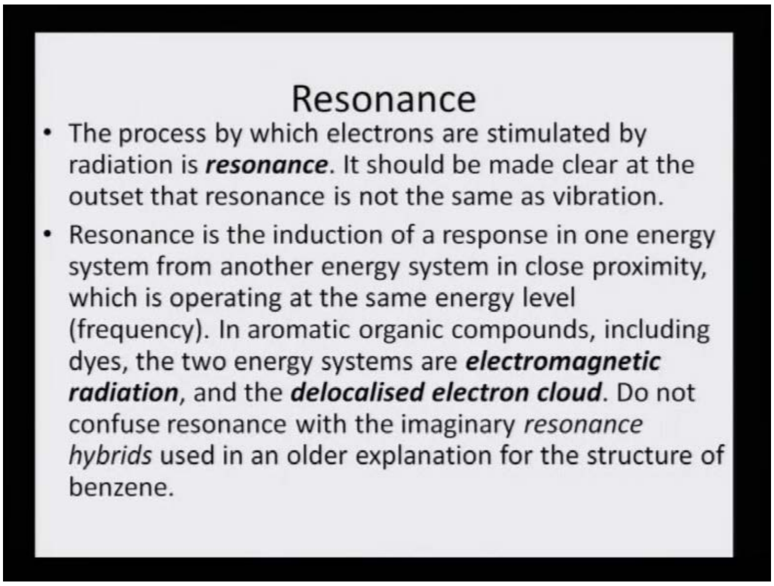

Resonance

- The process by which electrons are stimulated by radiation is ***resonance***. It should be made clear at the outset that resonance is not the same as vibration.
- Resonance is the induction of a response in one energy system from another energy system in close proximity, which is operating at the same energy level (frequency). In aromatic organic compounds, including dyes, the two energy systems are ***electromagnetic radiation***, and the ***delocalised electron cloud***. Do not confuse resonance with the imaginary *resonance hybrids* used in an older explanation for the structure of benzene.

Now, there is also because of the resonance structures that are created due to these electronic effects caused by either an auxochrome or a methyl group. The process by which the electrons are stimulated by radiation is resonance. It should be made clear at the outset that resonance is not the same as vibration. It is not like what we understand that you know it is not causing any kind of movement, but it is the delocalization and the stabilization of the electron within the molecule. Resonance is the induction of response in one energy system from another energy system in close proximity, which is operating at the same energy level or the frequency.

In aromatic organic compounds, including dyes, the two energy systems are electromagnetic radiation, and the delocalized electron cloud which absorbs this. Do not confuse resonance with the imaginary resonance hybrid used in an older explanation of the structure of benzene. So, it is not what we understand the resonance structure of benzene, it is the delocalization and the stabilization of these delocalized electrons and their affectivity towards the electromagnetic radiation absorption.

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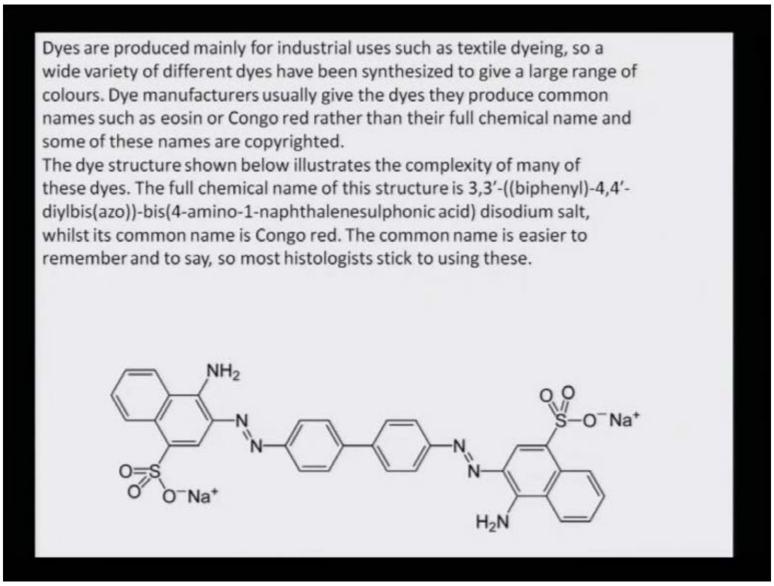

Dyes are produced mainly for industrial uses such as textile dyeing, so a wide variety of different dyes have been synthesized to give a large range of colours. Dye manufacturers usually give the dyes they produce common names such as eosin or Congo red rather than their full chemical name and some of these names are copyrighted.

The dye structure shown below illustrates the complexity of many of these dyes. The full chemical name of this structure is 3,3′-((biphenyl)-4,4′-diylbis(azo))-bis(4-amino-1-naphthalenesulphonic acid) disodium salt, whilst its common name is Congo red. The common name is easier to remember and to say, so most histologists stick to using these.

Dyes are produced mainly from industrial for industrial uses such as textile dyeing, so a wide verity of different dyes have been synthesized to give a large range of colors. Dye manufactures usually give the dyes they produce common name such as eosin or Congo red. Now, this is the structure of Congo red, because it has a big IUPAC nomenclature, the full name of that compound is 3, 3 prime-biphenyl-4, 4 prime diylbis(azo)-bis(4-amino-1-naphthalenesulphonic acid. Now, in order to and dye sodium salt of that is actually used and but the Congo red is the common name for this dying. And the common name is easier to remember. So therefore, one use one you can see how much of delocalization is there, these ionic groups contribute to their being a good you know adhering having the adhering ability and the conjugation in the benzene ring the azo linkages and all are creating very good chromophoric regions.

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The Colour Index

To overcome all of the confusion there is a standard list of all dyes, their synonyms and their structures. This is called the Colour Index (CI).

This is a monumental work of reference produced by The Society of Dyers and Colourists and each dye is given an individual number and listed along with its name(s) and properties. Since each dye on the list has a unique number to identify it, this list is the most reliable way of identifying a dye. When naming a dye in the description of a techniques, the CI number should be given to avoid ambiguity, e.g. eosin Y (CI 45380).

CI numbers are arranged according to their structure, with the most important feature being their chromophoric group. For example, all nitroso dyes have numbers between 10000 and 10299, nitro dyes have numbers between 10300 and 10999, monoazo dyes have numbers between 11000 and 19999, and so on.

There are 31 groups in all, with CI numbers up to 78000. Not all of these groups include important histological dyes; a few of the more important groups are listed below with examples of histological dyes from the group.

- Nitro dyes. These have the nitro group -NO2 as the chromophore, e.g. picric acid, martius yellow.
- Azo dyes. These have the -N=N- group (azo) as the chromophore, e.g. orange G

The color index: To overcome all the confusion, there is a standard list of all dyes, their synonyms and their structures. This is called Color Index or CI. This is a monumental work of reference produced by the Society of Dyers and Colourists and each dye is given an individual number, and it is listed along with its name and properties. Since each dye on the list has a unique number to identify it, this list is most reliable way of identifying a dye. When naming a dye in the description of techniques, the CI number should be given to avoid ambiguity. That means we are talking about eosin, it should be that eosin CI number is 45380. So that is like you know, you and I have a name and so it is a name, and it is a description, address also given.

CI numbers are arranged according to their structure, and the most important feature being their chromophoric group. For example, all nitroso dye have numbers between 10000 to 10299, nitro dyes have numbers between 10300 to 10099, monoazo dyes have numbers between 11000 to 19999, and so on. There are 31 groups in all, with CI numbers up to 78000. Not all of these groups include the dyes that are now available in the market. A few more important groups are listed below. There are azo dyes, nitro dyes where different groups are used as chromophores. So, you see that these are all a well understood science, the color index is the most important naming system of a dye.