

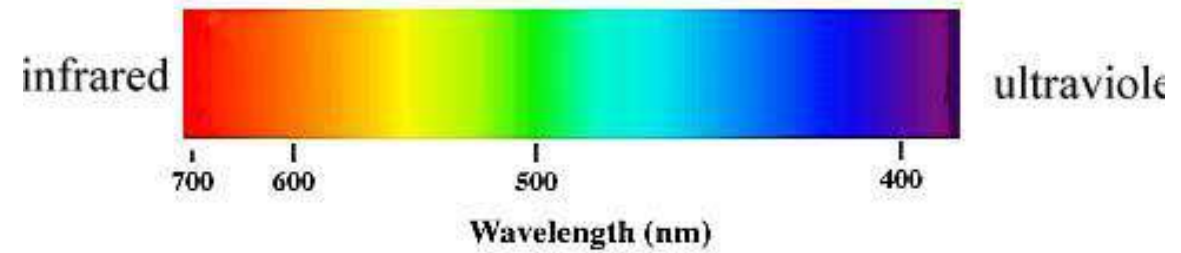
# Colorants

# Colorant

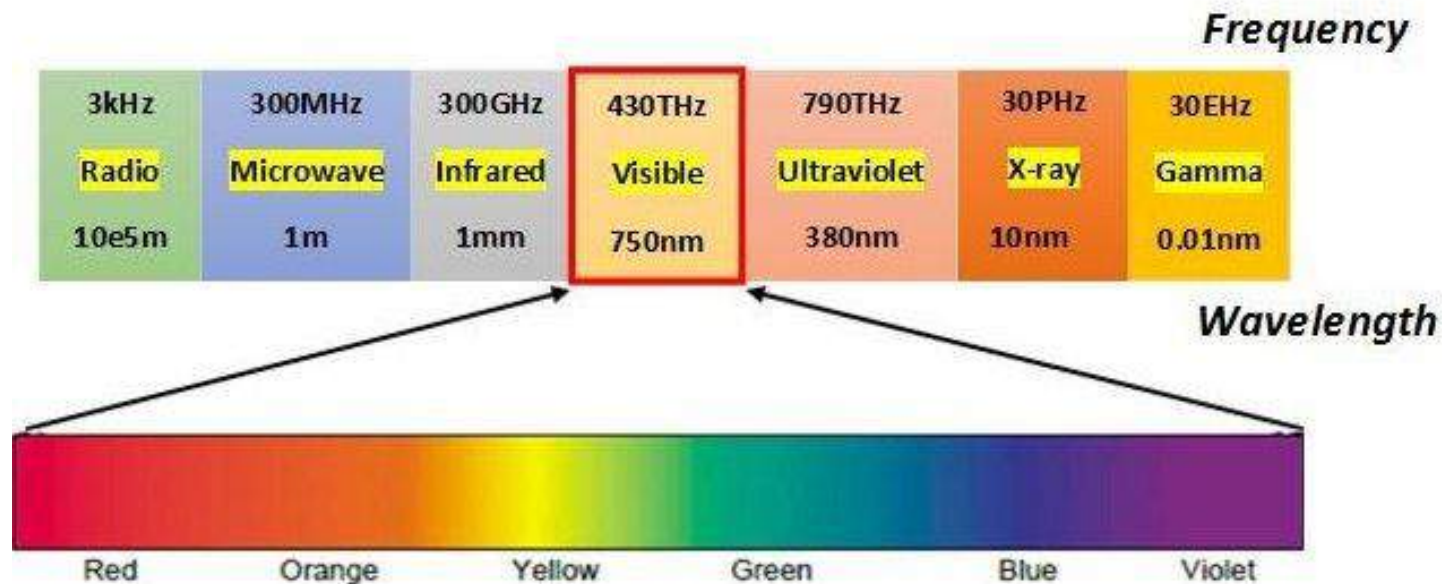
- is a substance that is added or applied in order to change the colour of a material or surface.
- Colourants can be used for many purposes including printing, painting, and for colouring many types of materials such as food and plastics.
- Colourants work by **absorbing** varying amounts of light at different wavelengths (or frequencies) of its spectrum, transmitting (if translucent) or reflecting the remaining light in straight lines or scattered.

# Visible Spectrum

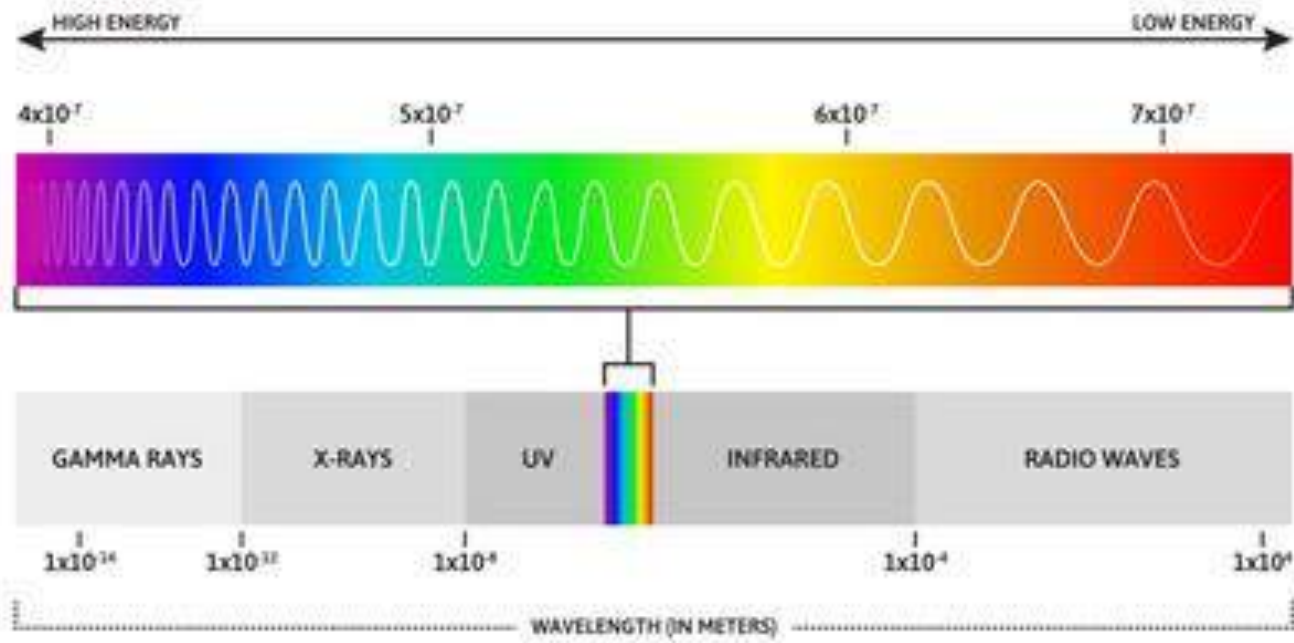
## The visible spectrum



- The visible light spectrum is the segment of the electromagnetic spectrum that the human eye can view. More simply, this range of wavelengths is called visible light. Typically, the human eye can detect wavelengths from **380 to 700 nanometers**.

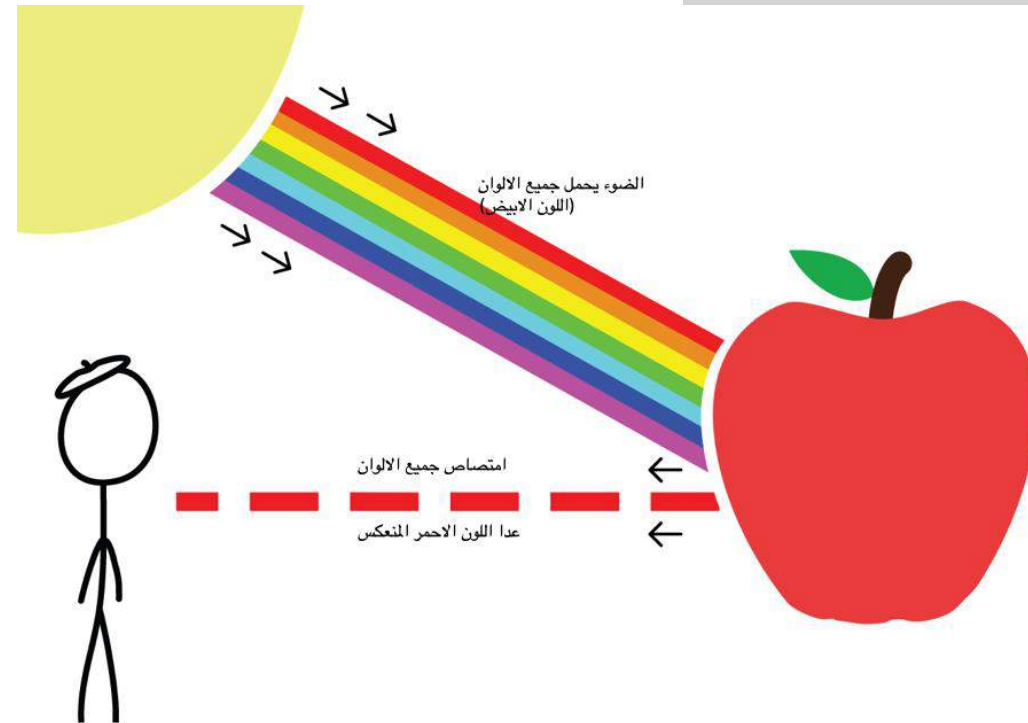
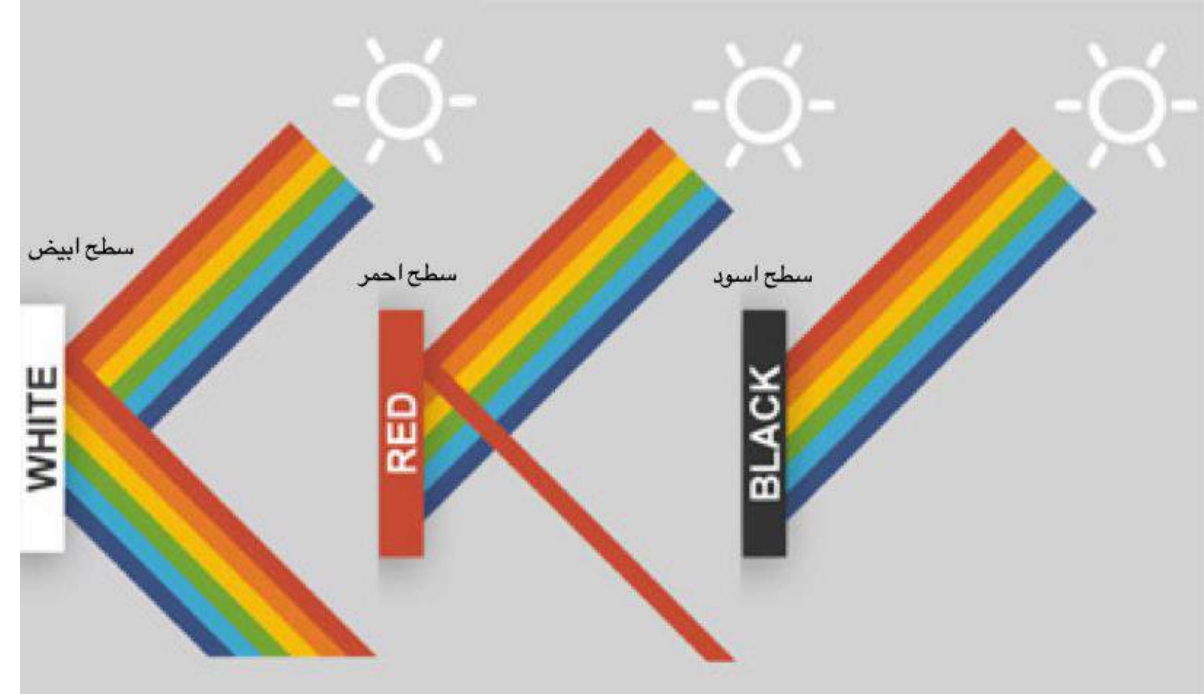
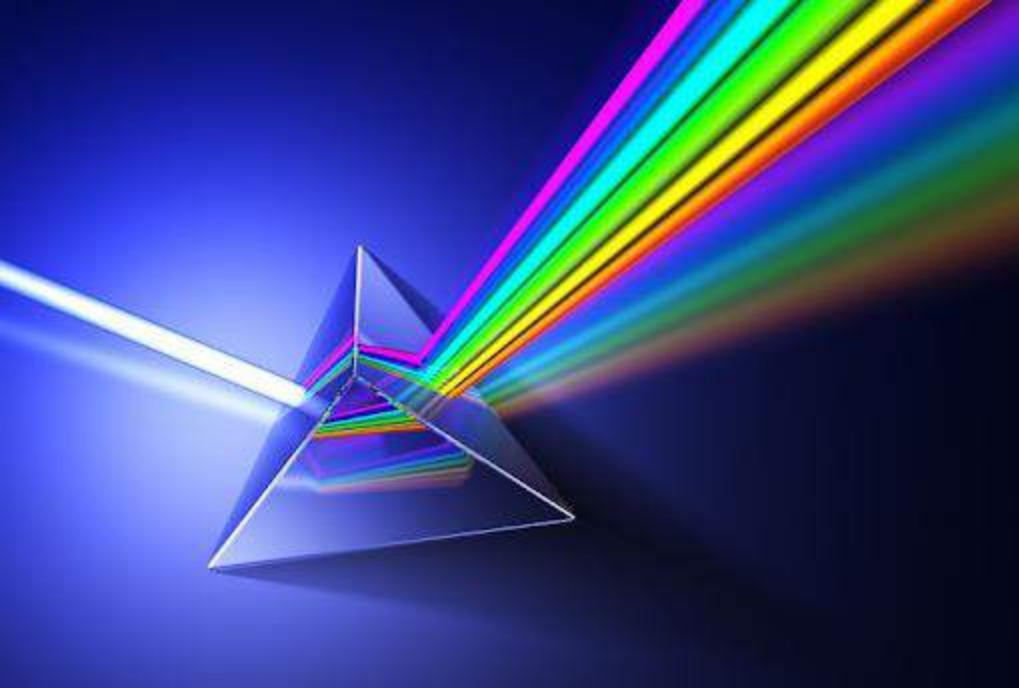


# VISIBLE SPECTRUM

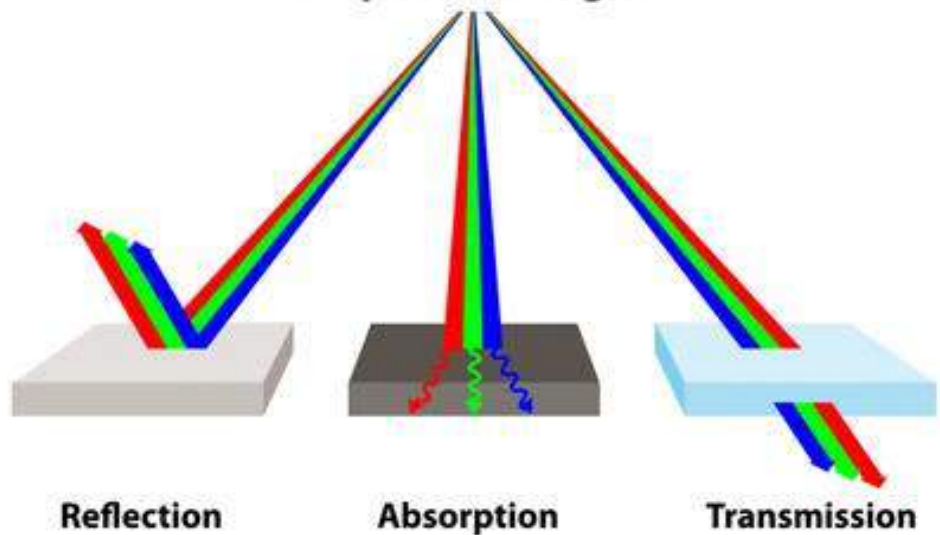


الألوان في منطقة طيف الضوء المرئي [3]

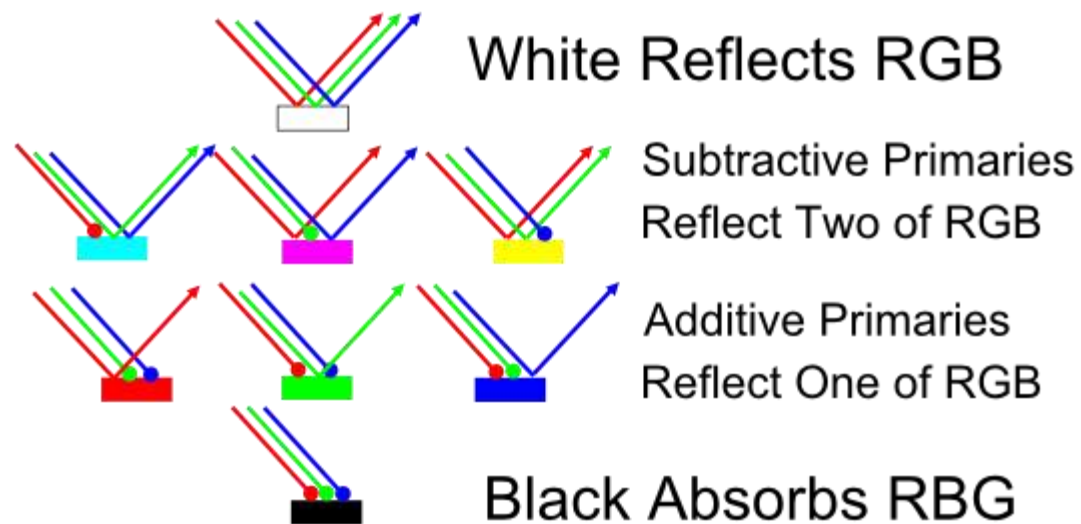
اللون	مدى الطول الموجي	مدى التردد
أحمر	nm 635–700 ~	THz 480–430 ~
برتقالي	nm 590–635 ~	THz 510–480 ~
أصفر	nm 560–590 ~	THz 540–510 ~
أخضر	nm 520–560 ~	THz 580–540 ~
سيان	nm 490–520 ~	THz 610–580 ~
أزرق	nm 450–490 ~	THz 670–610 ~
بنفسجي	nm 400–450 ~	THz 750–670 ~



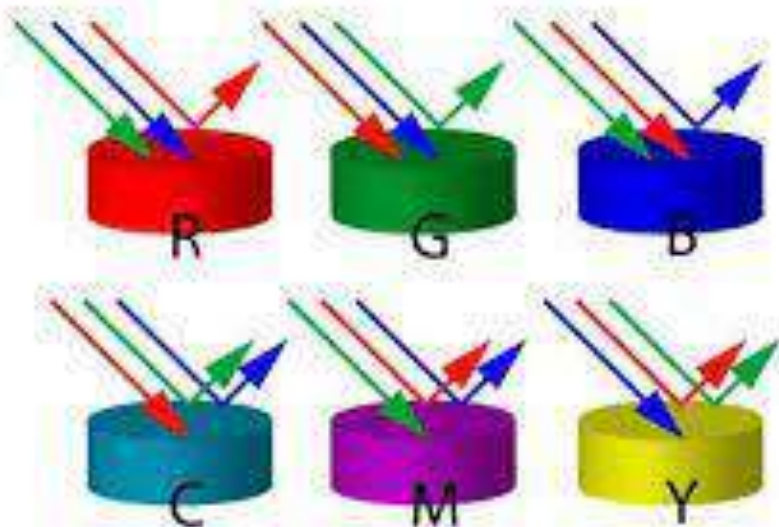
## Properties of Light



## Reflection and Absorption Detail



## Absorption and Reflection



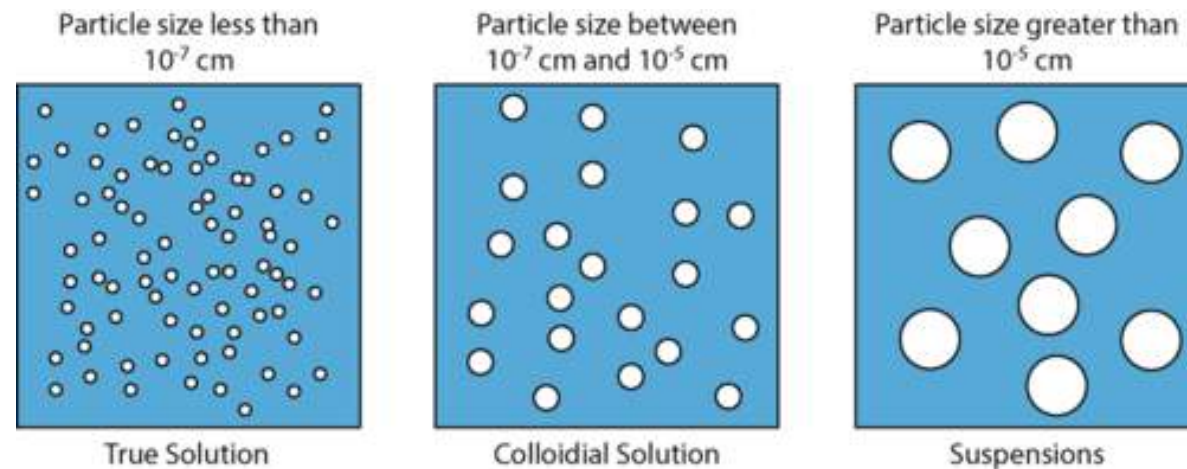
### Light Absorbed

#### Color

### Perceived Complementary (Subtraction) Color

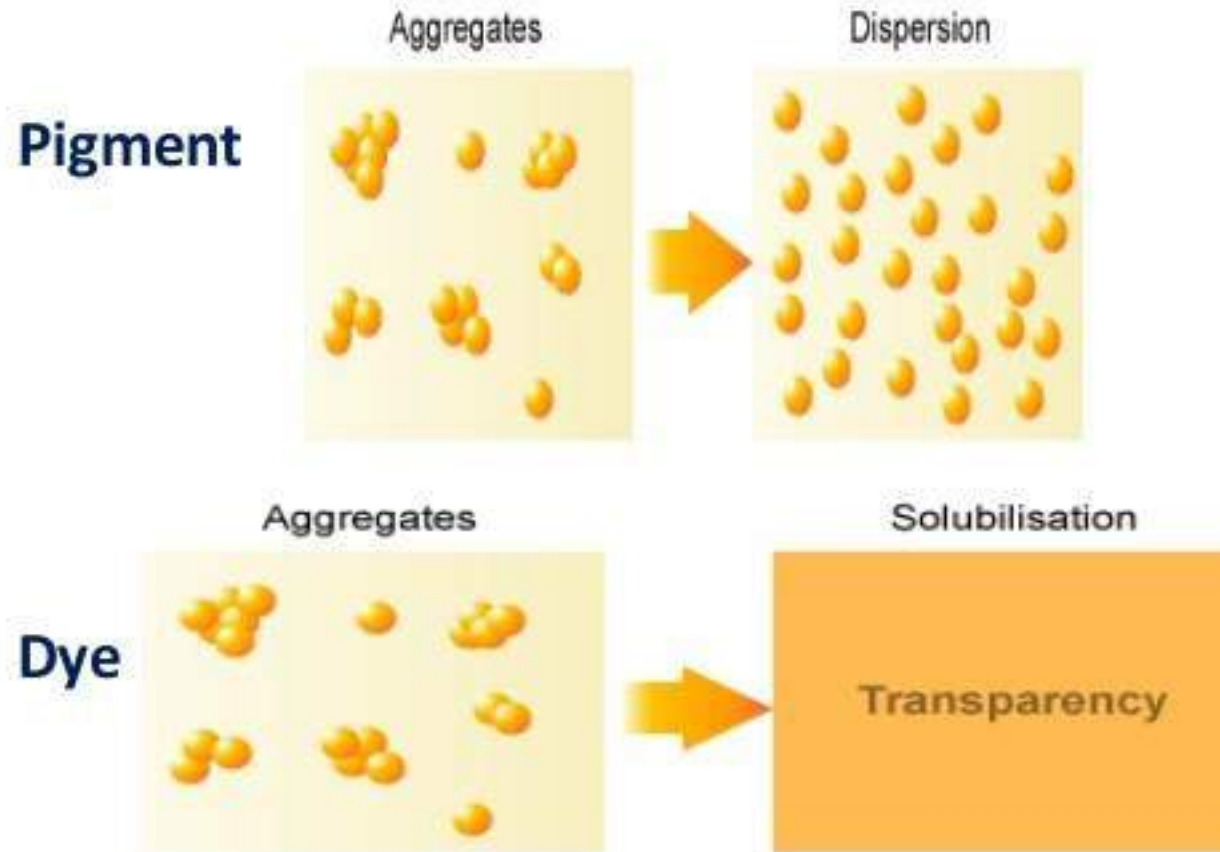
Violet		Green-yellow	
Blue		Yellow	
Green-blue		Orange	
Blue-green (cyan)		Red	
Green		Purple (magenta)	
Yellow-green		Violet	
Yellow		Blue	
Orange		Green-blue	
Red orange		Blue-green (cyan)	
Red		Green	

- Most colourants can be classified as dyes or pigments.
- Typical dyes are formulated as solutions, while pigments are made up of solid particles suspended and are generally suspended in a vehicle.



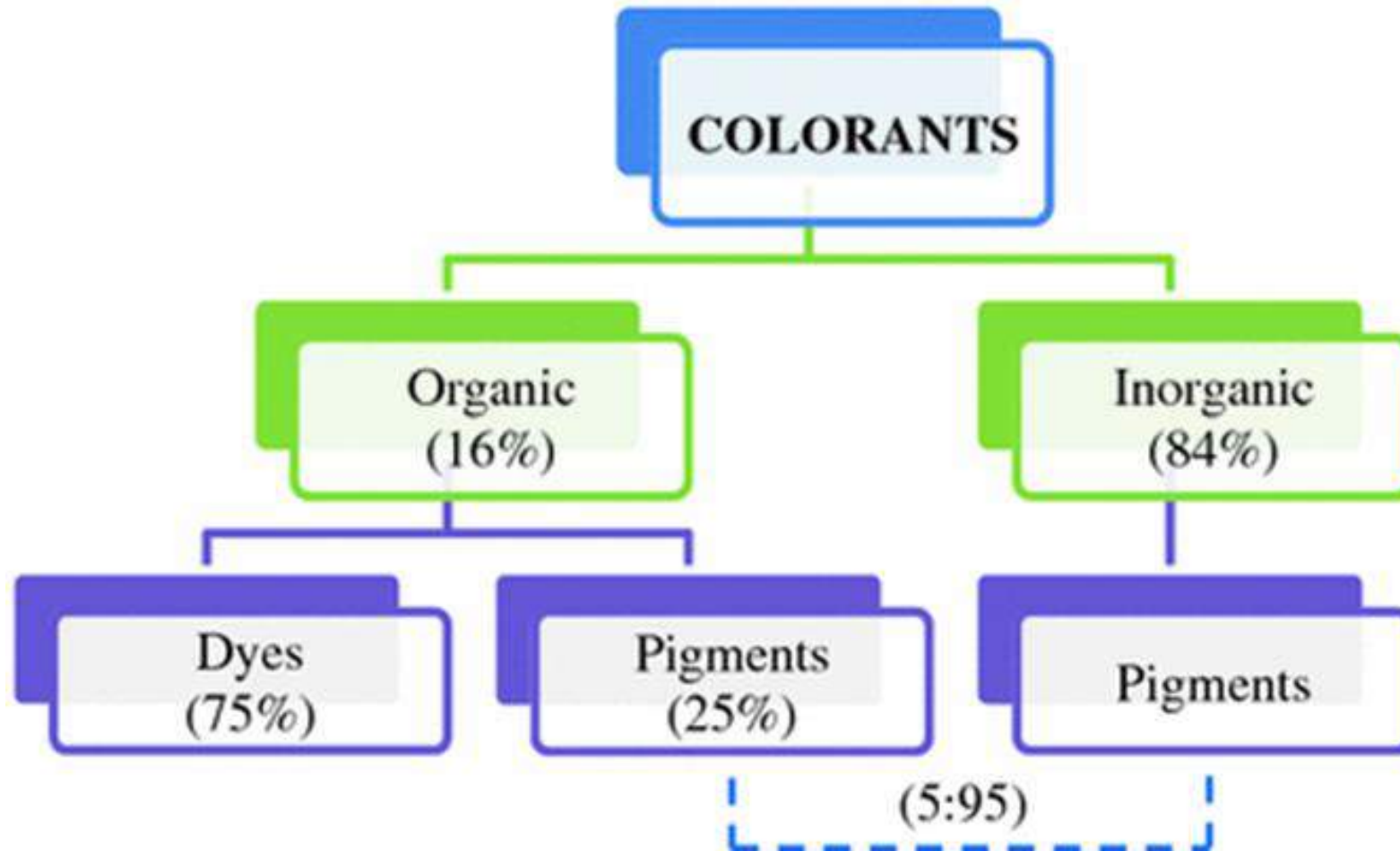
- The color a colorant imparts to a substance is mediated by other ingredients it is mixed with such as binders and fillers are added, for example in paints and inks. In addition, some colourants impart colour through reactions with other substances.

# Differences between Pigments and Dyes

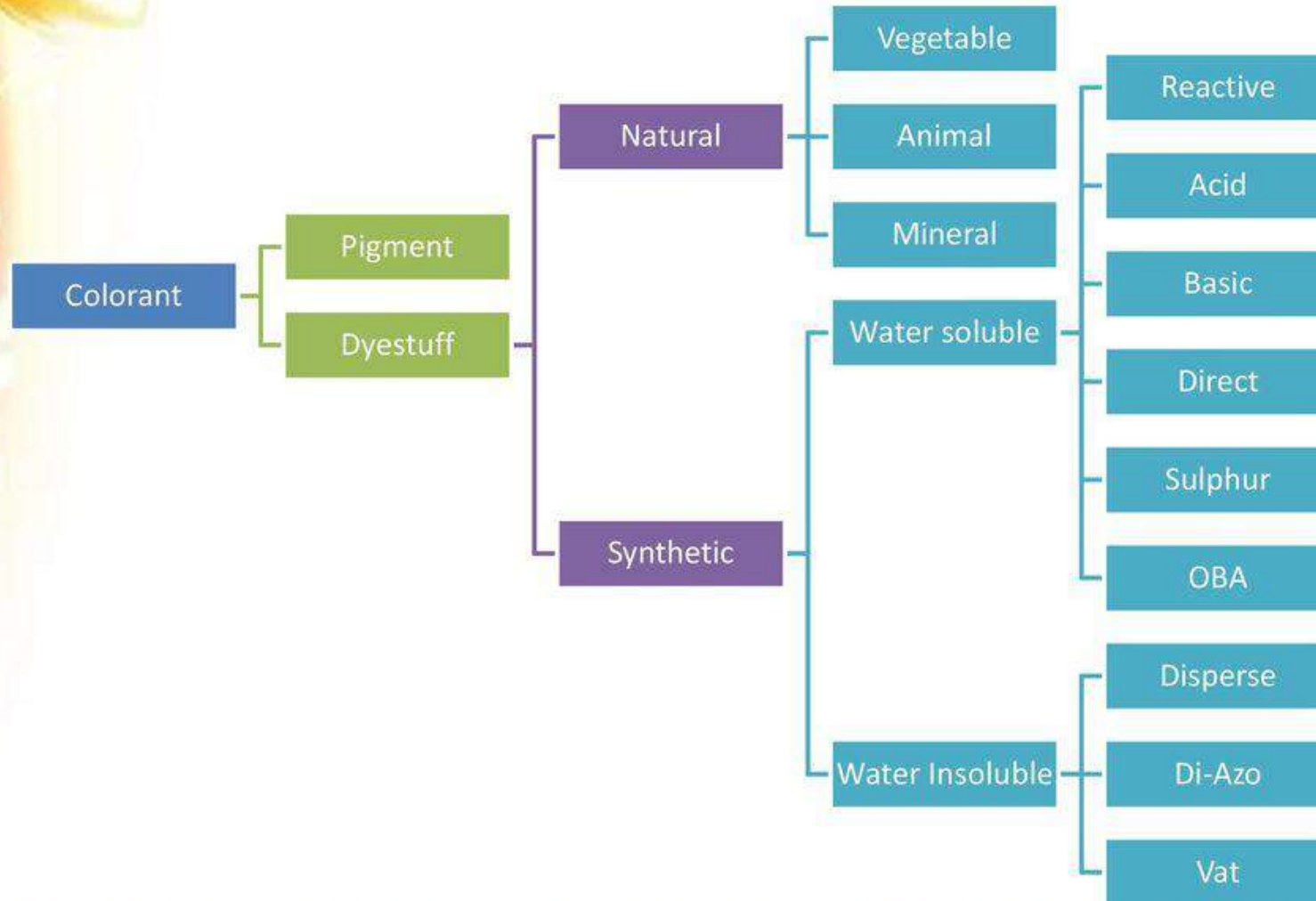




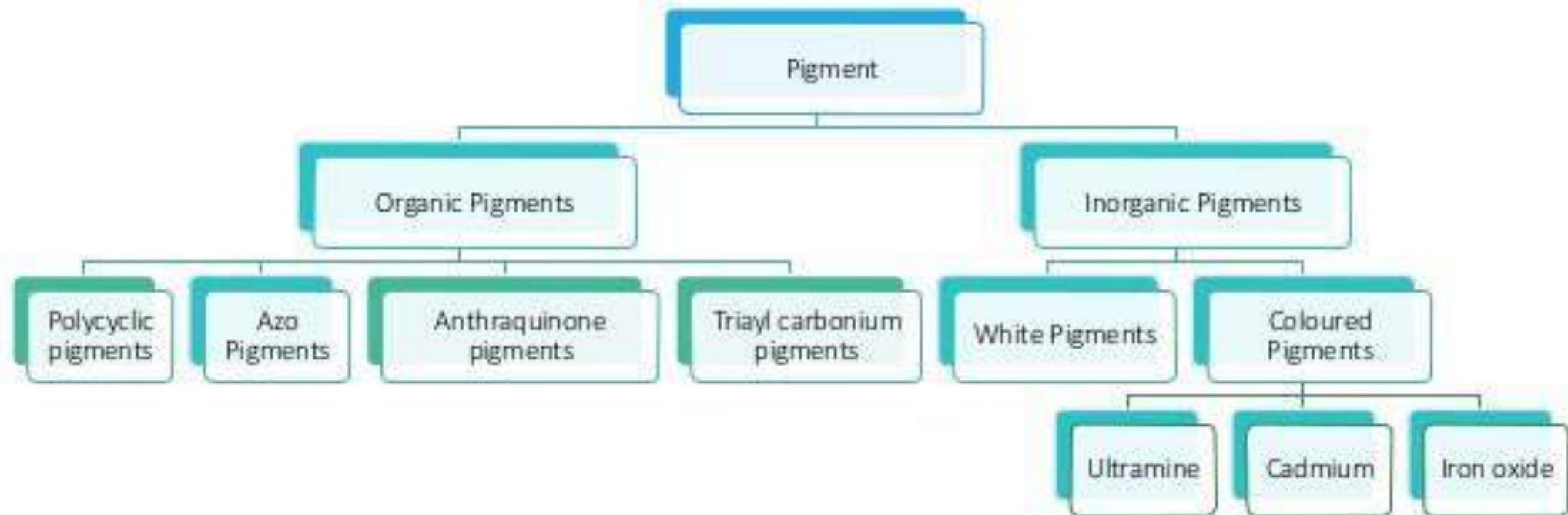
- Colourants, or their constituent compounds, may be classified chemically as inorganic (often from a mineral source) and organic (often from a biological source).



# Classification of COLORANT

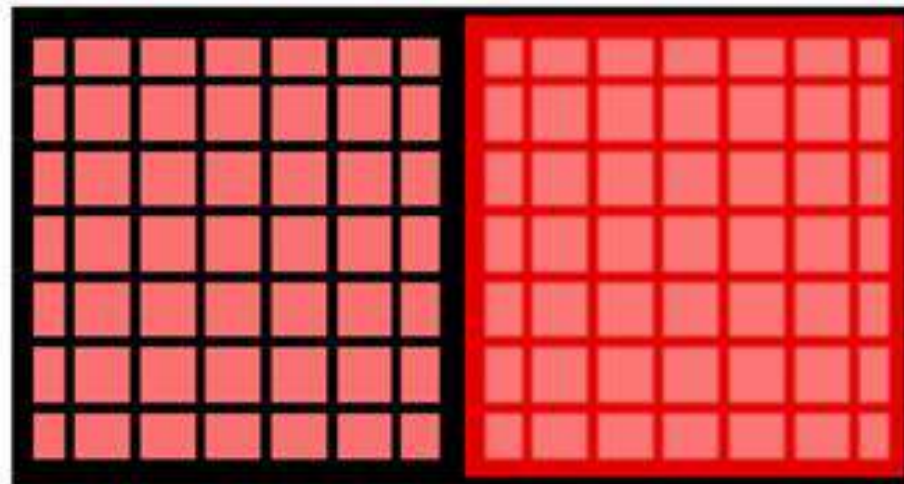


# Classification of Pigments



- Colorants are either dyes or pigments. Technically speaking, the difference is that dyes are soluble in the host material—typically water—while pigments are not. Another difference is that dyes do not scatter light and look transparent. On the other hand, pigments do scatter light and, thus, they are opaque

#### DIFFERENCE BETWEEN DYES AND PIGMENTS:



DYE

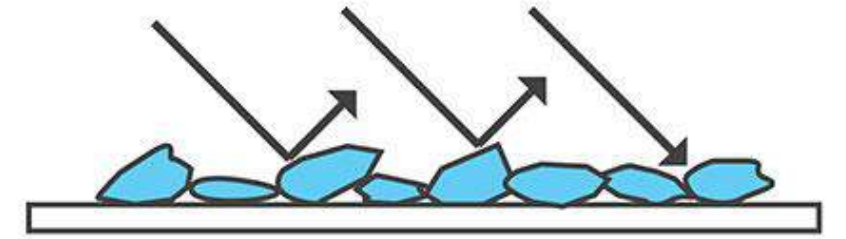
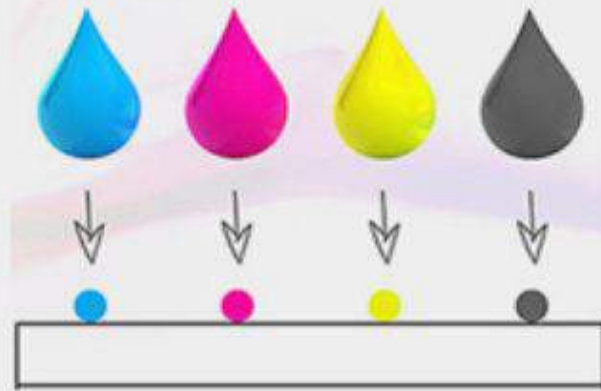
PIGMENT

DYES	PIGMENTS
1. Water solubility is 70%	1. 100% water insoluble
2. Dyes have direct affinity to textile material	2. They have no direct affinity to textile materials
3. Auxochrome groups are present	3. Auxochrome groups are absent
4. Most of the dyes are organic	4. Most of dyes are inorganic
5. Costly	5. Cheap
6. No binding agent is required	6. Binding agent is required
7. Dye diffusions in the fabric	7. Pigment diffusions on the fabric

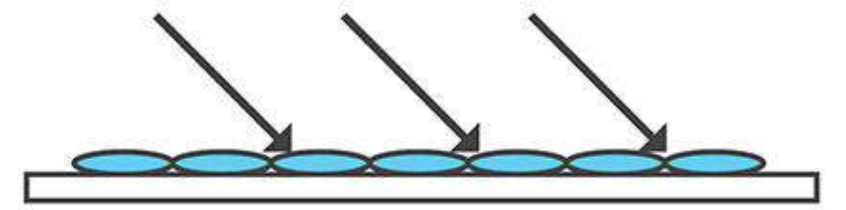
Dye-based ink



Pigmented ink



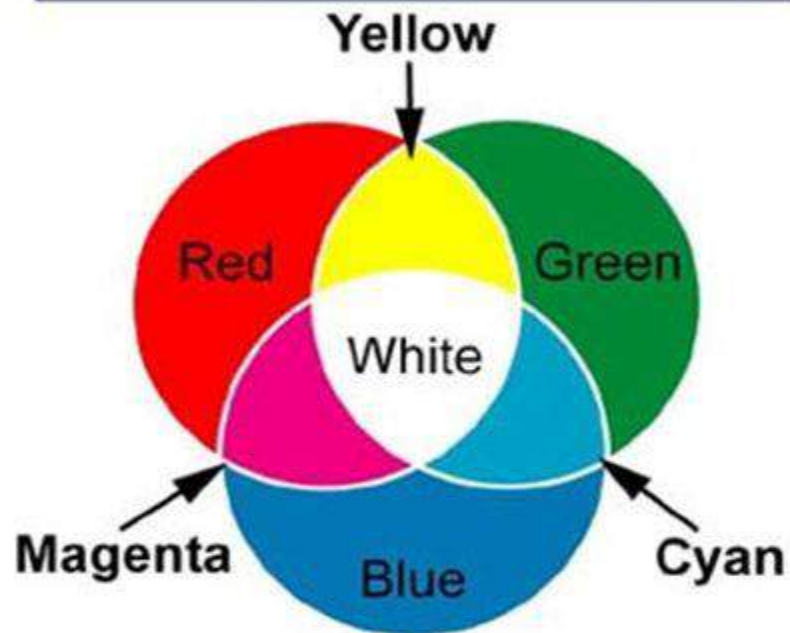
Light Reflecting off of Pigment Ink



Dye Based Ink Absorbing Light

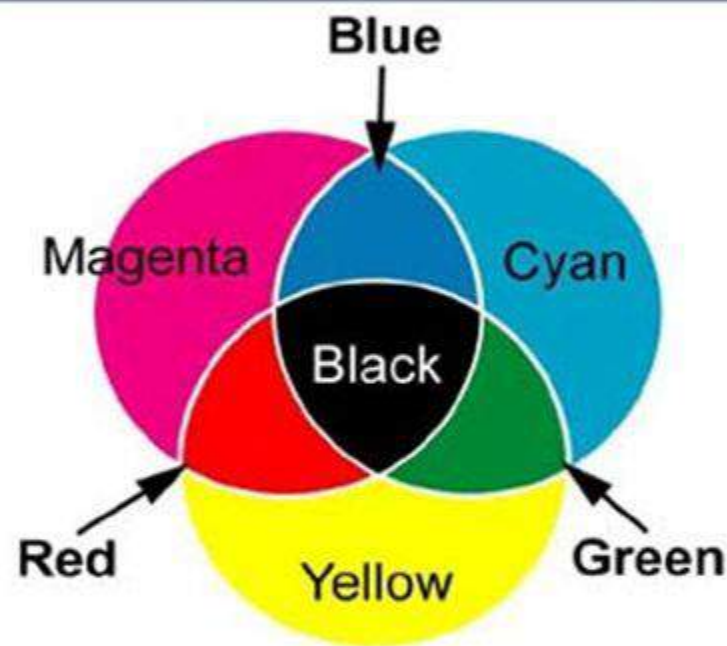
# Light V.S. Pigment

The additive primary colors



White = red + green + blue  
Yellow = red + green  
Magenta = red + blue  
Cyan = blue + green

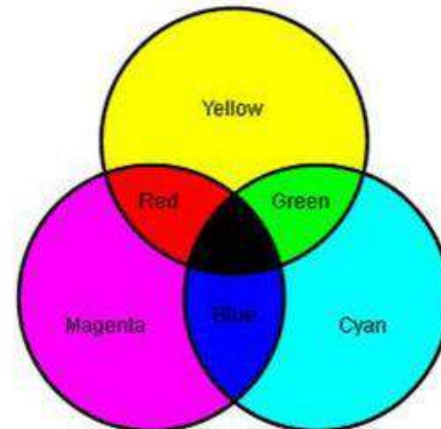
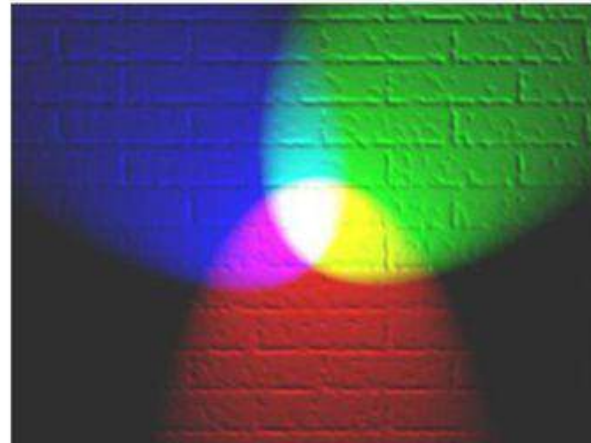
The subtractive primary colors



Black = magenta + yellow + cyan  
Red = magenta + yellow  
Green = cyan + yellow  
Blue = magenta + cyan

# Colors vs Pigments

- In science (especially physics) color refers to a wavelength of light.
  - The primary colors of light are : Red, Green, Blue
- A pigment is a colored material that is used to change the color of other substances. Ex: Paint
  - The primary pigments are : Magenta, Cyan, and Yellow



# Principles of tissue staining

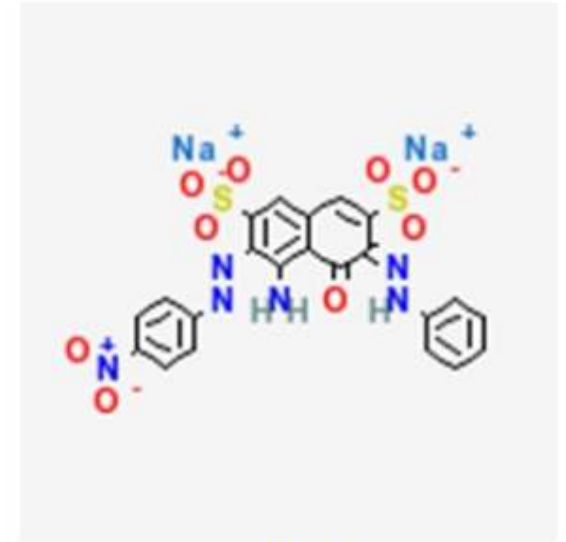
- Most cells and extracellular material are completely **colorless**, and to be studied microscopically sections must typically be stained.
- Methods of staining have been devised that not only make the various tissue components conspicuous but also permit distinctions to be made between them.
- Dyes stain tissue components more or less **selectively**, with many behaving like acidic or basic compounds and forming electrostatic (salt) linkages with ionizable radicals of molecules in tissues.



- Acid dyes are anionic, possess acidic groups, such as  $\text{SO}_3\text{H}$  and  $\text{COOH}$ , soluble in water.
- Acid dyes will stain acidophilic structures that have a net **positive charge** due to the fact that they have a negatively charged chromophore.
- Acid dyes are used to color basic tissue proteins.
- Acidophilic structures include the cytoplasm, collagen and mitochondria .
- Examples of acid dyes:
  - Lee's stain (stains reddish-pink).
  - PTAH stain (stains blue).
  - Eosin stain (stains pinkish-orange).

## Acidic Dyes

- Works best in acidic pH
- Ionizes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ )
- Creates Anionic (-) chromogen
- Attracted to (+) cell components [AA]
- Examples
  - Picric Acid
  - Nigrosin
  - India Ink
  - Eosin



Nigrosin

**Cationic** components, such as proteins with many ionized amino groups, have affinity for acidic dyes and are termed **acidophilic**.

## CHROMOPHORE

❖ It is a Greek word.

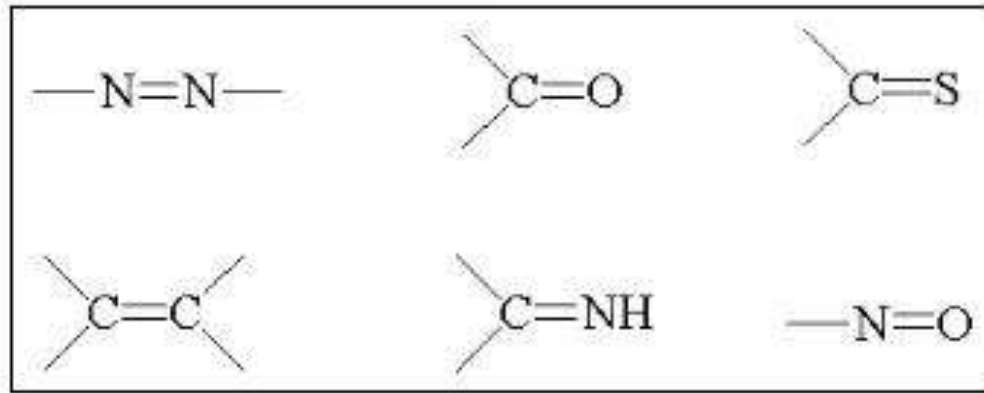
Chroma = “color” & phoros = “bearer”

❖ Defined as any isolated covalently bonded group that shows a characteristic absorption of Electromagnetic radiation in the UV or visible region.

❖ Compound containing chromophore is **CHROMOGEN**

Eg: C=C, C=O, NO<sub>2</sub>

**chromogen** is a colourless (or weakly coloured) chemical compound that can be converted by chemical reaction into a compound which can be described as "coloured"

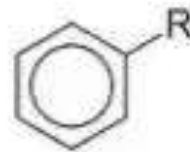


## Chromophores

(provides colour –  
unsaturated grps.)

## Chromogens:

(To which chromophore  
is attached)



Phenyl, Naphthyl, etc.

## Auxochromes:

(Modify the hue / solubility –  
Saturated functional groups attached to  
conjugated system)

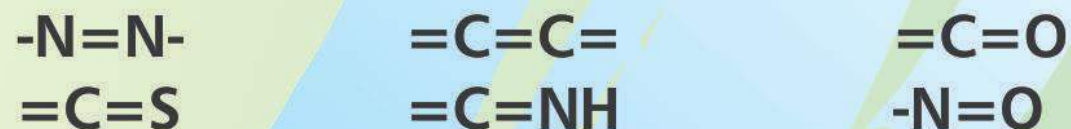
**-NH<sub>2</sub>**  
**-NHR**  
**-NR<sub>2</sub>**  
**-OH**  
**-OR**  
**-SO<sub>3</sub>**

# AUXOCHROME

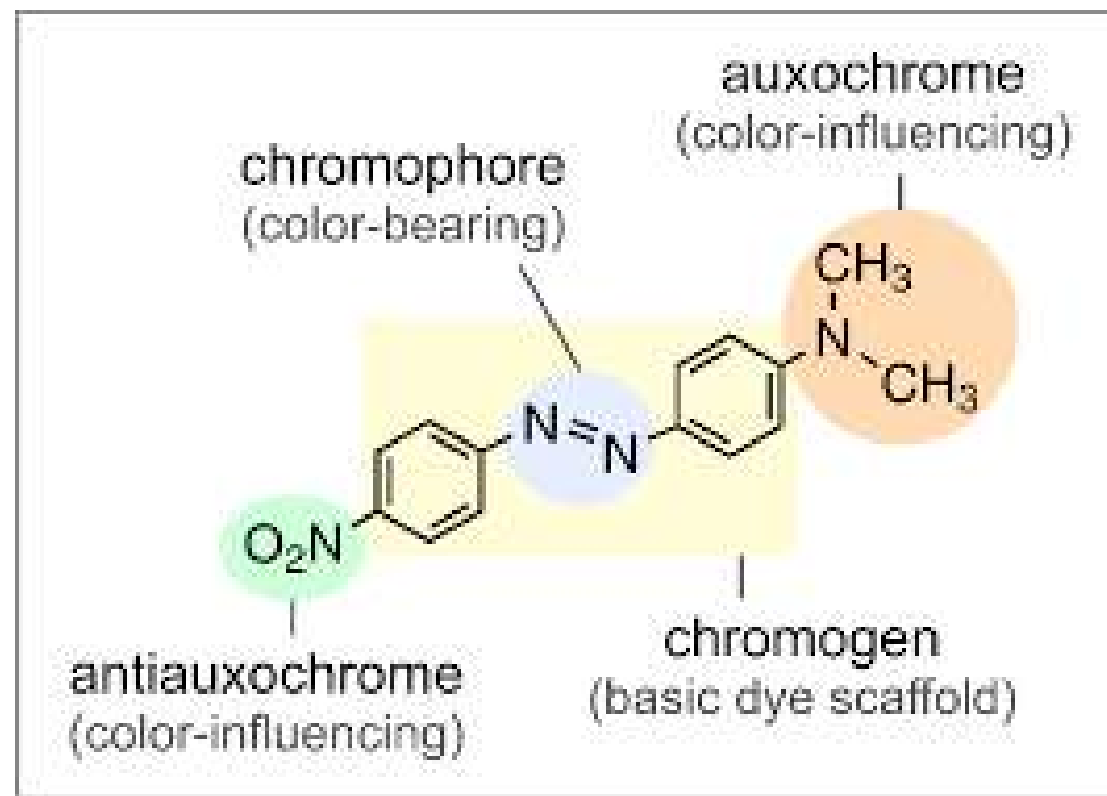
- **Auxochrome** is defined as any group, which does not itself act as a chromophore but whose presence brings about a shift of the absorption band towards the red end of the spectrum (longer wavelength)
- Chromophore + Auxochrome = newer chromophore
- ❖ Auxochrome is a colour enhancing group.
- ❖ The effect is due to its ability to extend the conjugation of a chromophore by sharing the nonbonding electrons.

# Chromophores and Auxochromes

In organic compounds, certain chemical groups cause absorption and give rise to colour. They are known as chromophores, eg.



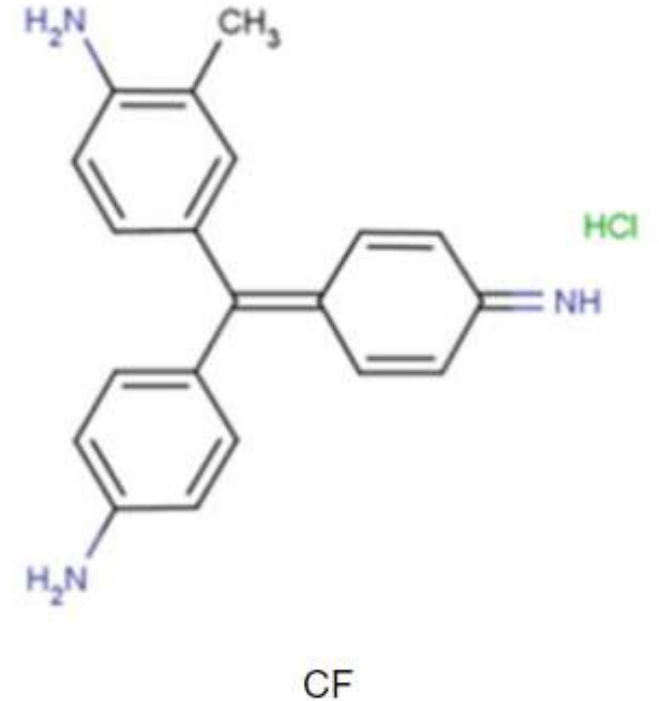
Other groups which intensify or modify colour are known as auxochromes, eg



# Basic Dyes

- Basic dyes are cationic, possess basic groups, such as -NH<sub>2</sub>.
- They are insoluble in water, When we dissolve them, we use alcohol or acetic acid and then dilute with water.
- Basic dyes will stain basophilic structures that have a net **negative charge** due to the fact that they have a positively charged chromophore.
- Basic dyes are used to color nucleic acids.

- Work best in basic pH
- Ionizes (Cl<sup>-</sup>, SO<sub>4</sub><sup>-</sup>)
- Creates (+) Cationic chromogen
- Attracted to (-) acidic cell components [DNA, proteins]
- Examples
  - Methylene Blue
  - Crystal Violet
  - Carbol Fuchsin
  - Safranin
  - Malachite Green



Cell components such as nucleic acids with a net negative charge (**anionic**) stain more readily with basic dyes and are termed **basophilic**.

# Tissue Staining

## Basophilic

- Basophilic
- Stain with basic dye [dye+Cl-]
- Toluidine blue, methylene blue, hematoxylin, alcian blue
- Nucleic acids, some cytoplasmic components (rRNA and rER), glycosaminoglycans and acidic glycoproteins

## Acidophilic

- Stain with acidic dye [Na+dye-]
- Orange G, eosin, acid fuschin
- Mitochondria, cytoplasm, secretory granules, ECM proteins