Colorimetry and spectrophotometry

**Basic Introduction:**

- **What is light?**
  - The light what we see is just a small region of electromagnetic spectrum, which human eyes are capable of sense. This region is called as “visible light”.

- **Where this light comes from?**
  - Due to fusion reaction in the sun. When two hydrogen atoms fused with each other, they form a helium molecule. In this process some mass is removed in the form of energy. This energy is the electromagnetic radiation. This is partly electric and partly magnetic, so it is called as electromagnetic radiation.

- **Why we can see any object and how could we define colors?**
  - When the visible light is reflected from any object and touches the sensors of our eyes, we can see the objects. In the process of reflection- out of multiple colors of the radiation, some colors are absorbed and some are reflected. The color which is reflected is the color what we can see.

- **Why black colored car is hotter than white colored car during summer?**
  - All the frequencies of white light (all colors) are absorbed by the black colored car while all the frequencies of white light (all colors) are reflected by white colored car. So, black colored car is hotter than white colored car during summer.

- **Draw and explain the spectrum of light.**
  - As we move from left to right side the frequency (vibrations/sec) of light decreases and wavelength (length of a wave) increases.
Some time we get line of colors so they are called as line spectra or discontinued spectra or emission spectra. Eg. Hydrogen spectra.

- **Max plank** = Light is having particle nature
- **Hugense** = Light is having wave nature
- **Einstine** = Light is having both (particle and wave) nature
- As an atom the smallest part of light is called as photon. It can be imagined as an energy packet.
- **Speed of light** = $3.0 \times 10^8$ m/sec
- **RGB** (red, green and blue) are basic colors and all other are the derivatives of these primary colors.
- Spectroscopy is dependent to emission while spectrophotometry is dependent to absorption of light.
- In the initial days, some radiation was expressed as a mysterious thing which could not be seen but one can experience it. Hence, the word “spectra” comes from a latin word “spectre”, which means ghost.

- **There are following possibilities when light is targeted to an object:**
  1. Transmission (if the object is transparent)
  2. Absorption (if the object is liquid/gas or capable of absorption)
  3. Reflection (if an opaque material is placed)
  4. Refraction (if medium is changed during the path of light)
  5. Scattering (if the surface is rough)
Colorimetry

- **Monochromatic Light**: A single colored light which is having a particular frequency is called as monochromatic light.
- **Monochromators**: Instruments used for getting a monochromatic light are called as monochromators. For eg. Prism, grating etc.
- Explain Beer and Lambert law.
- As a monochromatic light is passed through a solution, some intensity is absorbed in the solution. So, difference between the intensity of initial and transmitted light occurs.
- **Beer’s Law**: As the concentration increases the absorption increase and so intensity of transmitted light decreases exponentially.
- **Lambert’s Law**: As the thickness (or sometimes taken as cell length) increases the absorption increase and so intensity of transmitted light decreases exponentially.

\[ I_t = I_o \cdot e^{-kc} \]

Graph for Beer’s Law

\[ I_t = I_o \cdot e^{-kT} \]

Graph for Lambert’s Law
Here, $I_o =$ Intensity of initial (original) light
$I_t =$ Intensity of transmitted light
$k =$ constant
$C =$ concentration
$T =$ Thickness of the solution

As given in the above figure, as the transmission increases, absorbance decreases exponentially.
Deviation in Beer and Lambert's Law:
If a substance is following the beer's law, we get a straight line which is passing from the origin and the slope is “ab”. But some time we do not get a straight line. If we are getting much more value of absorbance than desired, than deviation is said as +ve deviation. But if we are getting much less value of absorbance than desired, than deviation is said as -ve deviation.

There are two reason for these deviations:

1. Instrumental Errors: The cause of instrumental errors are as follows:
   * Fluctuation in electricity
   * Source of light is weak or malfunction
   * Arrangement of filter/monochromator is not proper
   * Scattering of light is happening inside the instrument
   * Slit is not placed properly
   * Outside knob are not working according to inner instrumentation
   * Sensitivity of detector is low or malfunction

2. Chemical Errors: The cause of chemical errors are as follows:
   * Presence of bacteria
   * Solution is cloudy
   * Pigmentation is there in the solution
   * Acid-Base reaction is happening in the solution
   * Association-Dissociation reaction is happening in the solution
* Polarization reaction is happening in the solution
* If the color of the solution is changing with time
* If the absorption is happening due to solvent rather than solute
* This law is only applicable to some extent of concentration of the solution only. Below or above that concentration these laws are not applicable.
**Instrumentation of colorimeter:**

- Colorimeter is an instrument which compares the amount of light getting through unknown solution and the amount of light getting through a pure solvent.

**Instrumentation:**

The source light is passed through filter (to get desired frequency light) and concentrated using lens. Then this light is targeted on a sample (which is kept in cuvette). The light transmits through the sample and the transmitted light is measured by detector. As the intensity of the initial light is known, we can find the difference between - intensity of initial light and intensity of transmitted light.

**Practical application of colorimeter:**

- First of all, instrument is set to 100% transmission (0% absorption) for cuvette with the solvent only.
- Then known concentrations of the desired solution is prepared. (For eg. 1 ppm, 2 ppm, 3 ppm...)
- Then the absorptions related to the known concentrations are noted using the colorimeter.
- From the readings of absorption, a calibration curve is drawn. (graph: absorption vs concentration).
Then the absorption of the known concentration - of known solution is acquired using the colorimeter instrument and from the calibration curve the concentration of the unknown solution could be acquired.

Pre-requisite for a solution to be analyzed by colorimeter:
- The solution must be colored.
- The solution must not be having any contamination like Bacteria, Cloudy solution or Pigmentation. The solution must be clear (transparent).
- There must not be any reaction happening in the solution like acid-base, association-dissociation or polarization reaction.
- The solution must be having a particular concentration. Because lambert and beer laws can be applied only for particular range of concentration.
Difference between filter and monochromators: By filter a specific band of frequency can be acquired while by monochromators a specific ray of light can be acquired.

Difference between (monochromators) prism and grating:

<table>
<thead>
<tr>
<th>Prism</th>
<th>Grating</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.</td>
</tr>
<tr>
<td><img src="prism.png" alt="" /></td>
<td>![Grating.png]</td>
</tr>
<tr>
<td><img src="dispersion-angle.png" alt="" /></td>
<td></td>
</tr>
<tr>
<td>2. White Light</td>
<td>It is made up of aluminum or material with a bright surface. (2,500 to 60,000 lines per inch)</td>
</tr>
<tr>
<td>3. It can work between 400 to 1000 nm regions.</td>
<td>3. It can work between 200 to 800nm regions.</td>
</tr>
<tr>
<td>4. A spectrum is not as pure as grating.</td>
<td>4. A spectrum is much more pure as compared to prism.</td>
</tr>
<tr>
<td>5. 10 to 25 nm of ray band can be acquired.</td>
<td>5. 5 nm ray band can be acquired.</td>
</tr>
<tr>
<td>6. Dispersion of light is not sharp.</td>
<td>6. Dispersion of light is very sharp.</td>
</tr>
<tr>
<td>7. Ability of dispersion of light cannot be extended.</td>
<td>7. Ability of dispersion of light can be extended by increasing lines per inch.</td>
</tr>
<tr>
<td>8. No ghost spectrum is acquired.</td>
<td>8. Ghost spectrum is acquired when lines are not proper on grating.</td>
</tr>
</tbody>
</table>
Wave length selection method:

1. Use other filter than the color of the solution. Because the color of the solution is the color which is not absorbed by the solution. For eg. In case of CuSO₄ solution, use red colored filter.
2. Find the highest absorption using different filters of different colors.
3. The wavelength recommended for different solutions is given in the S.O.P. (standard operating procedure) manual of the instrument. So use that filter.
**Spectrophotometry**

- **Instrumentation of spectrophotometer:** There are two types of spectrophotometer available:
  1. Single beam spectrophotometer
  2. Double beam spectrophotometer

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**Single beam spectrophotometer**

**Double beam spectrophotometer**

- **Source of light:** The light source must be fulfilling the following requisitions:
  - The light coming from the source must be having proper intensity.
  - The light source must be having all the frequencies of light, so that the required frequency can be acquired.
  - The light source must be stable. It must not change with time.
  - For different region of light, following lamps (light sources) can be used:

<table>
<thead>
<tr>
<th>EM Region</th>
<th>Light Source</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. U.V. / near IR region</td>
<td>Hydrogen or Deuterium discharge Lamp</td>
<td>10-200 nm</td>
</tr>
<tr>
<td>2. Visible region</td>
<td>Tungsten Lamp</td>
<td>200-1000 nm</td>
</tr>
<tr>
<td>3. IR region</td>
<td>Nernst glover</td>
<td>1000-10,00,000 nm</td>
</tr>
</tbody>
</table>
Filter and Monochromators:
- From this part, the radiation with only specific wavelength or specific wavelength could be passed. Other radiations are absorbed.
- Filters:

![Color Wheel]

- Filters allow only a small section of frequency to pass through and all others are absorbed.
- Filters are used mostly in colorimeter. They are made up of glass or gelatin.
- By using different filters we can separate different regions of visible light as follows:

<table>
<thead>
<tr>
<th>Color of visible region</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Violet</td>
<td>380-470 nm</td>
</tr>
<tr>
<td>2. Blue</td>
<td>440-490 nm</td>
</tr>
<tr>
<td>3. Blue (slightly greenish)</td>
<td>490-500 nm</td>
</tr>
<tr>
<td>4. Green</td>
<td>500-560 nm</td>
</tr>
<tr>
<td>5. Yellow (slightly greenish)</td>
<td>560-580 nm</td>
</tr>
<tr>
<td>6. Yellow</td>
<td>580-600 nm</td>
</tr>
<tr>
<td>7. Orange</td>
<td>600-650 nm</td>
</tr>
<tr>
<td>8. Red</td>
<td>650-750 nm</td>
</tr>
</tbody>
</table>

- Monochromators: Optical devices used for selecting a specific wavelength from a range of frequency are called monochromators. They are of two types: 1. Slit 2. Dispersive element
  For dispersion of light, prism or grating or both can be used.
**Sample vessel (Cuvette):** In all the spectrophotometric and analysis the absorption of light is measured. So the cell used in the analysis must not absorb the light or the absorption must be minimum. Hence, for different region of spectrum - different material could be used as follows:

1. For UV range: Quartz cuvette
2. For visible range: glass cuvette
3. For IR range: NaCl, KBr, nujol cuvette

**Detector:** The light which is initiated from the source and pass through cuvette is measured by the detector. Absorption or transmission of the light can be measured by detector. Mainly three type of detectors can be used:

1. Photovoltaic Cell (Barrier layer Cell)
2. Photo tubes (Photo emissive tube)
3. Photo multiplier tubes

**Recorder:** The measurement of absorption or transmission is recorded in digital form in recorder. The graph of wavelength vs absorption can also be acquired. Figure for photo multiplier is given below:
Photo multiplier Tube:

- Due to very high amplification in Photo multiplier tube, it is much sensitive than simple photo cell tube. So It is very much used in spectrophotometer.
- There is a photo cathode, the surface of which is coated with light reflecting material.
- There are also symmetrically arranged poles which are called as dynode. Each dynode is also having light reflecting material. Which is having more potential than sequential cathode? Dynode is set to +ve voltage.
- So, when light indented on the surface of cathode, a primary electron is generated.
- These electrons flows to the neighboring dynode whose potential is 50-90 v more than cathode. Moreover each electron generates 4-5 secondary electrons.
- This process happens on almost 9 dynode. Thus, amplification happens in the tube and shower of electron \((4^9 = 2.6 \times 10^6)\) occurs.
- When this shower of electron is captured by detector, intensity of the light can be known and thus we can have strong signal.
- To start the tube, 500 to 900 V of electricity is necessary which is applied using no. of batteries in a sequence.
Numericals: