COLORIMETRY

• By VIJAYA BHARATHI GUDAPA REDDY
• JL IN MLT, KSRGJC (G)
• ANANTAPUR
Types

Colorimetric analysis is two types

- **Visual** colorimetry
- Photo-electric colorimetry

Visual colorimetry is one of the oldest form of color measuring technique which is not used now day, natural or artificial light is used as light is used as light source and determinations are made with a colorimetry or color comparator where human eye is used as detector.
Beer’s law:

- When a monochromatic light passes through a colored solution, amount of light transmitted decreases exponentially with increase in concentration of colored substance.
  - i.e. the amount of light absorbed by a colored solution is directly proportion to the conc. Of substance in the colored solution.

\[ A = \alpha C \]
Lambert’s law:

• The amount of light transmitted decreases exponentially with increase in path length (diameter) of the cuvette or thickness of colored solution through which light passes.
  - i.e. the amount of light absorbed by a colored solution depends on path length of cuvette or thickness or depth of the colored solution.
Combined Beer’s- Lambert’s law

- Combined Beer’s- Lambert’s law is thus expressed as amount of light transmitted through a colored solution decreases exponentially with increases in conc. Of colored solution & increase in conc. of colored solution & increase in the path length of cuvette or thickness of the colored solution.

- Combining the two laws:

\[ A \propto C \times L \]
\[ A = K \times C \times L \]

Let \( A_T \)= absorbance of the test solution
\( C_T \)= concentration of the test solution
\( A_S \)= absorbance of the standard solution
\( C_S \)= concentration of the standard solution
\[ A_T = K \times C_T \times L \]
\[ A_S = K \times C_S \times L \]

\[ \frac{A_T}{A_S} = \frac{K \times C_T \times L}{K \times C_S \times L} \]
\[ \frac{A_T}{A_S} = \frac{C_T}{C_S} \]

\[ C_T = \frac{A_T}{A_S} \times C_S \]
Concentration of TEST sol.:

\[ C_T = \frac{A_T}{A_S} \times C_S = \frac{OD_T}{OD_S} \times C_S \]

Concentration of TEST/100ml:

\[ \text{Concn of Std X 100} \times \frac{\text{Absorbance of TEST}}{\text{Absorbance of STANDARD}} \times \]
Concentration of TEST /100ml

\[ \text{Concentration of TEST /100ml} = \frac{\text{O.D of 'T' - O.D of 'B'}}{\text{O.D of 'S' - O.D of 'B'}} \times \frac{\text{Amount of 'S'}}{\text{Volume of 'T'}} \times 100 \]
Colorimeter
Components of Colorimetry

- **Light source**
- **Collimator** (Lens)
- **Monochromator** (Prism or Grating)
- **Wavelength Selector** (Slit)
- **Sample Solution** (in Cuvette)
- **Detector** (Photocell)
- **Digital Display or Meter**
1. **Tungsten lamp:**
   - Filament mode of tungsten sealed in a glass envelope.
   - Filed with inert gas.
   - Life time is limited due to gaseous tungsten formed by sublimation.
Light source

Carbon arc lamp

- If sufficient intensity of light is not obtained from tungsten lamp then carbon arc lamp can be use as a source for color measurement.
Sample Holder/ Cuvette

- Cuvettes are rectangular cell, square cell or circular one.
- Made up of optical glass for visible wavelength (quartz or fused silica for UV).
- Common one is square, rectangular to avoid refraction artifacts.
- Optical path (length) of cuvette is always 1 cm.
- Capacity may be 3 ml/2 ml/1 ml depending upon the thickness of the wall of the cuvette.
- For accurate and precise reading cuvette must be transparent, clean, devoid of any scratches and there should be no bubble adhering to the inner surface of the filled cuvette.
Preparation of solution for investigation

- In colorimetric estimation it is necessary to prepare 3 solutions:

- BLANK(B)
- STANDARD(S)
- TEST(T)
To eliminate the effect of light absorption by the reagent used

Water BLANK

Reagent BLANK
Solution of known concentration of the substance

Both O.D and concentration are known

So concentration of unknown can be calculated
Test solution is made by treating a specific volume of the test sample with reagents

As per procedure
Steps In Operation

1. Glass/gel filter is placed in the filter slot
2. 3/4\textsuperscript{th} of cuvette is filled with distilled water and placed in the cuvette slot
3. Instrument is switched ‘on’ and allowed to warm-up for 4-5 minutes
Button is adjusted using ‘coarse’ and ‘fine’ knobs to give zero optical activity in the galvanometer.

Blank solution is placed in an identical cuvette and the OD is read (‘B’).

Blank solution is transferred to the original test tube.
Test solution is taken in the same cuvette and O.D. is read (‘T’)

Test solution is transferred back to the original test tube

Standard solution is taken in same cuvette and O.D. is read (‘S’)

Standard solution is transferred back to the test tube

Cuvette is washed
Calculation

\[
\%\text{ conc. of test} = \frac{T - B}{S - B} \times \frac{Cs}{V} \times 100
\]

Cs \rightarrow \text{Conc. Of standard}

V \rightarrow \text{Volume of test sample}

NOTE :- Satisfactory results are obtained only when the O.D. values are in the range 0.1 – 0.7
Light spectrum and their wavelengths

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Region name</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;380</td>
<td>Ultraviolet</td>
<td>Invisible</td>
</tr>
<tr>
<td>380-440</td>
<td>Visible</td>
<td>Violet</td>
</tr>
<tr>
<td>440-500</td>
<td>Visible</td>
<td>Blue</td>
</tr>
<tr>
<td>500-580</td>
<td>Visible</td>
<td>Green</td>
</tr>
<tr>
<td>580-600</td>
<td>Visible</td>
<td>Yellow</td>
</tr>
<tr>
<td>600-620</td>
<td>Visible</td>
<td>Orange</td>
</tr>
<tr>
<td>620-750</td>
<td>Visible</td>
<td>Red</td>
</tr>
<tr>
<td>800-2500</td>
<td>Near-infrared</td>
<td>Not visible</td>
</tr>
</tbody>
</table>
Verification of Beer’s Law

- Prepare 1% standard solution of glucose, i.e. 1gm/dl 1000mg/100ml.
- Make different dilutions of standard solution using the general formula given as following for obtaining different concentrations of a solution by dilution with diluent (DW):

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>Conc. (mg%)</th>
<th>Amount of mL needed, ( V1 ) ( C1 \times V1 = C2 \times V2 )</th>
<th>DW (mL)</th>
<th>Total vol. V2 (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>( 1000 \times V1 = 50 \times 2, V1= 0.1 )</td>
<td>1.9</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>( 1000 \times V1 = 100 \times 2, V1= 0.2 )</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>( 1000 \times V1 = 150 \times 2, V1= 0.3 )</td>
<td>1.7</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>( 1000 \times V1 = 200 \times 2, V1= 0.4 )</td>
<td>1.6</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>( 1000 \times V1 = 250 \times 2, V1= 0.5 )</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>( 1000 \times V1 = 300 \times 2, V1= 0.6 )</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>350</td>
<td>( 1000 \times V1 = 350 \times 2, V1= 0.7 )</td>
<td>1.3</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>400</td>
<td>( 1000 \times V1 = 400 \times 2, V1= 0.8 )</td>
<td>1.2</td>
<td>2</td>
</tr>
</tbody>
</table>
Relationship between absorbance and transmittance
Beer's law
Applications Of Colorimeter

- Estimation of biochemical compounds in blood, plasma, serum, CSF, urine, etc.:
  - Glucose
  - Urea
  - Creatinine
  - Uric Acid
  - Bilirubin
  - Lipids
  - Total Proteins
  - Enzymes [e.g. ALT, AST, ALP]
  - Minerals [Calcium, Phosphorus etc.] etc....
Thank you