UV/VIS SPECTROPHOTOMETER

MECASYS, Co., Ltd.
What is UV/VIS?

- Electromagnetic Spectrum

<table>
<thead>
<tr>
<th>Type of Radiation</th>
<th>Type of Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Rays</td>
<td>Inner Electron</td>
</tr>
<tr>
<td>UV/VIS</td>
<td>Outer Electron</td>
</tr>
<tr>
<td>IR</td>
<td>Molecular vibration</td>
</tr>
<tr>
<td>Micro Wave</td>
<td>Molecular rotation</td>
</tr>
</tbody>
</table>

\[ \Delta E = h \nu \]

\[ \Delta E = E_1 - E_0 \]
Principle of UV/VIS SPECTROPHOTOMETER

- Principle
  - Light Intensity Change: By Absorbance or Transmittance
  - Quantity: Using Absorbance

\[ P_0 \xrightarrow{b} \lambda \xrightarrow{I} \lambda \]

![Diagram of UV/VIS Spectrophotometer Principle](image)
Lambert-Beer’s Law

- Transmittance, t
  \[ \frac{I_t}{I_0} = t \]

- Absorbance, A
  \[ \log \frac{1}{t} = A \]

- Lambert – Beer’s Law
  \[ A = abC, \quad A = \xi bC \]
  - b: Sample Path length, C: Sample Concentration,
  - a: Absorbance Constant
  - \( \xi \): Molecular Absorbance Constant
Standard Curve, Calibration curve

- **Lambert-Beer’s Law**
  - \( A = abc \)
  - Concentration determination of Sample

- **Standard Curve**
  - Standard Samples
  - Proportional constant
  - Absorbance measurement of Samples

![Absorbance vs Concentration graph](image)

Concentration determination of Sample
Limitation of Lambert-Bear’s Law

- Deviations in absorptivity coefficients
  - at high concentrations (>0.01M) due to electrostatic interactions between molecules in close proximity
- Scattering of light
  - due to particulates in the sample
- Fluorescence or Phosphorescence of the sample
- Changes in refractive index at high analysis concentration
- Shifts in chemical equilibria as a function of concentration
- Stray light
Application of UV/VIS Spectrophotometer

Quantity analysis
(Using a Spectrum)

Quality analysis
(Standard Curve)

Chemical analysis
Food safety analysis
Blood analysis
DNA/RNA Conc. analysis
Residual Pesticide analysis
Residual chlorine analysis
Functions of Spectrophotometer

- **ABS/\%T/CONC**
  - Measuring Absorbance/Transmittance/Concentration

- **Survey Scan**
  - Measuring Absorbance(Transmittance) Spectrum

- **Standard Curve (Calibration Curve)**
  - Determining Proportional Constant between ABS and Conc

- **Simple Kinetics**
  - Measuring Time Dependent ABS Variation

- **ABS Ratio**
  - Measuring ABS Ratio (DNA/Protein)
Structure of UV/Visible Spectrophotometer

- Light source
- Lens or Mirror
- Monochromator
- Sample
- Detector
- Amplifier
- Readout
Example

Spectrophotometer

Light Source

Monochromator

Sensing Part

Sample Compartment

Applied Technology Development Division
Light Source

- **Deuterium Arc Lamp**
  - UV Region
  - Wavelength Range: 190~420nm

- **Tungsten Lamp**
  - Wavelength Range: Part of the UV and the whole of the Visible range (약 350 ~ 2,500nm)

- **Xenon Lamp**
  - Wavelength Range: 190~800nm
Monochromator

- Accepts polychromatic input light from a lamp and outputs monochromatic light
- Components: Entrance slit, Dispersion device, Exit slit

![Diagram of a monochromator showing the path of light through the components: entrance slit, dispersion device, and exit slit.]

White Light ➔ Entrance slit ➔ Dispersion device ➔ Exit slit ➔ Monochromatic Light
Dispersion Device (I)

- **Filter**
  - Absorptive Filter
  - Interference Filter: FWHM 10 – 50 nm
Dispersion Device (II)

- Prism
Dispersion Device (III)

- Grating
  - Plane Grating, Concave Grating
Monochromator structure

Littrow mounting

Czerny-turner mounting

Mirror

Grating

Slit

Exit slit

Grating

Entrance slit
Monochromator Design

- **Modified Czerny-Turner Type**
  - Focal Length: 200mm
  - Sine Drive Mechanism
  - 1nm Bandwidth

- **Czerny-Turner Type**
  - Focal Length: 100mm
  - Direct Drive Mechanism
  - 4nm Bandwidth
Absorption Cell

- Receptacle for Sample.
- Material
  - Quartz, Fused Silica (Using over 190nm)
  - Glass (Using Over Visible Region)
Detector

- PMT (Photo Multiplier tube)
- Photodiode
- Photodiode & CCD
Type of UV/VIS Spectrophotometer

- Single Beam Type
- Double Beam Type
Type of UV/VIS Spectrophotometer

- **Scanning type:**

- **PDA type:**
Specifications Example

- **Spectral Bandwidth**: 1 nm
- **Wavelength**
  - Range: 190 – 1100 nm
  - Accuracy: ± 0.5 nm
  - Reproducibility: ± 0.1 nm
- **Photometric**
  - Range: -0.3 – 3.0 Abs (0 – 125 %T)
  - Accuracy: < 1% at 1 Abs
  - Reproducibility: ± 0.001 Abs
- **Stray Light**: <0.05% at 220 nm & 340 nm
Accessories (I)

- Multi-Cell Holder
- Sipper System
Accessories (II)

◆ Micro Volume Cell  ◆ Round Cell  ◆ Long Path Cell

◆ Temperature Cell Holder, Film/Thin-Layer Holder
Thank You!