UV/visible spectrophotometry: selected readings from textbook

Chapter 19 (inclusive)
Chapter 20 (section 20.1 only)
Chapter 21 (sections 21.2 thru 21.6)

Exercises: 19-B, 19-C

Problems: 19.9, 19.10, 19.16, 19.19, 19.28
21.16, 21.17, 21.20

Notes on UV/visible spectrophotometry

Use wavelength of maximum absorbance (λ_{max});

i) greater sensitivity of analysis since greater absorbance signal for given analyte concentration (ε_{max} at λ_{max})
ii) absorption curve is generally flat near λ_{max}, therefore variations in wavelength have little effect and larger slit widths can be used

Best results (minimum uncertainty) obtained between 0.4 and 0.9 absorbance units; Abs > 1, less than 10% transmittance and detector response is low with an associated high relative error

Abs < 0.1, greater than 90% and detector has response of reference (blank) is similar sample and again the relative error increases

Using UV/visible spectrometry

Masking Agents are used to selectively react with a known interferent. Masking agents act by immobilizing or chemically binding to an interferent in a form that no longer contributes to or attenuates the signal from the analyte.

Example; masking Fe^{3+}

To mask Fe^{3+} interference in spectrophotometric analysis of Mn^{2+}
Use phosphate to precipitate $FePO_4(s)$, filter and analyze supernatant

To mask Fe^{3+} interference in volumetric analysis of Ca^{2+} using EDTA
Use cyanide to complex iron as $Fe(CN)_6^{3-}$

Direct Calibration:

Zero instrument with reagent blank
Record absorbance for series of standards to check linear range
Abs α $Conc_x$; therefore $A_{unk}/A_{std} = C_{unk}/C_{std}$ (in linear range)
Or use calibration curve to interpolate unknown concentration from linear regression ($y = mx + b$)

Standard Addition:

Use single or multiple spikes of standard to compensate for matrix effects (i.e., interferences and/or material losses)

Analyzing Mixtures of Absorbing Species:

If both ε and Abs are known at two wavelengths ($\lambda 1$ and $\lambda 2$) for two absorbing species (x and y), then the concentration of each can be calculated (C_x and C_y).

$$\text{Abs}(\lambda 1) = \varepsilon_x(\lambda 1)\, b\, C_x + \varepsilon_y(\lambda 1)\, b\, C_y$$

$$\text{Abs}(\lambda 2) = \varepsilon_x(\lambda 2)\, b\, C_x + \varepsilon_y(\lambda 2)\, b\, C_y$$

Since $\varepsilon_x(\lambda 1)$, $\varepsilon_y(\lambda 1)$, $\varepsilon_x(\lambda 2)$, $\varepsilon_y(\lambda 2)$ are known and Abs ($\lambda 1$), Abs ($\lambda 2$) are measured, solve two equations for two unknowns C_x and C_y (see handout).

UV/visible light detectors

1) Photomultiplier tube (PMT) – A photoejected electron cascades through a series of dynodes each of which amplifies the signal by ejecting several electrons for each electron absorbed. (see figure)

2) Photodiode Array – A doped silicon semi-conductor chip that creates charge separation with the absorption of a photon. Charge can be measured as a current required to compensate charge separation. Current to frequency converters amplify signal. Output is measured as a frequency (cycles per unit time α light intensity).

   Diode arrays are have many microchip photodiodes arranged as a linear array, each acting as an independent microdetector. The resulting mutichannel analyzer can be used to generate an entire absorption spectra simultaneously without the need for wavelength scanning. Photodiode arrays are not as sensitive as PMT's but they generate a quick spectrum and require no moving parts, since dispersed light impinges directly on multichannel diode array.