

# Absorption by Organic Compounds:

Two types of electrons are responsible for the absorption of ultraviolet and visible radiation by organic molecules:

- (1) shared electrons that participate directly in bond formation and
- (2) unshared outer electrons that are largely localized on atoms such as oxygen, the halogens, sulfur, and nitrogen.

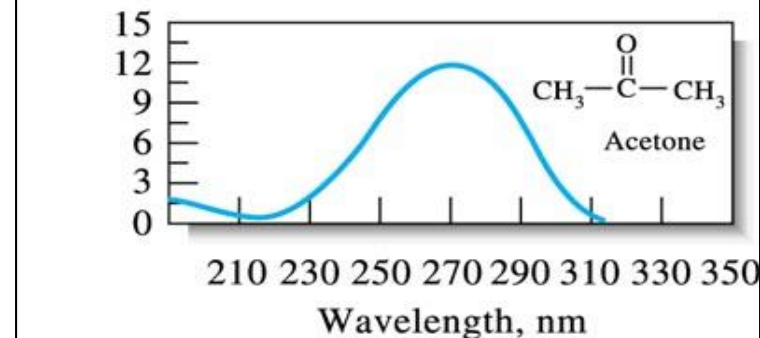
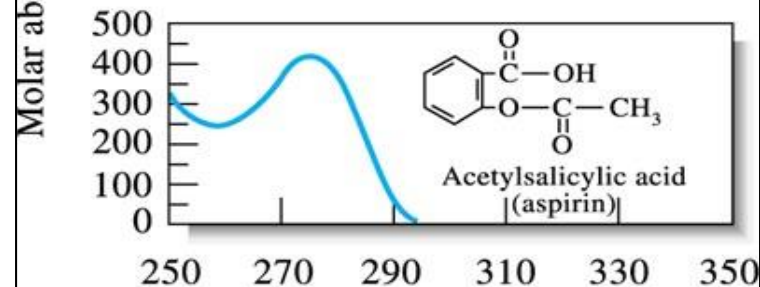
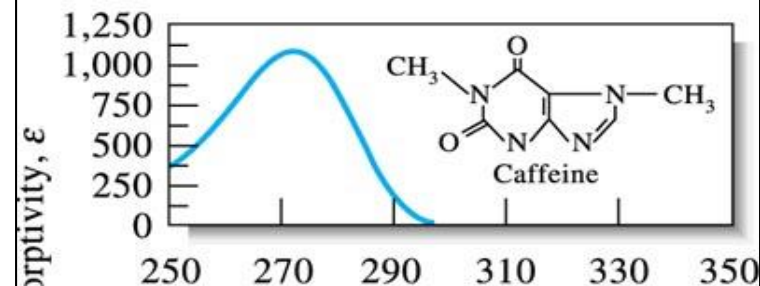
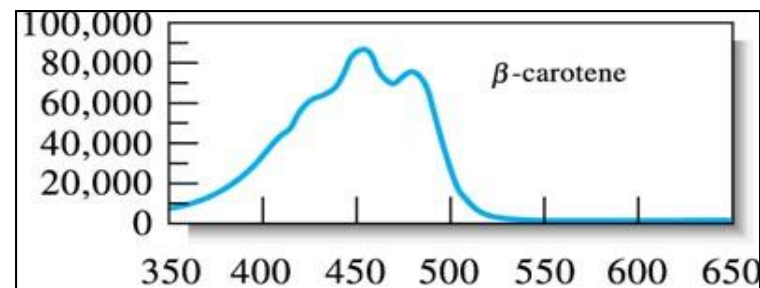


TABLE 26-1

## Absorption Characteristics of Some Common Organic Chromophores

Chromophore	Example	Solvent	$\lambda_{\max}$ , nm	$\epsilon_{\max}$
Alkene	$C_6H_{13}CH=CH_2$	<i>n</i> -Heptane	177	13,000
Conjugated alkene	$CH_2=CHCH=CH_2$	<i>n</i> -Heptane	217	21,000
Alkyne	$C_5H_{11}C\equiv C-CH_3$	<i>n</i> -Heptane	178	10,000
			196	2,000
			225	160
Carbonyl	$\begin{array}{c} O \\    \\ CH_3CCH_3 \end{array}$	<i>n</i> -Hexane	186	1,000
			280	16
	$\begin{array}{c} O \\    \\ CH_3CH \end{array}$	<i>n</i> -Hexane	180	Large
			293	12
Carboxyl	$\begin{array}{c} O \\    \\ CH_3COH \end{array}$	Ethanol	204	41
Amido	$\begin{array}{c} O \\    \\ CH_3CNH_2 \end{array}$	Water	214	60
Azo	$CH_3N=NCH_3$	Ethanol	339	5
Nitro	$CH_3NO_2$	Isooctane	280	22
Nitroso	$C_4H_9NO$	Ethyl ether	300	100
			665	20
Nitrate	$C_2H_5ONO_2$	Dioxane	270	12
Aromatic	Benzene	<i>n</i> -Hexane	204	7,900
			256	200

- $\lambda$ s of these characteristic absorptions and their  $\epsilon$  are changed due to the presence of other chemical gps (*OH, NH<sub>2</sub>, X*-auxochromes), unshared es)  $\rightarrow$  absorption at longer  $\lambda$   $\rightarrow$  increase of  $\epsilon$
- Compounds with several chromophores and auxochromes are likely to be coloured

**TABLE 26-2**

**Absorption by Organic Compounds Containing Unsaturated Heteroatoms**

Compound	$\lambda_{\max}$ , nm	$\epsilon_{\max}$
CH <sub>3</sub> OH	167	1480
(CH <sub>3</sub> ) <sub>2</sub> O	184	2520
CH <sub>3</sub> Cl	173	200
CH <sub>3</sub> I	258	365
(CH <sub>3</sub> ) <sub>2</sub> S	229	140
CH <sub>3</sub> NH <sub>2</sub>	215	600
(CH <sub>3</sub> ) <sub>3</sub> N	227	900

# Electronic transitions in metal complexes

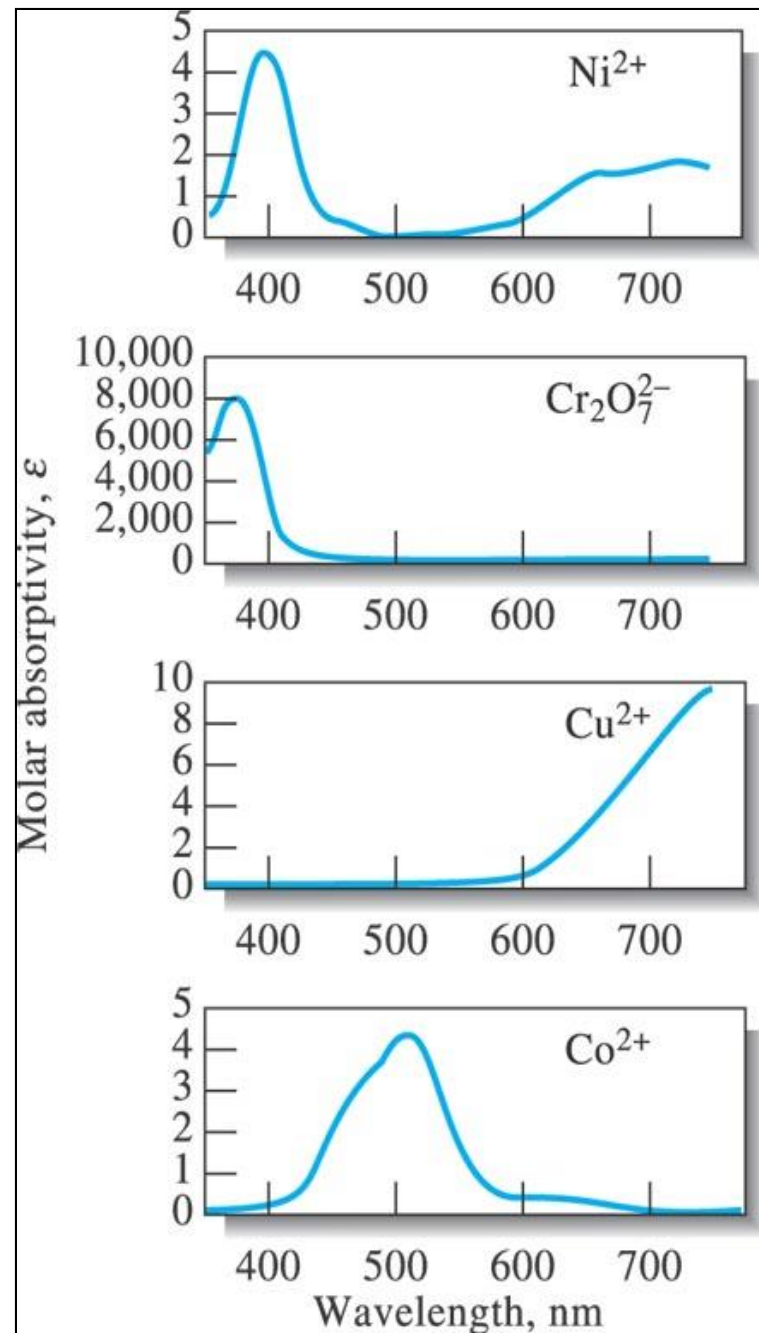
- M–L (complex) band is different from that of M and of ligand.
- Ligands, acid, base (Lewis)

• *There are 3 types of basic electronic transitions which are considered to explain the absorption spectra of M–L.*

- 1- Excitation within the transition metal ions in complex (*d-d transition*).
- 2- Excitation related to *charge-transfer transition* (M → L, L → M).
- 3- Excitation within the ligands in complex.

# 1- $d-d$ ( $f-f$ ) transition

- Transition metal complexes with a  $d^n$  ( $f^n$ ) electron configuration of the central M ion.
- In general, the ions and complexes of elements in the first two transition series absorb broad bands of vis radn in at least one of their oxidn states  $\rightarrow$  they are colored.
- $L \rightarrow$  splitting of M ion's basic levels  $\rightarrow$  e's are found at those energy levels.
- They are sometimes donated as L-field absorption bands.



- The  $\Delta E$  Between those d (f) orbitals is *not very great*  $\rightarrow$  transition metal complexes (also their aquo ions) are *coloured*  $\rightarrow$  absorption spectra in the *visible* region.
- Theoretically, *d-d* transitions are "*forbidden*"  $\rightarrow$  *very low intensity* ( $\epsilon \sim 10-200$ )  $\rightarrow$  their use in spectrophotometric detn is limited, but they are important in *theoretical studies*.

## 2- Charge – transfer (CT)

- Electron transfer betn L & M ion
- Atomic / molecular orbital with higher e density → transfer to orbital of another atom / molecule with lower e density.
- 2 types L → M, M → L
- CT transition intensity > d-d,
- $\epsilon = 10^2 - 10^4$
- CT bands are observed in the complexes existing 2 oxdn states differing by one.
- $\text{Fe}^{3+} / \text{Fe}^{2+}$ ,  $\text{Ti}^{4+} / \text{Ti}^{3+}$

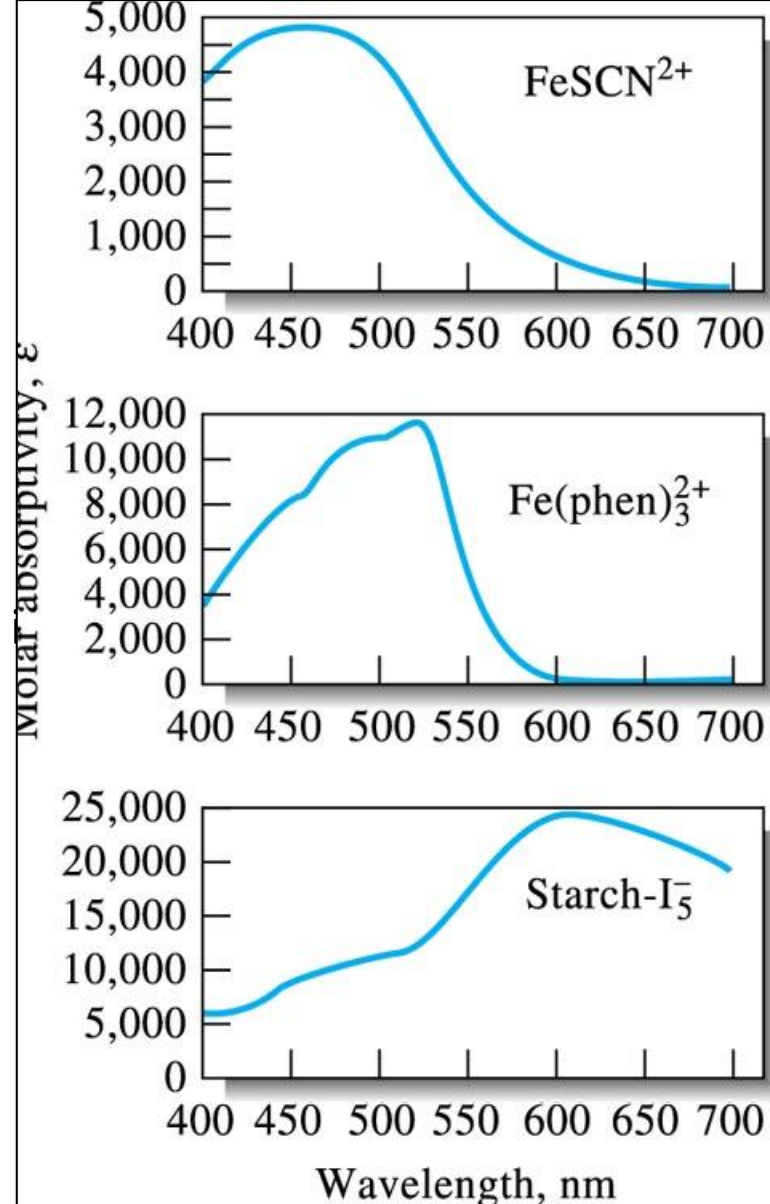
For quantitative purposes, C-T A is important because  $\epsilon$  are large, → high sensitivity.

Many inorganic and organic complexes exhibit this type of absorption and are therefore called C-T complexes.

A C-T complex consists of an e-donor gp bonded to an e acceptor.

When absorbs radn, an e is transferred from the donor to an orbital of the acceptor.

The excited state is the product of a kind of internal oxidation/reduction process.

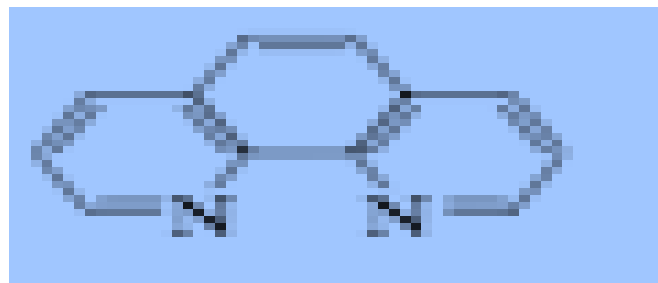


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## 3- Excitation within the ligands in complex.

## 2- Quantitative Methodology

- Let us suppose that you are familiar with the method for determining the iron content of water, based on the formation of the red-orange complex of Fe(II) with 1,10-phenanthroline (the most often used complexing agent for iron when present at *low concentrations*).
- **Fe(1,10-phen)<sub>3</sub><sup>2+</sup>**
- **Charged complexes**
- **Solubility in organic solvents.**



*Can you suggest what details would be specified in a typical procedure involving a spectrometric method of analysis of an inorganic component in a sample of tap water?*

- (a) the amount of material to be used
- (b) the amounts of reducing agent, complexing agent (1,10-phen), buffer, time.
- (c) the prepn of calbn soln
- (d) the wavelength and cell dimensions.
- (e) the method of calculations.



- *What other steps have to be specified for the determination of an inorganic component in an organic matrix such as a food product, or in a water sample with a high organic content?*

- Organic matrix may cause severe interference problems → 2 additional steps are:
- (f) Removal of the organic matter

Wet oxidation

ashing

- (g) solution of the residue by and acid dissolution procedure

- *For the determination of the iron content of water, some questions that might be asked include:*
- Is the *1,10-phen* is the *best reagent* for iron?
- Is a *reducing agent* needed?
- Why do we add the *buffer* solution?
- If *time* is important does that mean that the colour of the complex is unstable?
- Do we always use  $\lambda_{\max}$ ?
- Can we use plastic *cells*?

- When a sample is to be analysed by uv/vis consider the following, when designing an analytical procedure,
- Sample preparation,
- Solution conditions of measurement,
- Instrumental parameters.

## 2.1 solution preparation, solvents and cells

- When we have a solution of an absorbing constituent, the absorption either being a property of the original analyte or of a chemical derivative of that analyte.

### 2.1.1 Stability and solubility

- (a) Absorbing species should be stable for sufficient time.
- *Instability can arise from: air oxidation, photochemical decomposition, solution conditions (solvent, pH, T)*
- (b) Colloidal or insoluble material must not develop due to slow hydrolysis or other reaction with the solvent. If the product is insol. → extract into another solvent.
- *The presence of colloidal or suspended material → light scattering → an increase in absorbance*

## 2.1.2 Choice of solvent

- (a) good solubilising power.
- (b) stable interactions with the absorbing species.
- Solvent should be transparent in the region of measurement and pure.

- Water is the cheapest, most transparent solvent for water-soluble substances.
- Closed cells and air bubbles.
- Light scattering / boiling water.
- Distilled / deionised H<sub>2</sub>O is pure.

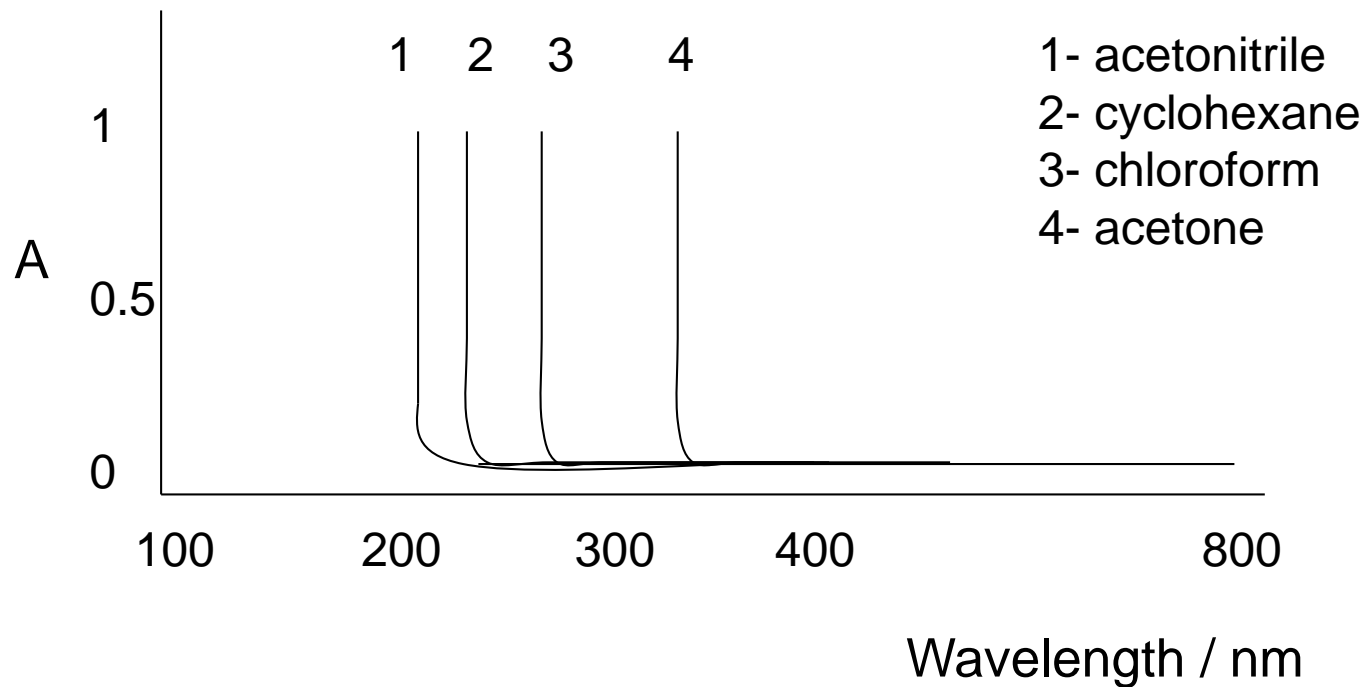
### **Other solvents**

- Alcohols (methanol, ethanol, propan-2-ol) can be used at low UV region (transparent).
- Hexane, cyclohexane (UV)
- Trichloromethane, tetrachloromethane, ethers (diethylether) are used in solvent extraction (metal complexes) –they have limited transparency in the UV<300nm.
- Diethyl ether is also unsuitable because of its high volatility.

- *Cut-off wavelengths for common solvents. Values at which the transmittance falls to 25% ( $A=0.602$ ) measured in 10 mm cell using water as the reference.*

<b>solvent</b>	<b><math>\lambda</math>(nm)</b>
<b>Hexane</b>	<b>199</b>
<b>Heptane</b>	<b>200</b>
<b>Isoctane</b>	<b>202</b>
<b>Diethylether</b>	<b>205</b>
<b>Ethanol</b>	<b>207</b>
<b>Propan-2-ol</b>	<b>209</b>
<b>Methanol</b>	<b>210</b>
<b>Cyclohexane</b>	<b>212</b>
<b>Acetonitrile</b>	<b>213</b>
<b>Dioxan</b>	<b>216</b>
<b>Dichloromethane</b>	<b>233</b>
<b>Tetrahydrofuran</b>	<b>238</b>
<b>Trichloromethane</b>	<b>247</b>
<b>Tetrachloromethane</b>	<b>257</b>
<b>Dimethyl sulphoxide</b>	<b>270</b>
<b>Dimethyl formamide</b>	<b>271</b>
<b>Benzene</b>	<b>280</b>
<b>Pyridine</b>	<b>306</b>
<b>propanone</b>	<b>331</b>

*The electronic spectra of some common solvents in 1 cm cell, for acetonitrile  $\lambda_{max} = 167$  nm, and for acetone  $\lambda_{max} = 279$  nm*



## 2.1.3 Sample Cells (Cuvettes)

- Visible region – glass, transparent plastic (aq. solution).
- UV - < 330 nm quartz, fused silica cells.
- Choose 2 matching pair one for the blank and the other for the sample (s)

### For measurements

- Adjust  $\lambda$
- Zeroing ( $A=0$ ,  $T\% = 100$ )
- Wash your cells then fill it with blank, be sure it is dry and clean. Avoid having any air bubbles.
- If  $A$  is  $> 0.02$  you can not set the reading to zero.

A biochemical enzymatic analysis is being carried out at 340 nm by spectrometric measurements. Indicate which of the following would result in a small (S) and which would result in a large (L) effect on the measured absorbance.

- (i) cloudy sample
- (ii) glass cell
- (iii) contamination with propanone.
- (iv) tungsten source.
- (v) the pH is not adjusted.

- L
- S
- L
- S
- L



## 2.2 Reagents, complexation techniques, soln conditions

Some analysed materials

have absorption bands

require selective reagent (high  $\epsilon$ )

### CuSO<sub>4</sub>

H<sub>2</sub>O

amm

DEDIC (diethyl  
dithiocarbamate)

$\lambda = 800$  nm

C = 0.01 mol/L

A = 0.2

$\lambda = 600$  nm

C = 0.01

mol/L

A = 0.8

$\lambda = 436$  nm

C = 10<sup>-4</sup> mol/L

A = 1.0

## 2.2.1 The ideal reagent

- best available reagent and solution conditions
- 1,10-phen / Fe, DEDC / Cu
- (a) stability in soln
- (b) reproducible
- rapid reaction, rate of reaction.
- (d) selectivity or specificity
- (e) solvent compatibility.
- (f) Linear calibration

## 2.2.2 Choice of reagent

- Though DEDC is the best for Cu we can use
- $\text{NH}_4\text{OH}$  and this depends on the
- nature of the sample.
- e.g.  $\text{NH}_4\text{OH}$  for Cu in steel
- concentration is high
- no interfering species (Ni)

- $\text{SCN}^-$  for  $\text{Fe}^{3+}$
- simple but the resulting complex is
- non-stoichiometric and the colour is unstable.
- The stability of colour is influenced by:
  - (a) concentration
  - (b) ionic strength
  - (c) interference ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ )

## Some of the best known reagents for the determination of Fe (II)

• Reagent	$\epsilon_{\max}$	$\lambda_{\max}/\text{nm}$
• 2,2'-bipyridyl	800	522
• 1,10-phen	1100	510
• 4,7diphen-10-phen	2240	533
• 2,4,6-tri(2-pyridyl)- 1,3,5-triazine (TPTZ)	2260	595

## 2.2.3 Solution conditions for analysis

- Check: accuracy, precision and detection limit.
- (a) solvent polarity
- (b) pH, ionic strength (aq soln)
- (c) temp

*Other factors which might well influence the analysis include:*

- (d) order of addn of reagents,
- (e) mixing or stirring rate,
- (f) time for colour development.

## 2.3 Choice of wavelength and calibration data

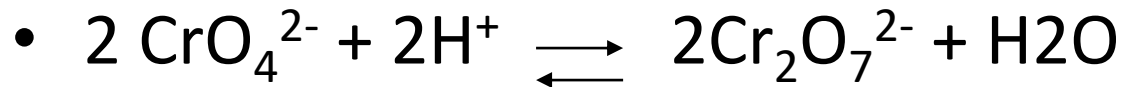
- adjust  $\lambda$ .
- Scan  $\lambda$  range
- Calibration measurements.
- $\lambda_{\max}$  or at the top of an alternative absorption peak.
- Detmn of Mn in steel
- The sample blank

# Limitations in the application of the Beer-Lambert Law

- (a) optically homogeneous soln,
- (b) monochromatic light,
- (c) low concentration

## Chemical deviation

- (1) The comp in soln must not dissociate or associate.
- $AB \rightleftharpoons A^+ + B^-$
- Picric acid (pale yellow)  $\xrightleftharpoons{H_2O}$  dissociation (yellow)
- (2) No interaction between solute and solvent.
- (3) pH should be constant

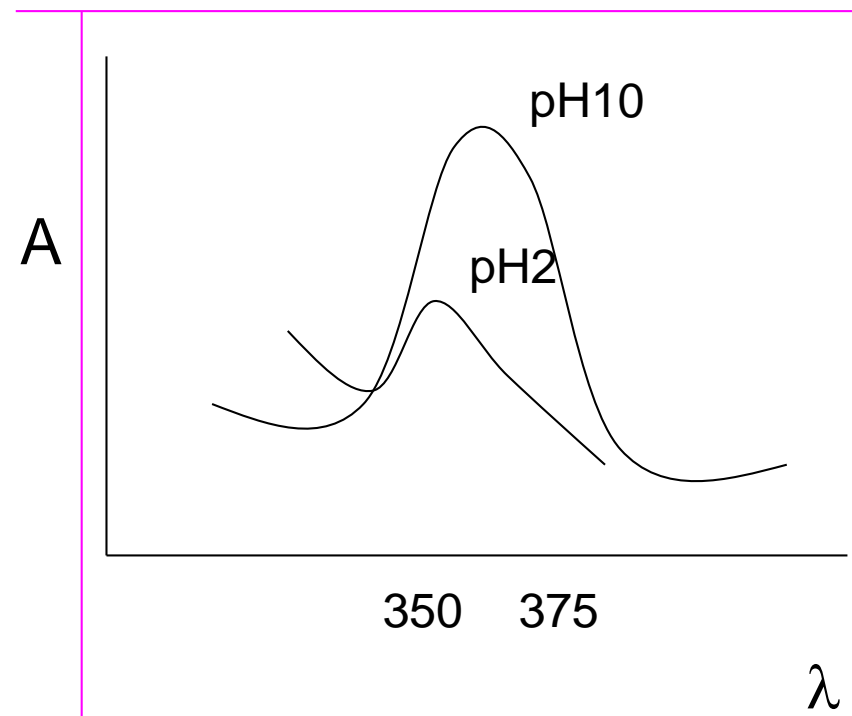


- Suggest 2 ways to overcome this problem when preparing Cr (VI) standard solution ( $\text{K}_2\text{Cr}_2\text{O}_7$ )

- One of the ways is chemical and the other is spectral.

- (a) Buffer all soln at  $\text{pH} < 3$ ,  $\lambda = 348$

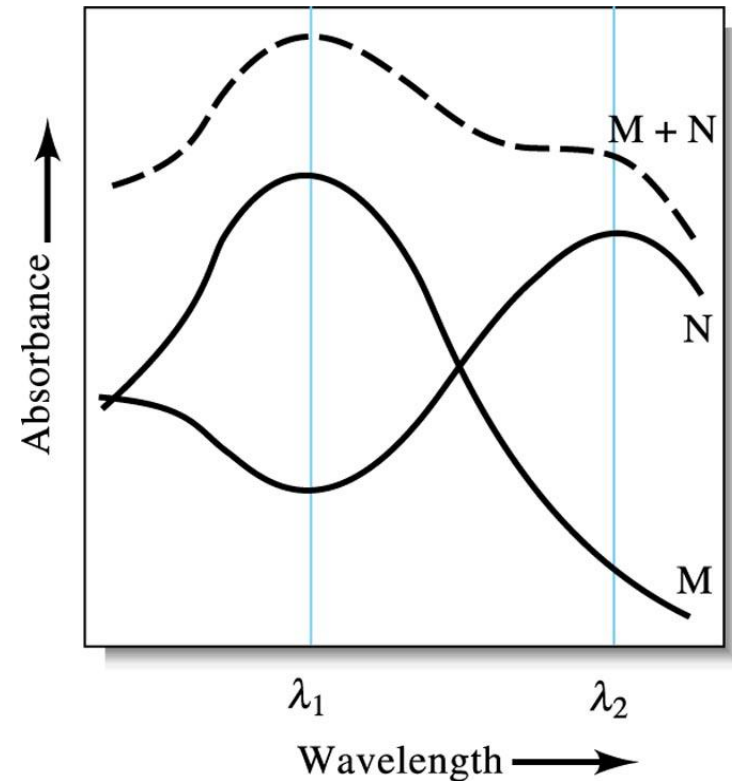
- (b) Un-buffered soln at isobestic point ( $\lambda = 340$ ), A is independent of pH at this  $\lambda$ .





# Binary and multi-component systems

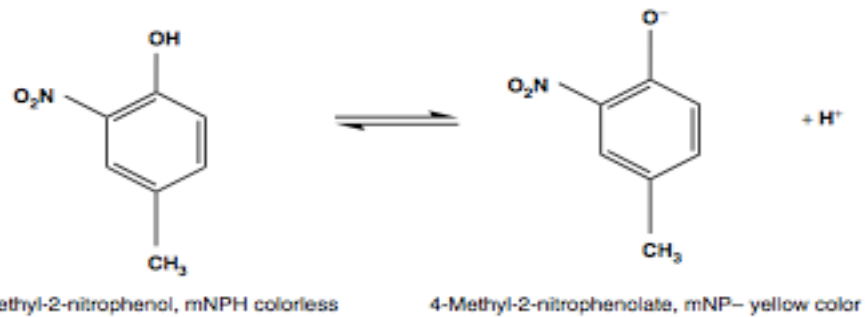
- $A_m = A_x + A_y$
- $= \epsilon_x c_x l + \epsilon_y c_y l$
- At  $\lambda_1$   $A_m^1 = \epsilon_x^1 c_x l + \epsilon_y^1 c_y l$
- At  $\lambda_2$   $A_m^2 = \epsilon_x^2 c_x l + \epsilon_y^2 c_y l$
- A of the components in a
- mix are additive.
- They continue to obey the
- Beer-Lambert Law.



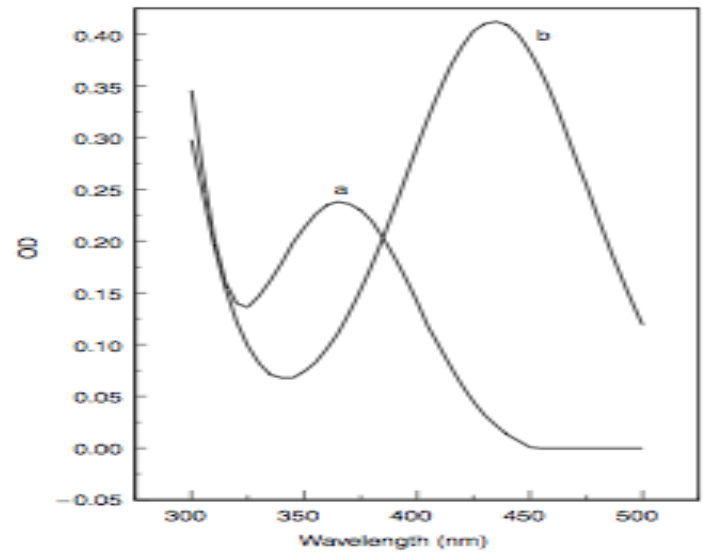
# Applications

- 1- structure determination. Identification (unsat gps) or elucidation of their str (direct comparison of the spectrum with others of known str.)
- 2- analysis of mix.
- 3- Evaluation of pK values of indicators.  
 $\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$  at isobestic point  $\text{pH} = \text{pK}_a$
- 4- quantitative analysis.  
 $A_1/A_2 = c_1/c_2$  or calibration curve.
- 5- mol-wt-det.  $A = \epsilon cl \rightarrow c = M, S = M \times \text{mol-wt}$
- 6- complexes composition  
 $nM + pL \rightarrow M_nL_p$
- (a) the molar ratio method.
- (b) the continuous variation method

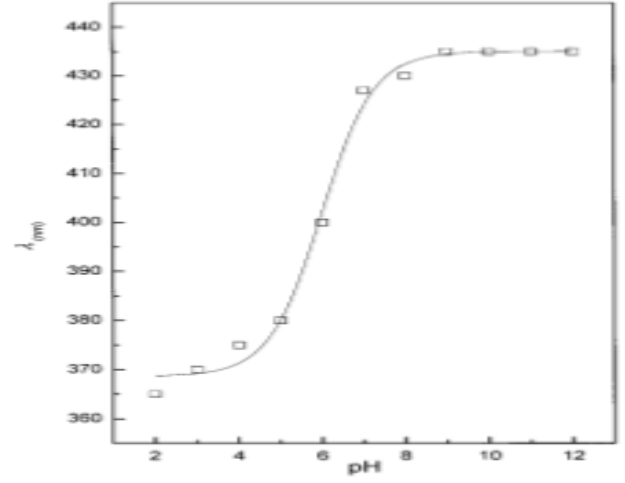
# Determination of the pK of 4-Methyl-2-Nitrophenol



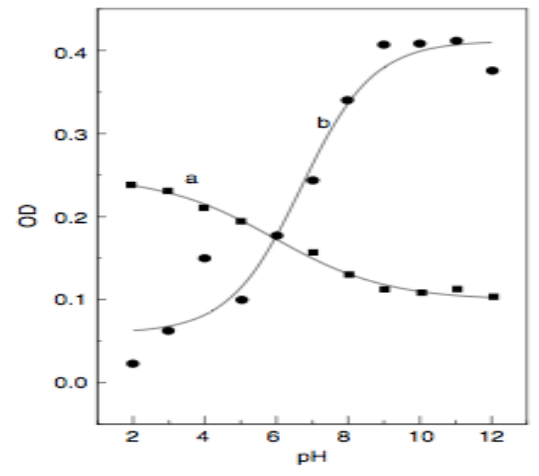
Ionization of 4-methyl-2-nitrophenol to 4-methyl-2-nitrophenolate.



Absorption spectra of methylnitrophenol in Tris 10 mM, at pH 2 (a) (365 nm) and pH 11 (b) (435 nm). An isobestic point is observed at 385 nm (both forms of methylnitrophenol display equal A).

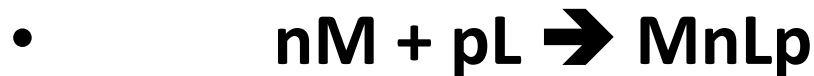


Position of the absorption peak of methylnitro-phenol with pH.



Variation in A with pH at the peaks of protonated (365 nm) (curve a) and deprotonated (435 nm) (curve b) forms of methylnitrophenol.

## •Complexes composition



- *The molar ratio method.*

- Concentration of one component is kept fixed,
- the other is varied,
- then record A

- *The continuous variation method.*

- The molar ratio is varied by changing the concn of both component (e.g total concn 2.5mM),
- while the total no of moles of both components are kept const (Job's method)

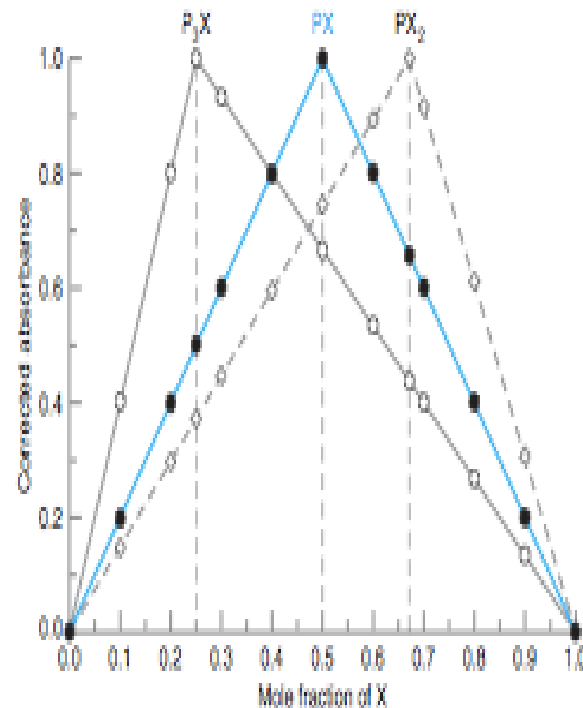
measured absorbance minus the absorbance that would be produced by free P and free X alone

$$\text{Corrected absorbance} = \text{measured absorbance} - \epsilon_P b P_T - \epsilon_X b X_T$$

**TABLE 18-1** Solutions for the method of continuous variation

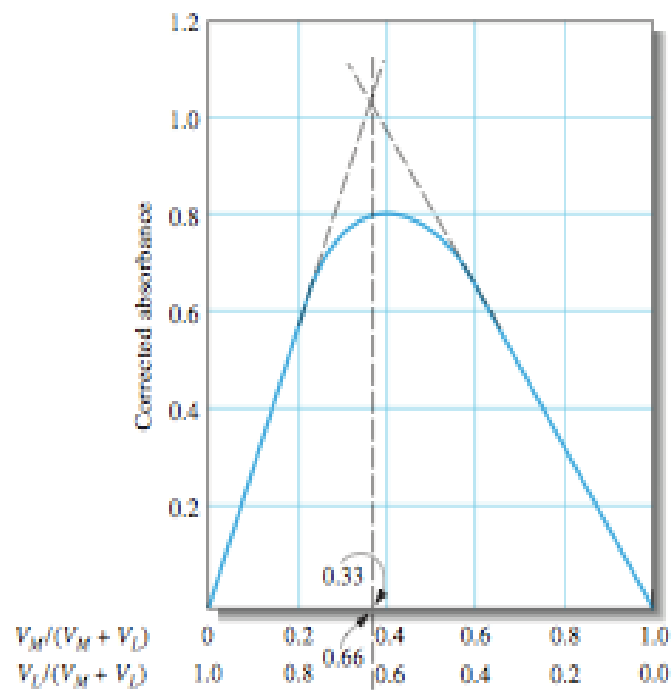
mL of 2.50 mM P	mL of 2.50 mM X	Mole ratio (X:P)	Mole fraction of X $\left(\frac{\text{mol X}}{\text{mol X} + \text{mol P}}\right)$
1.00	9.00	9.00:1	0.900
2.00	8.00	4.00:1	0.800
2.50	7.50	3.00:1	0.750
3.33	6.67	2.00:1	0.667
4.00	6.00	1.50:1	0.600
5.00	5.00	1.00:1	0.500
6.00	4.00	1:1.50	0.400
6.67	3.33	1:2.00	0.333
7.50	2.50	1:3.00	0.250
8.00	2.00	1:4.00	0.200
9.00	1.00	1:9.00	0.100

*NOTE: All solutions are diluted to a total volume of 25.0 mL with a buffer.*



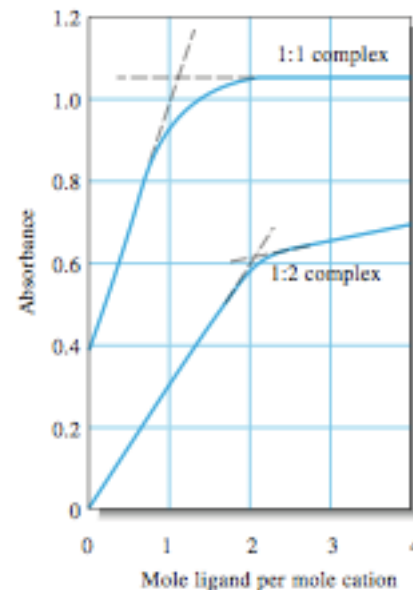
**FIGURE 18-8** Ideal behavior of Job plots for formation of the complexes P<sub>2</sub>X, PX, and PX<sub>2</sub>.

continuous variations method  
 cation and ligand solutions with identical analytical concentrations are mixed in such a way that the total volume and the total moles of reactants in each mixture are constant but the mole ratio of reactants varies systematically (for example, 1:9, 8:2, 7:3, and so forth).



Continuous-variation plot for the 1:2 complex  $ML_2$ .

In the mole-ratio method, a series of solutions is prepared in which the analytical concentration of one reactant (usually the metal ion) is held constant while that of the other is varied. A plot of absorbance versus mole ratio of the reactants is then prepared.



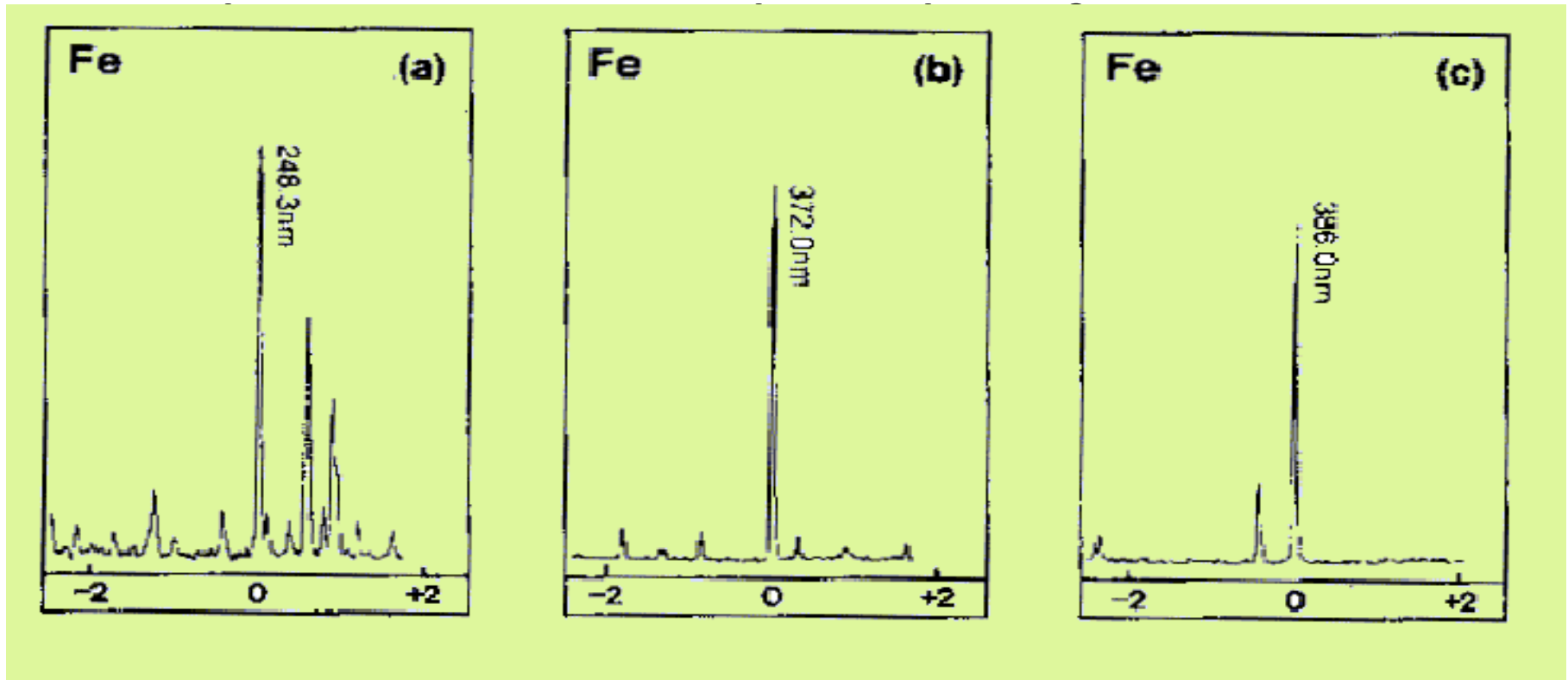
Mole-ratio plots for a 1:1 and a 1:2 complex. The 1:2 complex is the more stable of the two complexes as indicated by closeness of the experimental curve to the extrapolated lines. The closer the curve is to the extrapolated lines, the larger the formation constant of the complex; the larger the deviation from the straight lines, the smaller the formation constant of the complex.

# Atomic absorption spectrometry

## Basic principles

- Molecular absorption                      Atomic absorption

## Absorption Wavelengths of Iron

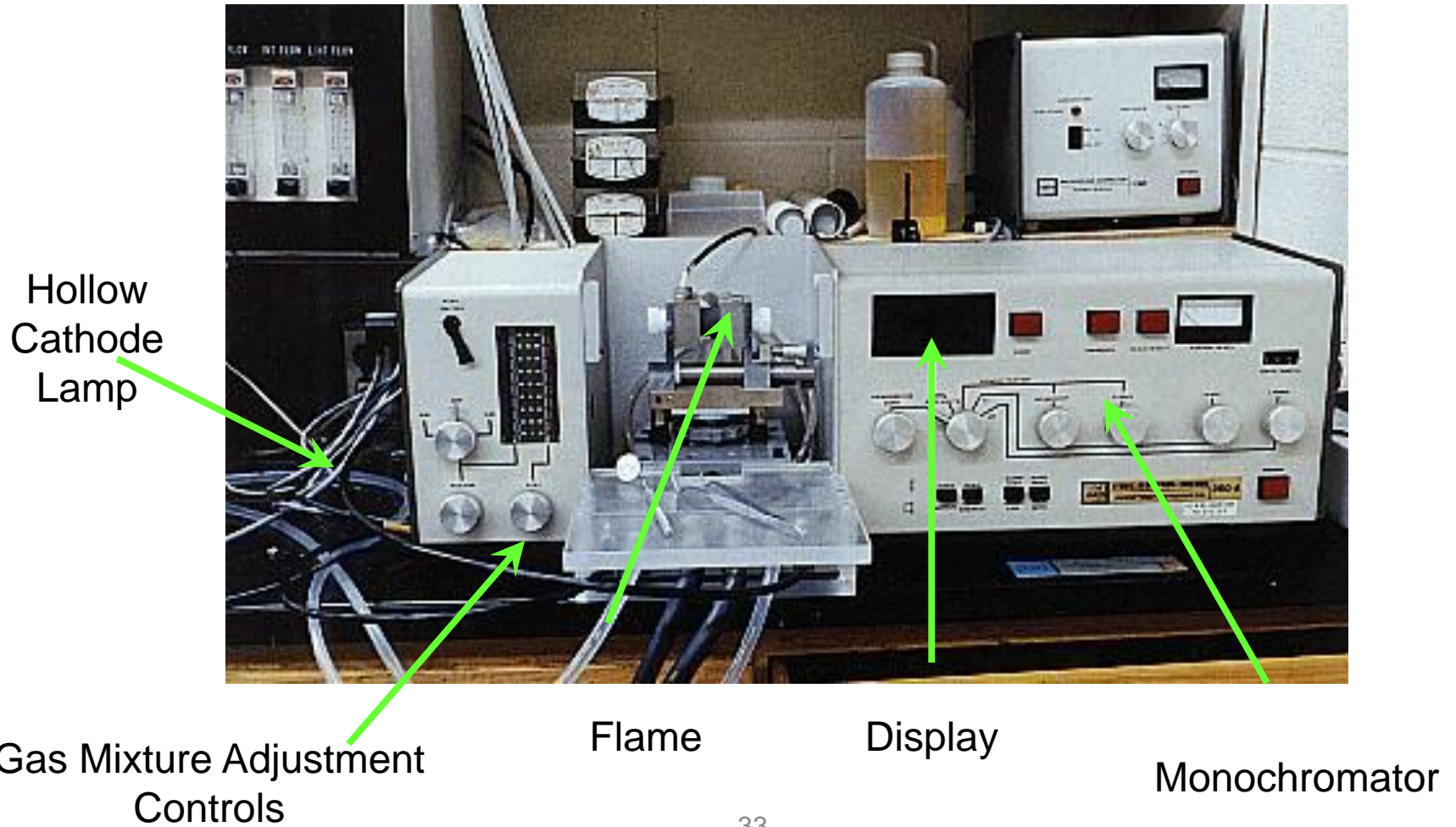


# Atomic Absorption Spectrophotometer (AAS)





# FAAS Operation



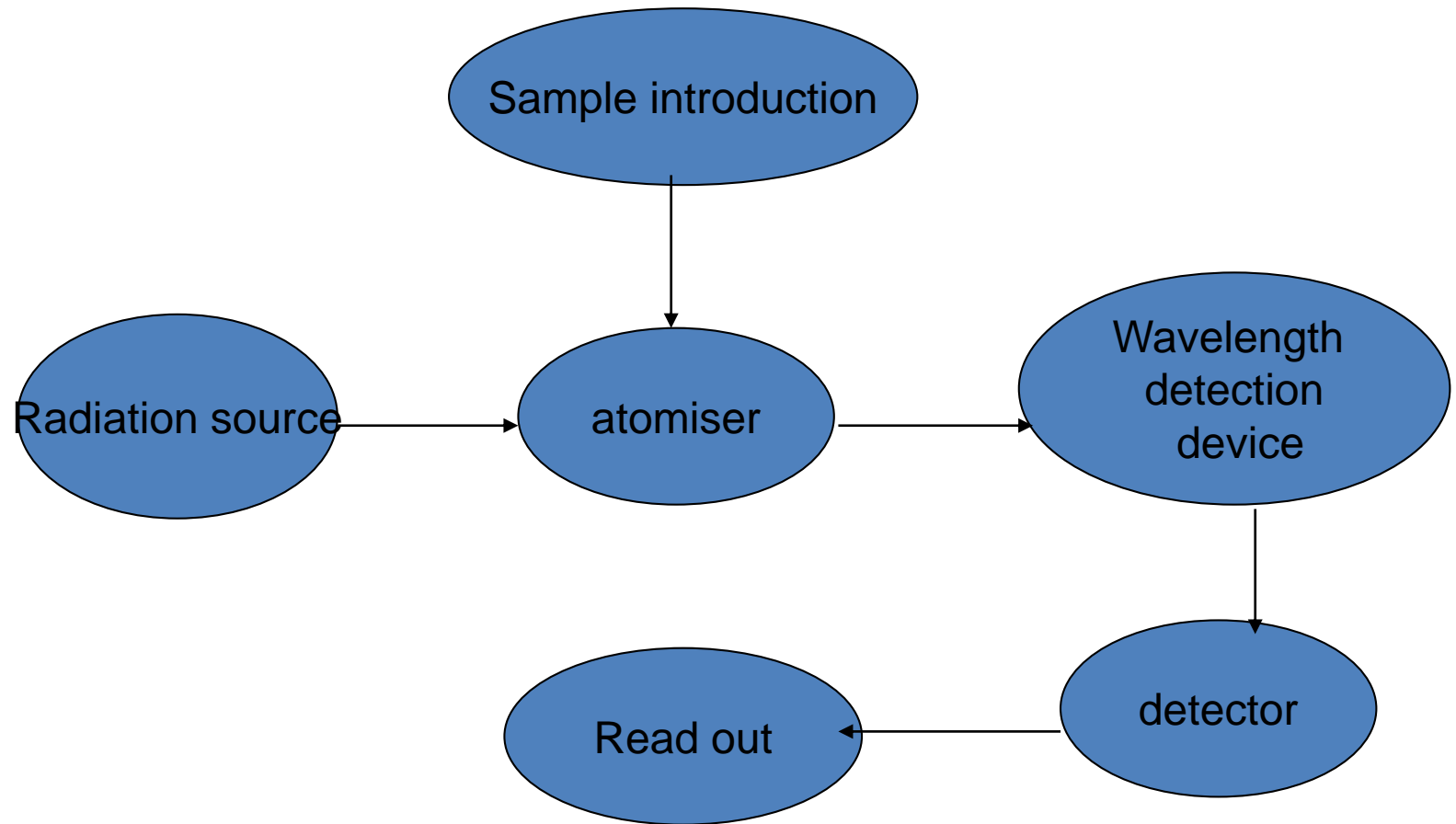
# Atomic Absorption Spectrometry

- small concentrations
  - Al, As, Au, B, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ge, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Si, Sr, Ti, V, W and Zn
- used in
  - industry
  - quality control of metals in steel
  - Water, food, ores and pharmaceuticals analysis for metals ions

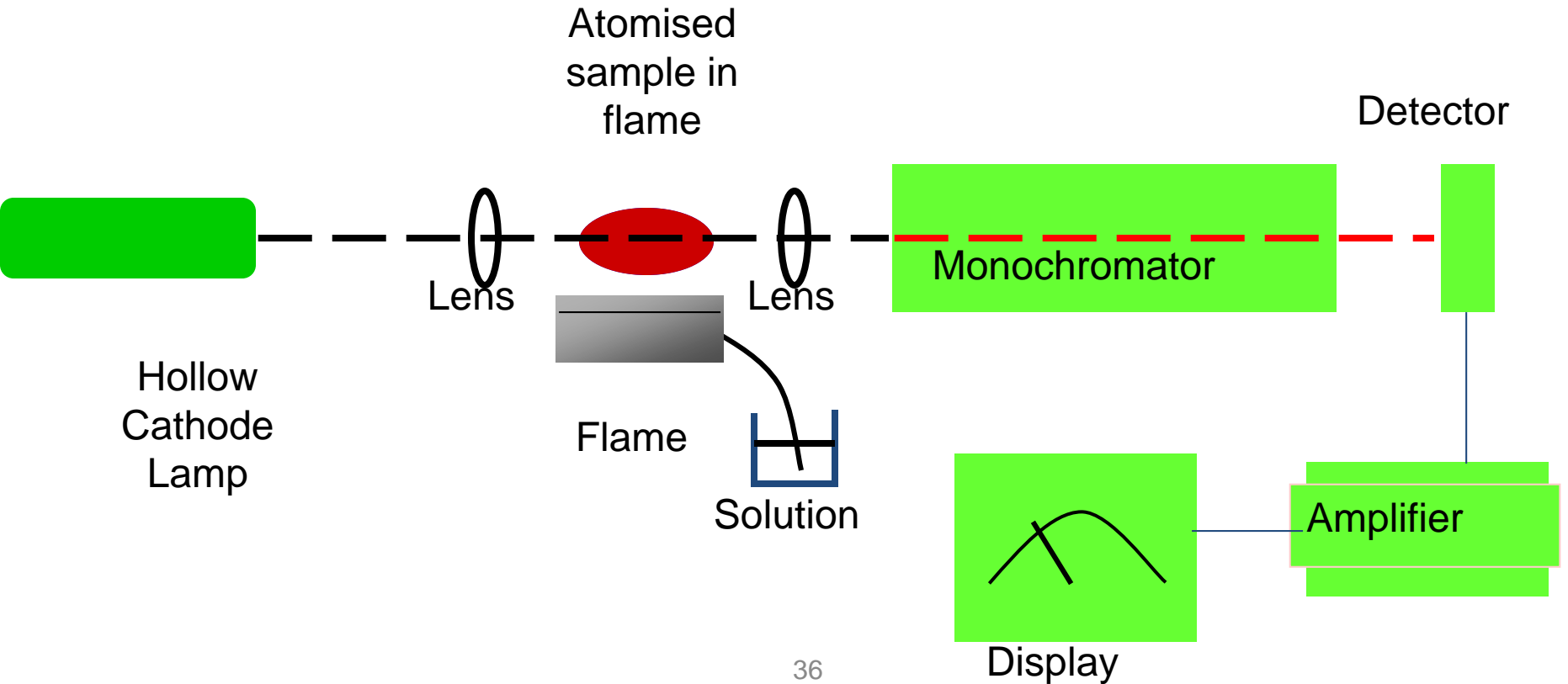
## AAS Advantages

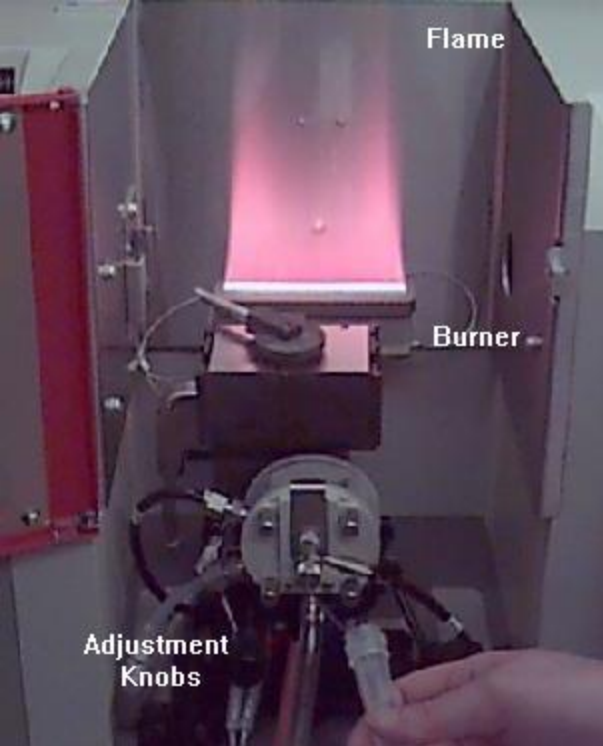
- very sensitive (ppm-ppb)
  - specific:
    - $\lambda$  is strongly absorbed by only the  $M^{n+}$
- A Source of Error
- Another species may be absorbing at the same  $\lambda$

# instrumentation

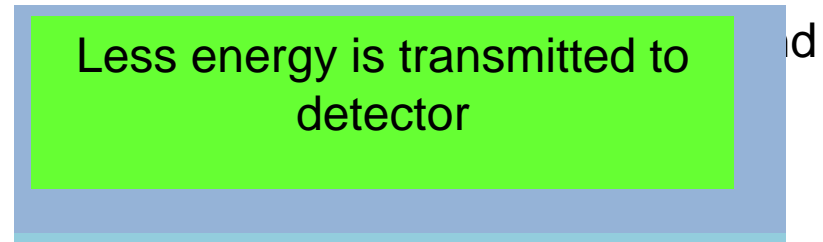


# Atomic Absorption Spectrometer



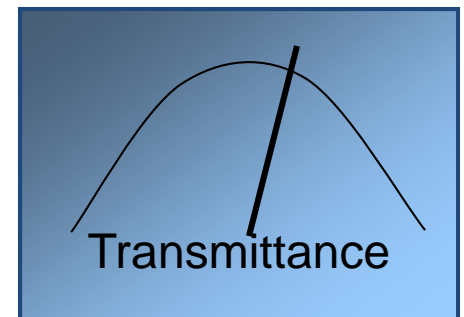


# Close-up view of AAS



Hollow Cathode Lamp emits several unique wavelengths of light

Ions in Flame



# Radiation source

- Mainly the hollow cathode lamp (HCL)
- To lesser extent electrode discharge lamp (EDL)

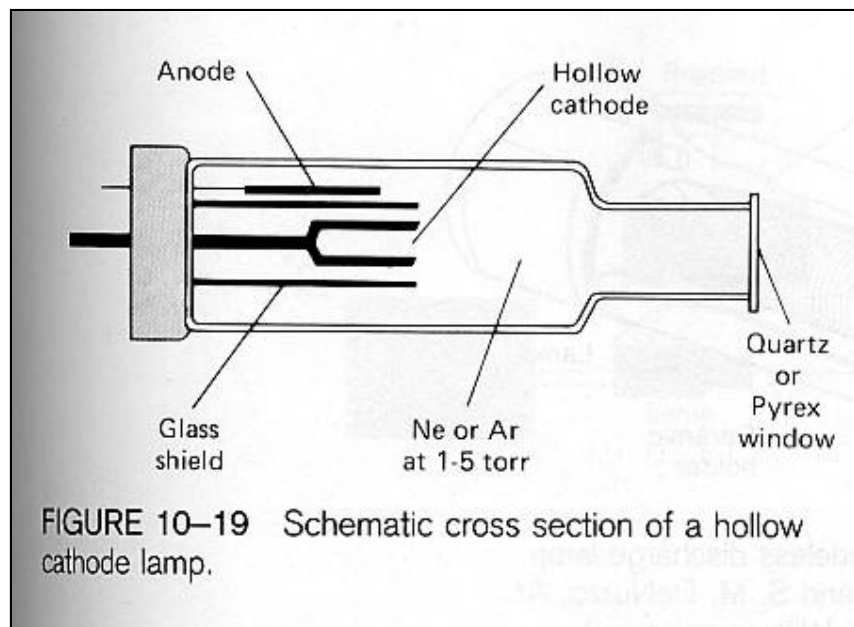
## *Hollow Cathode lamp*

Apply  $\sim 300$  V across electrodes.

$\text{Ar}^+$  or  $\text{Ne}^+$  travel toward the cathode.

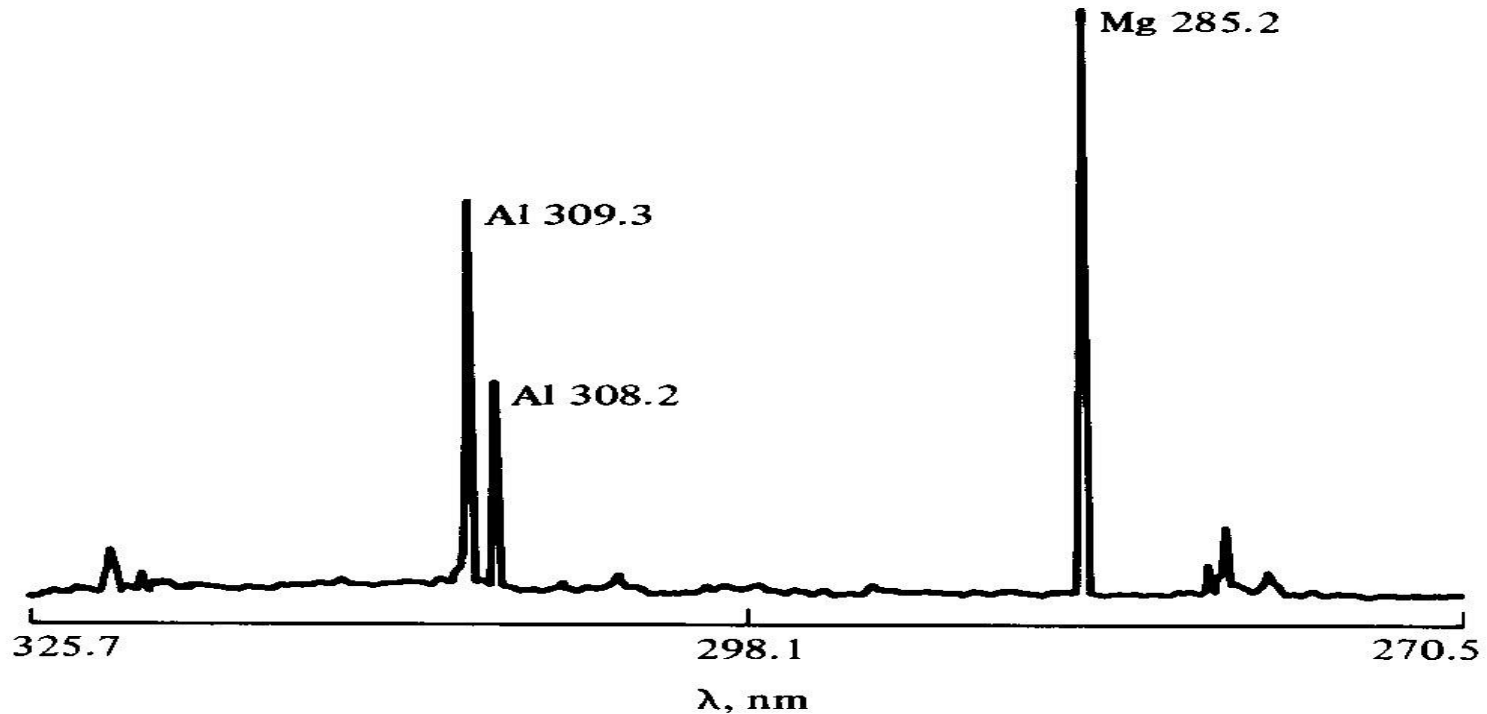
If potential is high enough cations will sputter metal off the electrode.

Metal emits photons at characteristic atomic lines as the metal returns to the ground state.



Douglas A. Skoog and James J. Leary,  
*Principles of Instrumental Analysis*,<sup>38</sup>  
Saunders College Publishing, Fort  
Worth, 1992.

# *Hollow Cathode Discharge Tube.*



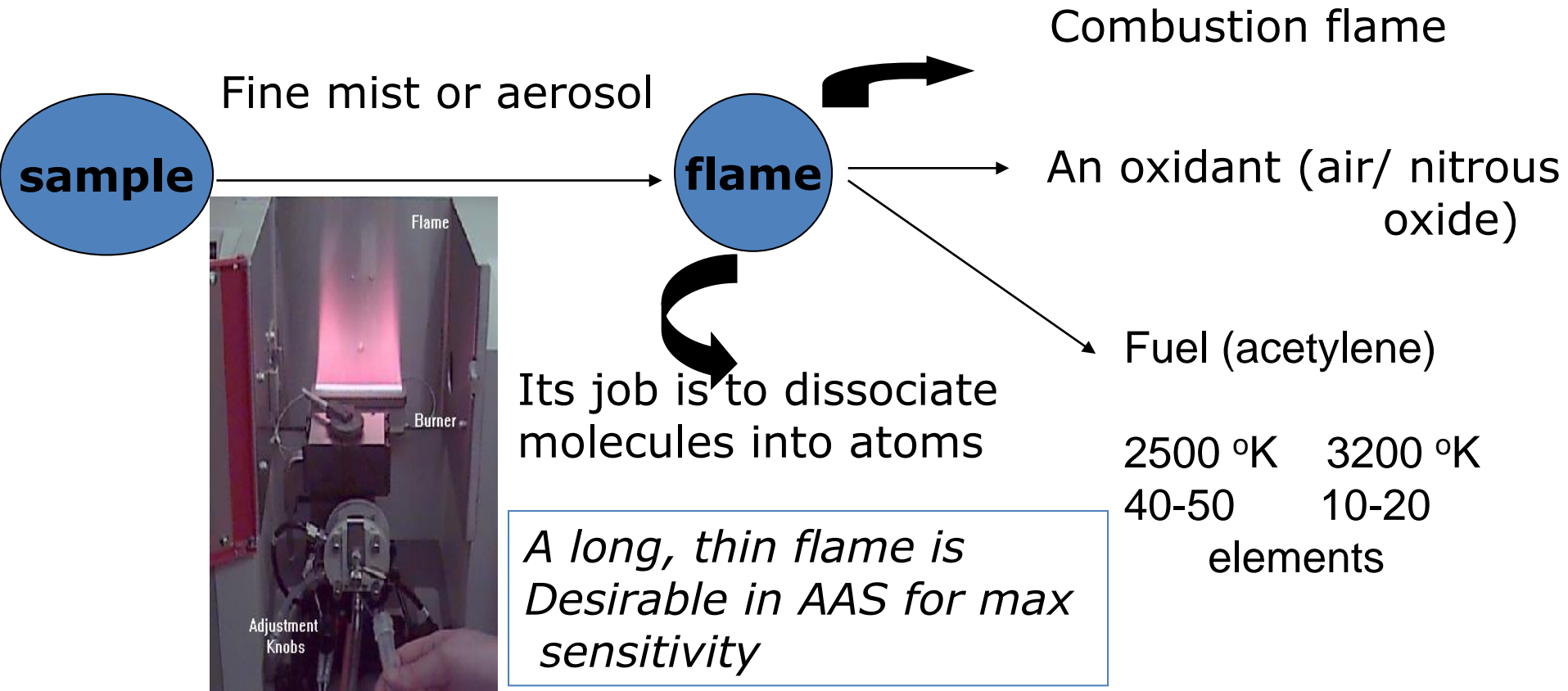
**FIGURE 4-8** Portion of spectrum from a dual-element hollow cathode lamp.

Line Widths are typically  $0.01 - 0.02 \text{ \AA}$ .

Life time, warm up

# Flame atom cell

## What is its job?





## Sample introduction system

- Should reproducibly and efficiently transfer a sample to the atomiser
- Pneumatic nebulisers
- The sample is sucked through a capillary tube
- The sample is surrounded by the oxidant gas
- Fine mist (tiny droplets) into flame
- Large droplets into drain (waste)

## Monochromator

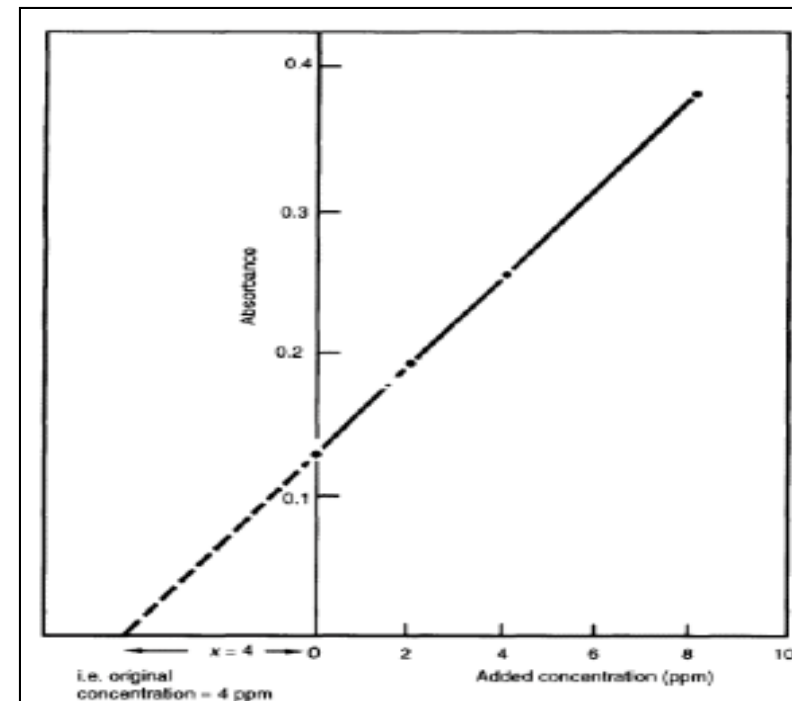
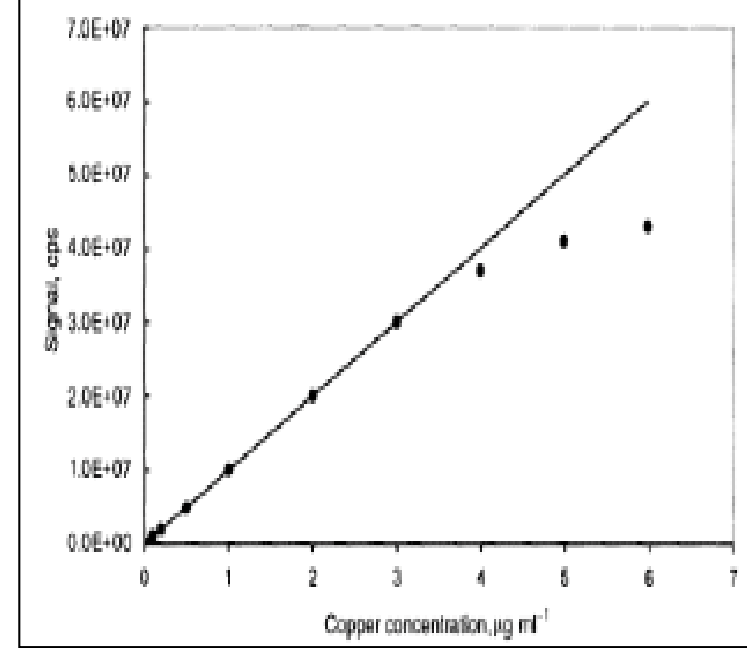
- After the flame
- Its function is to isolate the  $\lambda$  (radn) of the interest from the other  $\lambda$ s from the radn source and light emitted by other elements in the flame

## Furnace AAS

- Atomisation in an electrically heated graphite furnace is sometimes advantageous for higher sensitivity

# Quantitative analysis

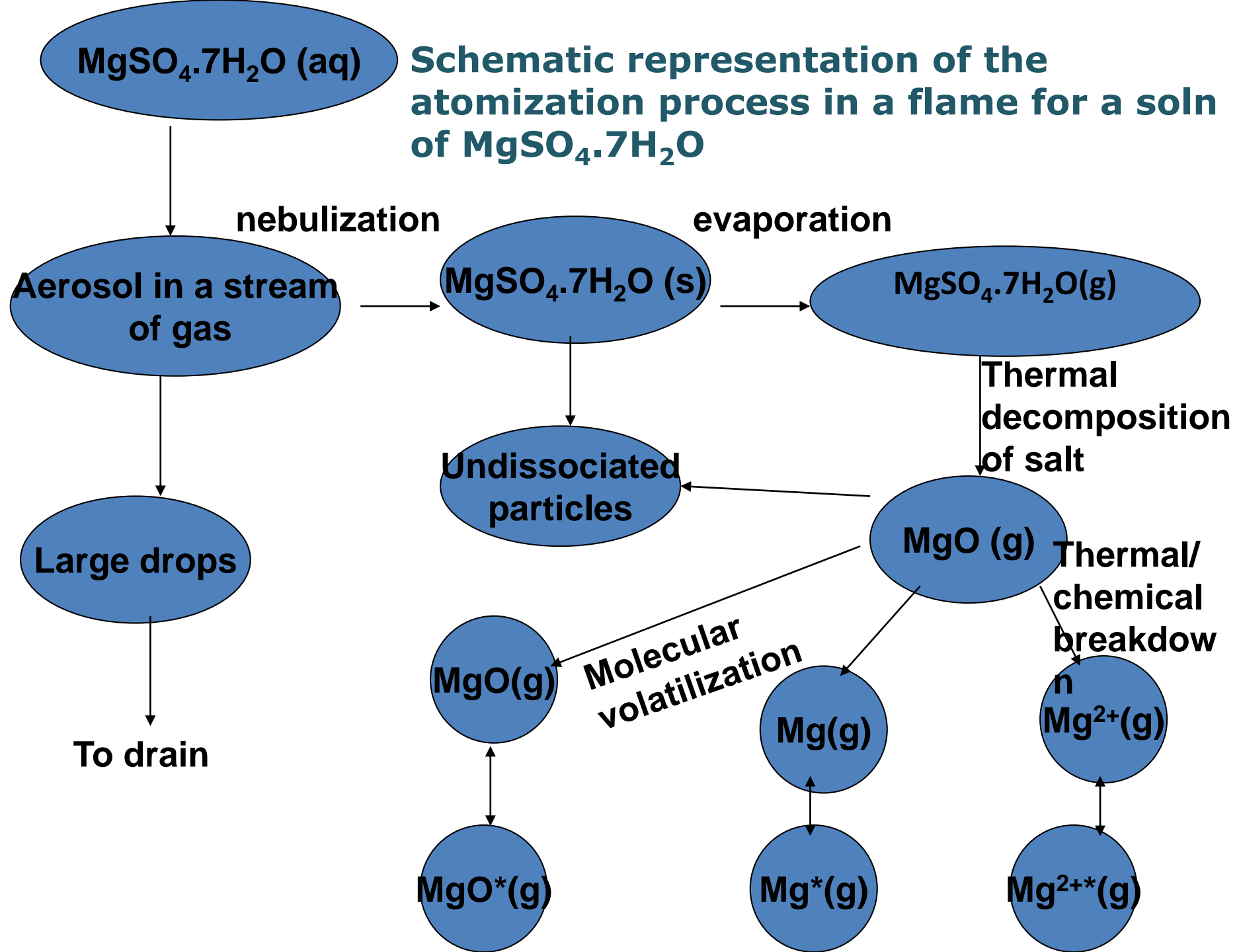
- A calibration curve is constructed  $A, c$
- 4 standards and a blank
- Use dilun or concen to fit your unkown into stand solns
- Matrix matching
- Standard addn method



# Interferences in FAAS

- An interference is a chemical or physical effect that causes the signal to be reduced or increased compared to the signal from the calibrated soln

# Schematic representation of the atomization process in a flame for a soln of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$



- Chemical interferences are the most common ones
- Analyte → thermally stable comp → no dissociation → no atoms
- A chemical interference can prevent, enhance or suppress the formation of ground state atoms in the flame

- M – O compounds
- $\text{Ca} + \text{PO}_4 \rightarrow \text{Ca} - \text{PO}_4$  (in the flame)
- During evaporation of liquid droplets in the flame  
 $\rightarrow$  Ca pyrophosphate  $\text{Ca}_2\text{P}_2\text{O}_7$  (very stable at air – acetylene flame  $\rightarrow$  reduces free Ca atoms (compared to that obtained in the absence of  $\text{PO}_4$

That type of interference can be controlled by one of the following methods

*1-complexation*

*2- releasing agent method*

*3- using higher temp*

- **1-complexation**
- EDTA + analyte → complex → preventing the formation of a refractory oxide.
- The addn of HF improves A of Ti, Zr

- **2- releasing agent method**
- La chloride + Ca + PO<sub>4</sub> → La – PO<sub>4</sub> → Ca free
- The good releasing agents are the metals which themselves form stable oxysalts.
- Sr and La are the most commonly used releasing agents

- **3- using higher temp**
- It helps to breakdown the comp

# Ionization interference

- Flame (T) → ionization of analytes
- It can be controlled by the addn of an excess of an easily ionised element to both sample and standards
- K, Na, Rb, Cs
- La for Al, Ca, Mg, Si, etc.