Chapter 13
An Introduction to Ultraviolet/Visible Molecular Absorption Spectrometry

13A Measurement Of Transmittance and Absorbance

- Absorption measurements based upon ultraviolet and visible radiation find widespread application for the quantitative determination of a large variety of species.
- Ordinarily, the concentration of an absorbing analyte is linearly related to absorbance as given by Beer's law:

–Beer's Law:

$$A = -\log T = \log P_0/P = \varepsilon bc$$

A = absorbance

ε = molar absorptivity [M^{-1} cm^{-1}]

c = concentration [M]

P_0 = incident power

P = transmitted power (after passing through sample)

TABLE 13-1 Important Terms and Symbols for Absorption Measurements

Term and Symbol*	Definition	Alternative Name and Symbol
Incident radiant power, P_0	Radiant power in watts incident on sample	Incident intensity, I_0
Transmitted radiant power, P	Radiant power transmitted by sample	Transmitted intensity, I
Absorbance, A	$\log(P_0/P)$	Optical density, D; extinction, E
Transmittance, T	P/P_0	Transmission, T
Path length of sample, b	Length over which attenuation occurs	l, d
Concentration of absorber, c	Concentration in specified units	
Absorptivity,† a	$A/(bc)$	Extinction coefficient, k
Molar absorptivity,‡ ε	$A/(bc)$	Molar extinction coefficient

Transmittance and absorbance, as defined in Table 13-1, cannot normally be measured in the laboratory because the analyte solution must be held in a transparent container or cell.

- Reflection occurs at the two air-wall interfaces as well as at the two wall-solution interfaces.
- The resulting beam attenuation is substantial, as we demonstrated in Example 6-2, where it was shown that about 8.5% of a beam of yellow light is lost by reflection in passing through a glass cell containing water.
- In addition, attenuation of a beam may occur as a result of scattering by large molecules and sometimes from absorption by the container walls.

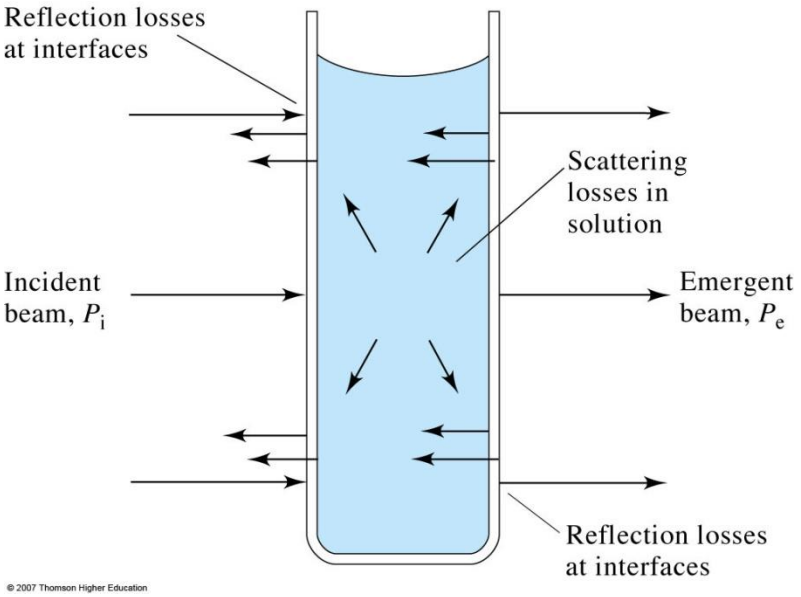


FIGURE 13-1 Reflection and scattering losses with a solution contained in a typical glass cell. Losses by reflection can occur at all the boundaries that separate the diffferent materials. In this example, the light passes through the air-glass, glass-solution, solution-glass, and glass-air interfaces.

Measurement of Transmittance and Absorbance:

The power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an identical cell containing only solvent. An experimental transmittance and absorbance are then obtained with the equations.

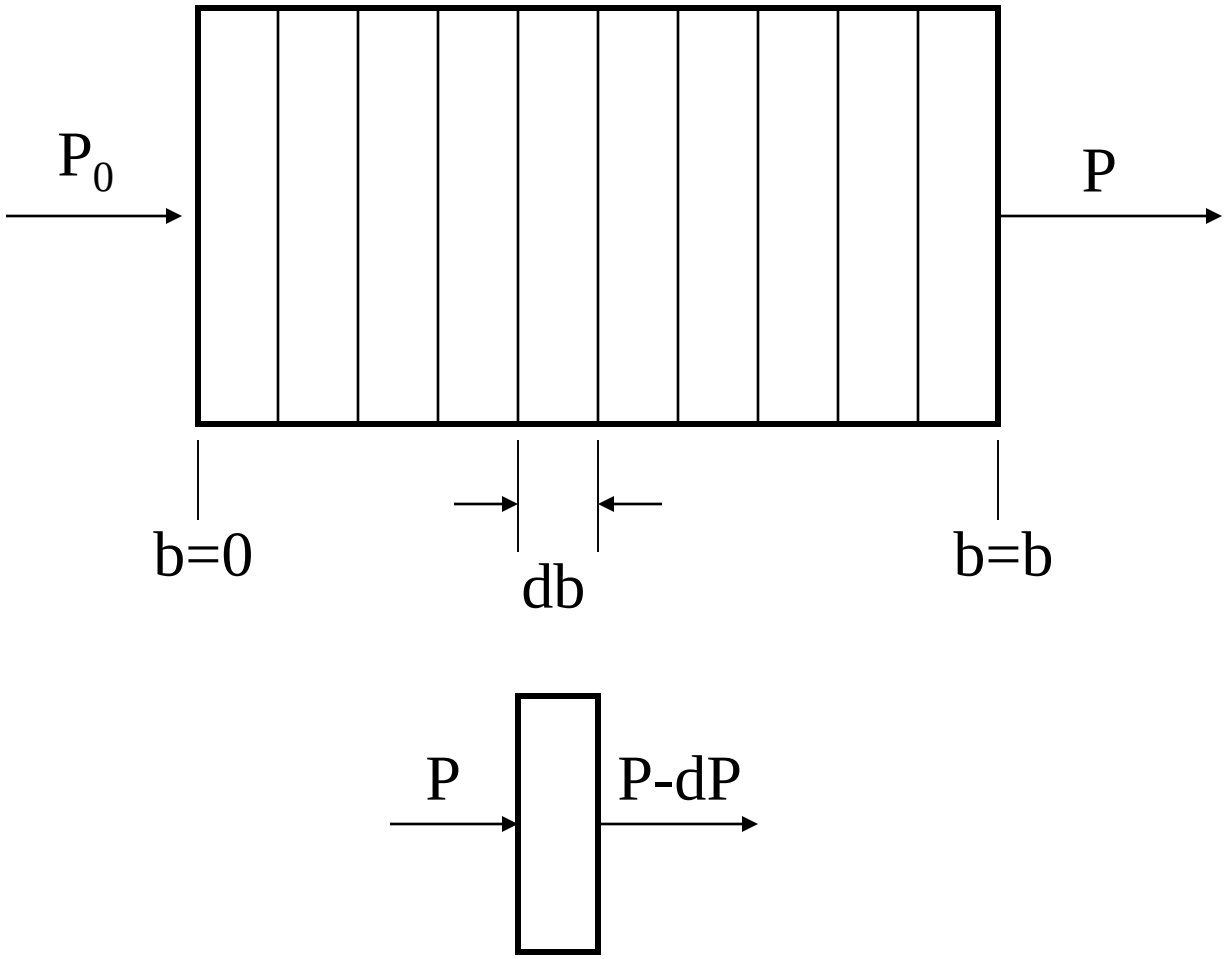
P_0 and P refers to the power of radiation after it has passed through the solvent and the analyte.

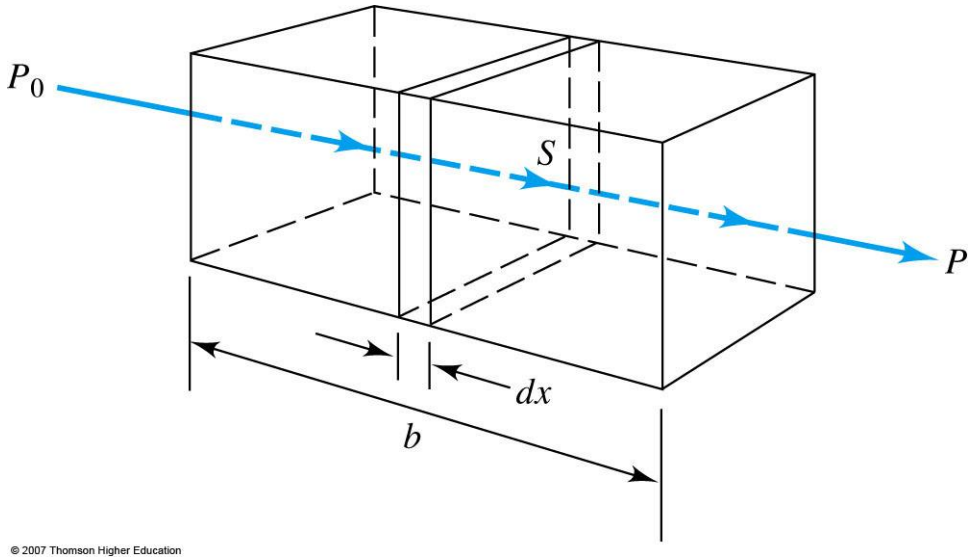
$$T = \frac{P_{solution}}{P_{solvent}} = \frac{P}{P_0}$$

$$A = \log \frac{P_{solvent}}{P_{solution}} \approx \log \frac{P_0}{P}$$

- # Derivation of Beer's Law:

Sample cell with absorbing molecules





dP $\propto$ P Incremental power lost $\propto$ power in; i.e., increase power in, increase power absorbed

dP $\propto$ db Longer pathlength, greater number of molecules in incremental slice and more power absorbed

Therefore, dP $\propto$ Pdb dP = -kPdb

k = proportionality constant (function of λ, c)

negative sign: because power is lost (i.e., absorbed)

Rearrange:

$$\frac{dP}{P} = -kdb$$

Integrate: $\int_{P_0}^{P} \frac{1}{P} dP = -k \int_{0}^{b} db$

$$InP - InP_0 = -kb - (-k)(0)$$

$$In\frac{P}{P_0} = -kb$$

Factor out concentration part of k: k = k”c

$$In\frac{P}{P_0} = -k"bc$$

Convert fraction (remove –sign) and change In to log:

$$\log\frac{P_0}{P} = \frac{1}{2.303} k:"bc$$

$$A = \log\frac{P_0}{P} = \varepsilon bc$$

$$(1/2.303)\text{k”} = \varepsilon$$

13B-1 Application of Beer's Law to Mixtures

Beer's law applies to a medium containing more than one kind of absorbing substance. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by

$$A_{total} = A_1 + A_2 + \ldots + A_n$$
$$= \varepsilon_1 b c_1 + \varepsilon_2 b c_2 + \ldots + \varepsilon_n b c_n$$

where, the subscripts refer to absorbing components 1, 2, …, n.

Assumptions in derivation of Beer's Law

- incident radiation is Monochromatic(all molecules absorb light of one λ)
- Absorbing molecules act independently of one another i.e, low c
- Pathlength is uniform (all rays travel the same distance in sample)
- No scattering
- Absorbing medium is optically homogeneous
- Incident beam is not large enough to cause saturation
- All rays should be parallel to each other and perpendicular to surface of medium.

13B-2 Limitations (deviations) to Beer's Law

* *Real Limitations*

  High concentration > 0.01 M

- the extent of solute-solvent interactions, solute-solute interactions, or hydrogen bonding can affect the analyte environment and its absorptivity.

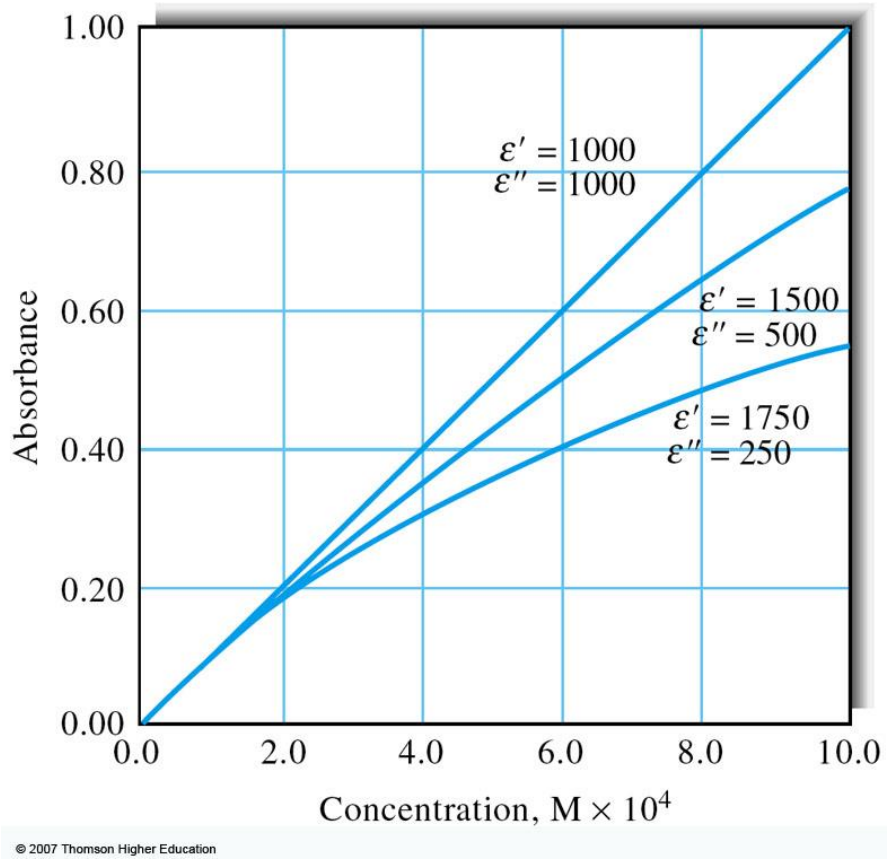
* *Chemical Deviations*

- Analyte dissociates, associates or reacts to give molecule with different absorption characteristics (e.g., pH-dependent indicators)
  - Example 13-1

* *Instrumental Deviations*
  - Polychromatic radiation
  - Stray Radiation

Instrumental Deviations :

– In the presence of Polychromatic radiation (i.e., light of more than one λ)



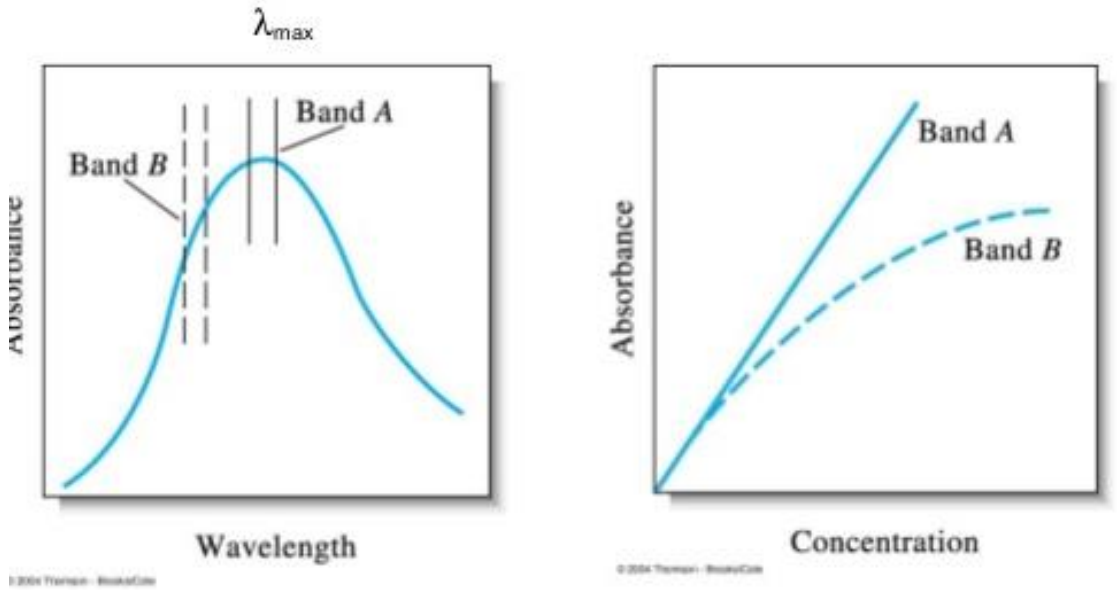
$$A_{meas} = \log \frac{\left(p'_0 + P''_0\right)}{\left(P' + P''\right)}$$

Where P' and P" are powers for λ' and λ", respectively

- Negative deviation = lower absorbance than predicted because higher transmittance
- Higher T because molecules don't absorb one λ as well as other

The absorber has the indicated molar absorptivities at the two wavelengths λ' and λ".

The effect of polychromatic radiation on Beer's law. In the spectrum on the left, the absorptivity of the analyte is nearly constant over band ***A*** from the source. Note in the Beer's law plot at the bottom that using band ***A*** *gives* a linear relationship. In the spectrum, band *B* corresponds to a region where the absorptivity shows substantial changes. In the lower plot, note the dramatic deviation from Beer's law that results.



To avoid deviations, it is advisable to select a wavelength band near the wavelength of maximum absorption where the analyte absorptivity changes little with wavelength

Instrumental Deviations :

–In the presence of Stray radiation

- the radiation exiting from a monochromator is usually contaminated with small amounts of scattered or stray radiation. This radiation, commonlv called *stray light, is defined as radiation from the instrument that is outside the nominal wavelength band chosen for the determination.*

- This *stray radiation often is the result of scattering and reflection off the surfaces of gratings, lenses or mirrors. filters, and windows*.

- The wavelength of stray radiation often differs greatly from that of the principal radiation and, in addition, the radiation may not have passed through the sample.

Instrumental Deviations :

–In the presence of Stray radiation

$$A' = \log \frac{(p'_0 + P_s)}{(P' + Ps)}$$

Ps = power from stray radiation

Extra light hits detector → higher T; lower A

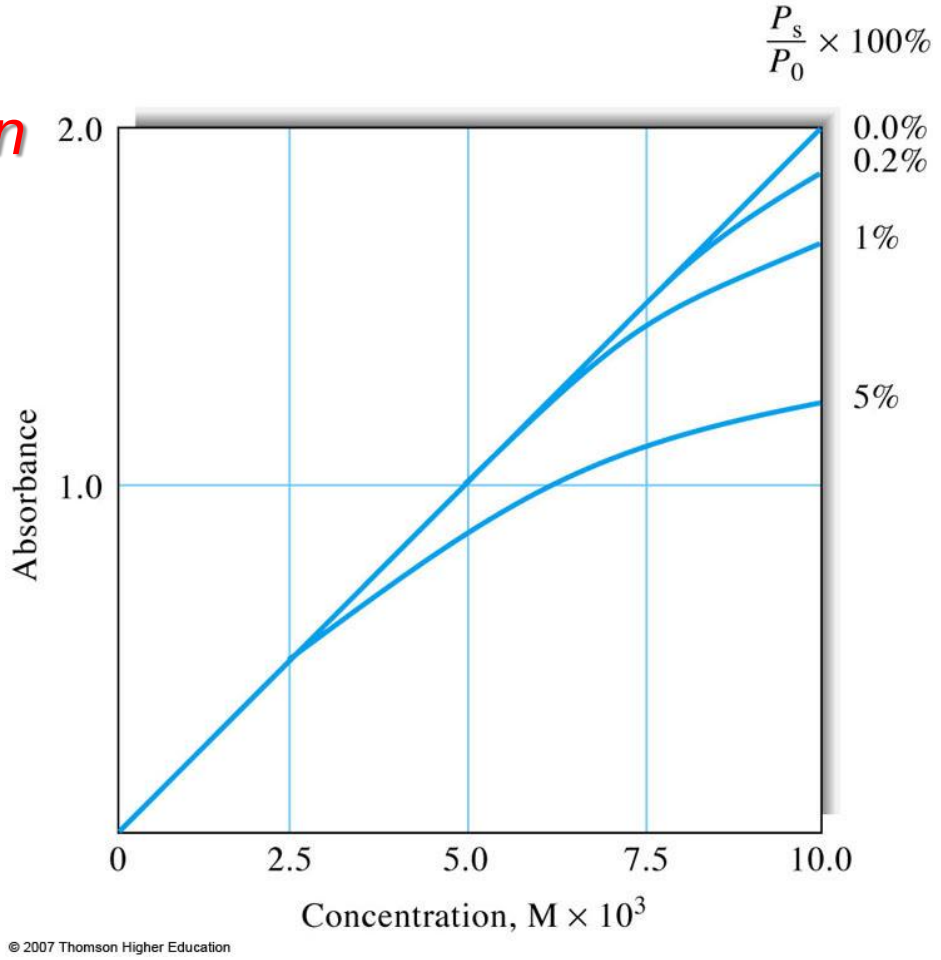
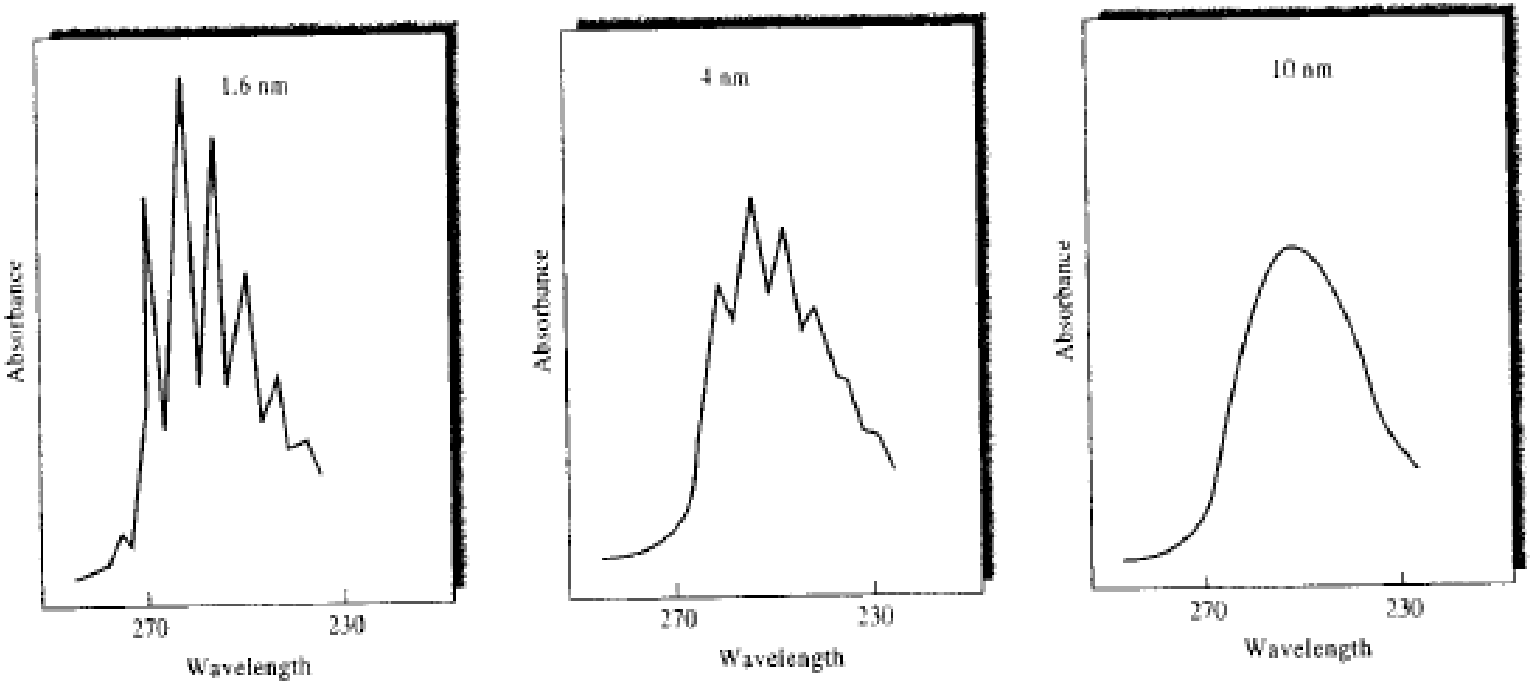


FIGURE 13-6 Apparent deviation from Beer's law brought about by various amounts of stray radiation. Note that the absorbance begins to level off with concentration at high stray-light levels. Stray light always limitsthe maximum absorbance that can be obtained because when the absorbance is high, the radiant power transmitted through the sample can become comparable to or lower than the stray-light level.

13.C Effect of Slit Width on Absorbance Measurements



- Figure 13-8 illustrates the loss of detail that occurs when slit widths are increased from small values on the left to larger values in the middle and right. In this example, the absorption spectrum of benzene vapor was obtained at slit settings that provided effective bandwidths of 1.6, 4, and 10 nm.
- For qualitative studies, the loss of resolution that accompanies the use of wider slits is often important because the details of spectra are useful for identifying species. Narrow slits widths are required to resolve complex spectra

13.C Effect of Slit Width on Absorbance Measurements

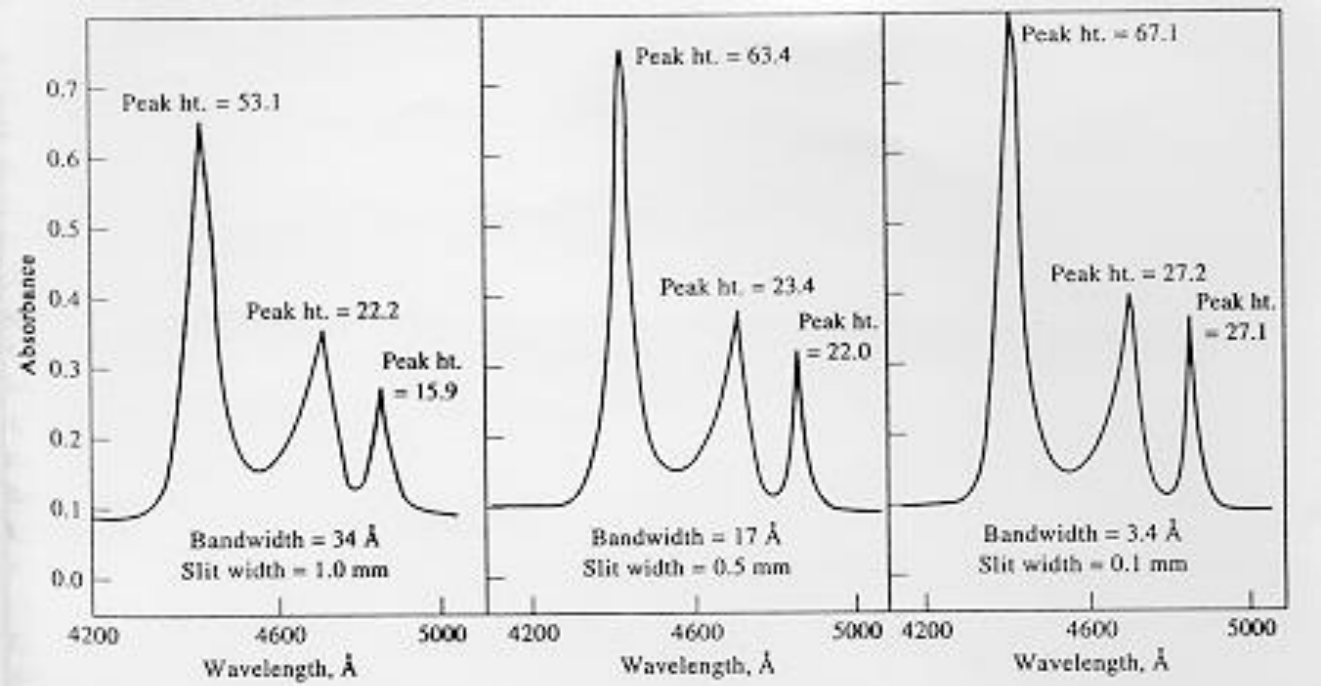


Figure 13-3 Effect of slit width and bandwidth on peak heights. Here, the sample was a solution of praseodymium chloride. *(From Optimum Spectrophotometer Parameters, Application Report AR 14-2. Cary Instruments: Monrovia, CA. With permission.)*

- quantitative measurement of narrow absorption bands requires using narrow slit widths or, alternatively, very reproducible slit-width settings. Unfortunately, a decrease in slit width by a factor of 10 reduces the radiant power by a factor of 100 because the radiant power is proportional to the square of the slit width.
- There is thus a trade-off between resolution and signal-to-noise ratio.
- For quantitative measurements, slit width needs to be kept large enough to provide high signal throughput.

Instrumentation

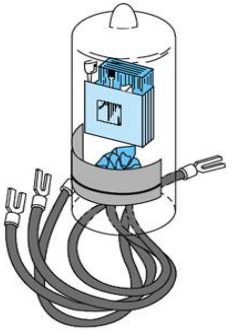
Light Source	λ - selector	Sample container	Detector	Signal processing

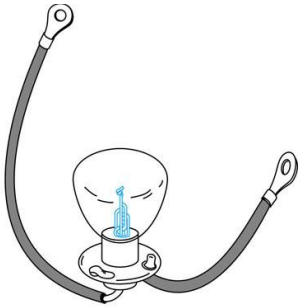
- Light Sources (commercial instruments)
  - D_2 lamp (UV: 160 – 375 nm)
  - W lamp (vis: 350 – 2500 nm)

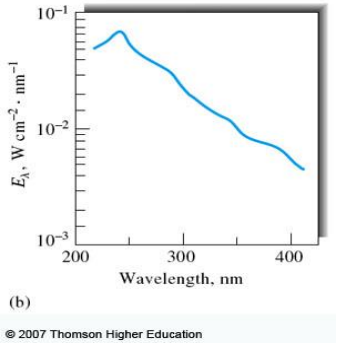
- λ - selection (monochromators)

- Sample holders
  - Cuvettes (b = 1 cm typically)
  - Glass (Vis)
  - Fused silica (UV 350 nm)

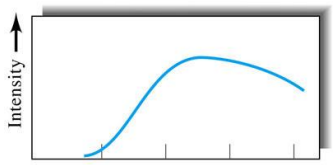
- Detectors
  - Photodiodes
  - PMTs







Deuterium and Hydrogen Lamps



Tungsten Filament Lamps

Types of Instruments

(1) single beam,

(2) double beam,

- double beam in space,
- double beam in time,

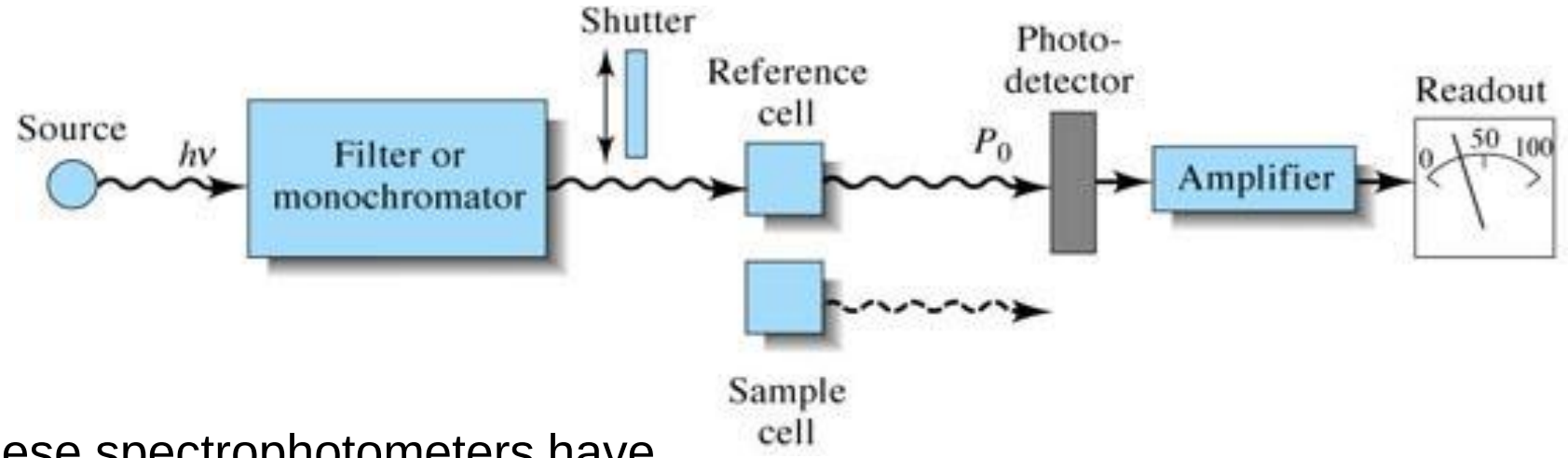
(3) multichannel

Types of Instruments

- *Single beam*

– Place cuvette with blank (i.e., solvent) and take a reading → 100% T

– Replace cuvette with sample and take reading → % T for analyte

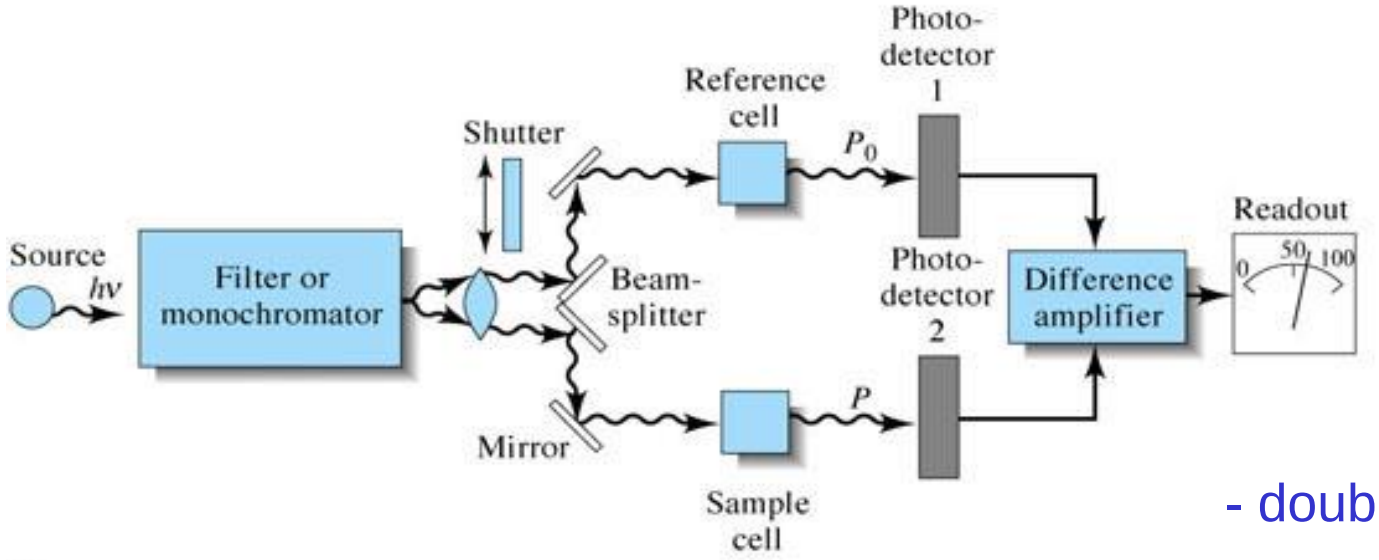


These spectrophotometers have

- inter changeable W and D2 lamp sources,
- rectangular silica cells,
- a high-resolution grating monochromator with variable slits.
- Photomultiplier tubes as transducers, and
- the output is often digitized, processed, and stored in a computer

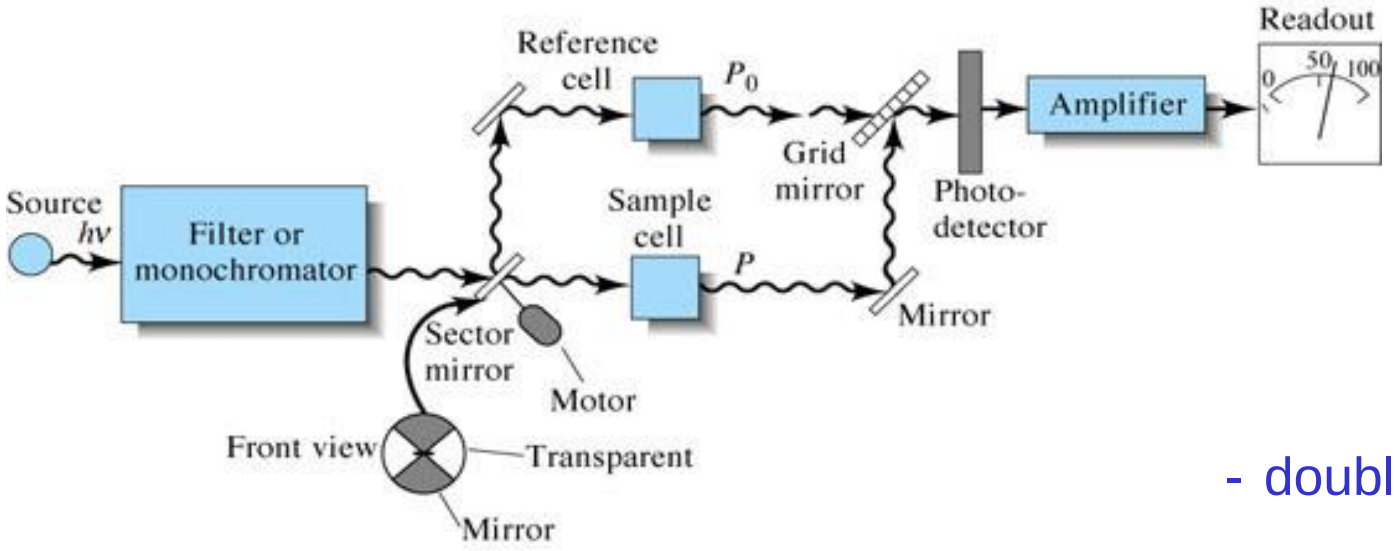
•Double beam (most commercial instruments)

–Light is split and directed towards both reference cell (blank) and sample cell



- double beam in space

(b)



- double beam in time

– Double-beam instruments advantages;

- Compensates for fluctuations in radiant source and drift in the detector
- Better design for continuous recording of spectra
- double beam in space; requires two ***matched*** photodetectors. electronics measure ratio (or the logarithm of their ratio) and displayed by the readout device.
- Double beam in time, the beams are separated in time by a rotating sector mirror that directs the entire beam from the monochromator first through the reference cell and then through the sample cell.
- Double beam in time, generally preferred because of the difficulty in matching the two detectors needed for the double-beam-in-space design.

Multichannel Instruments

•The dispersive system is a grating spectrograph placed after the sample or reference cell. The array detector is placed in the focal plane of the spectrograph, where the dispersed radiation strikes it.

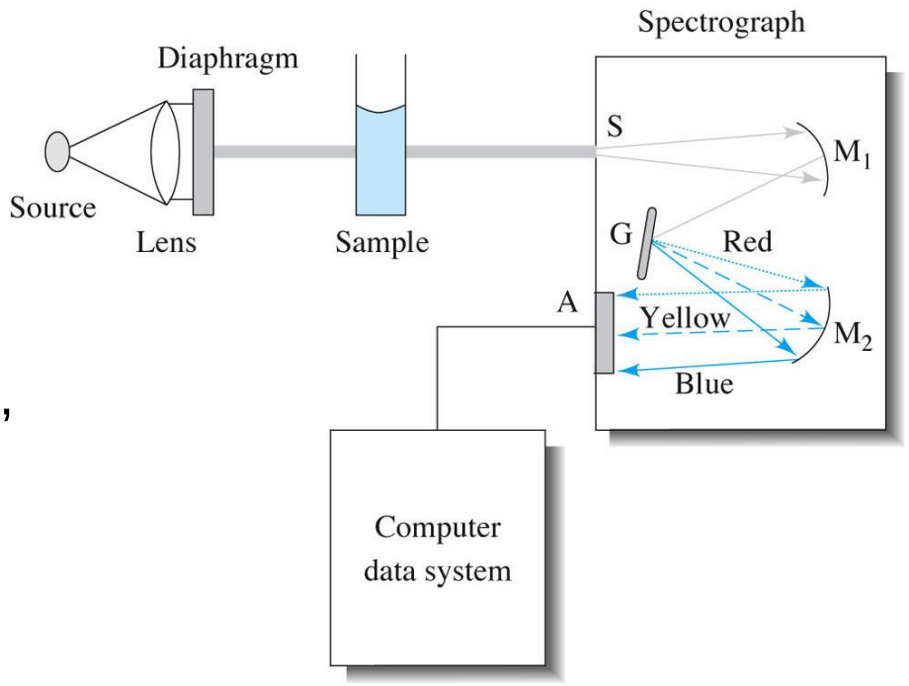
•Photodiode array multichannel detector, can measure all wavelengths dispersed by grating simultaneously.

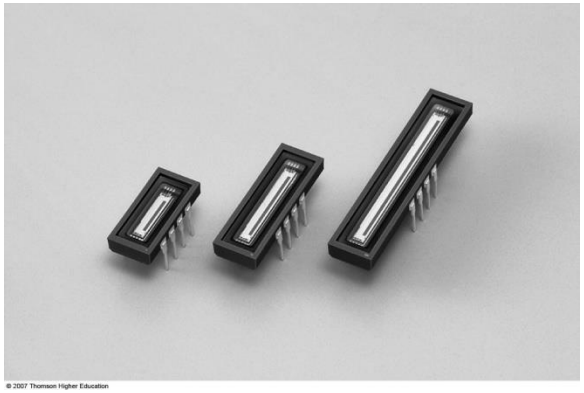
•Advantage: scan spectrum very quickly "snapshot" < 1 sec.

•Powerful tool for studies of transient intermediates in moderately fast rxn's .

• Useful for kinetic studies.

• Useful for qualitative and quantitative determination of the components exiting from a liquid chromatographic column.





13D-3 Some Typical Instruments

Photometers: Filter

- simple, inexpensive tools for performing absorption measurements
- Filter photometers: more convenient, more rugged and easier to maintain and use than the more sophisticated spectrophotometers.
- Photometers have high radiant energy throughputs and thus good S/N ratios even with relatively simple and inexpensive transducers and circuitry.
- Filter photometers are particularly useful in portable instruments intended for field use or for use in measuring the absorbances of flowing streams.
- These photometers are also used for quantitative determinations in clinical laboratories.

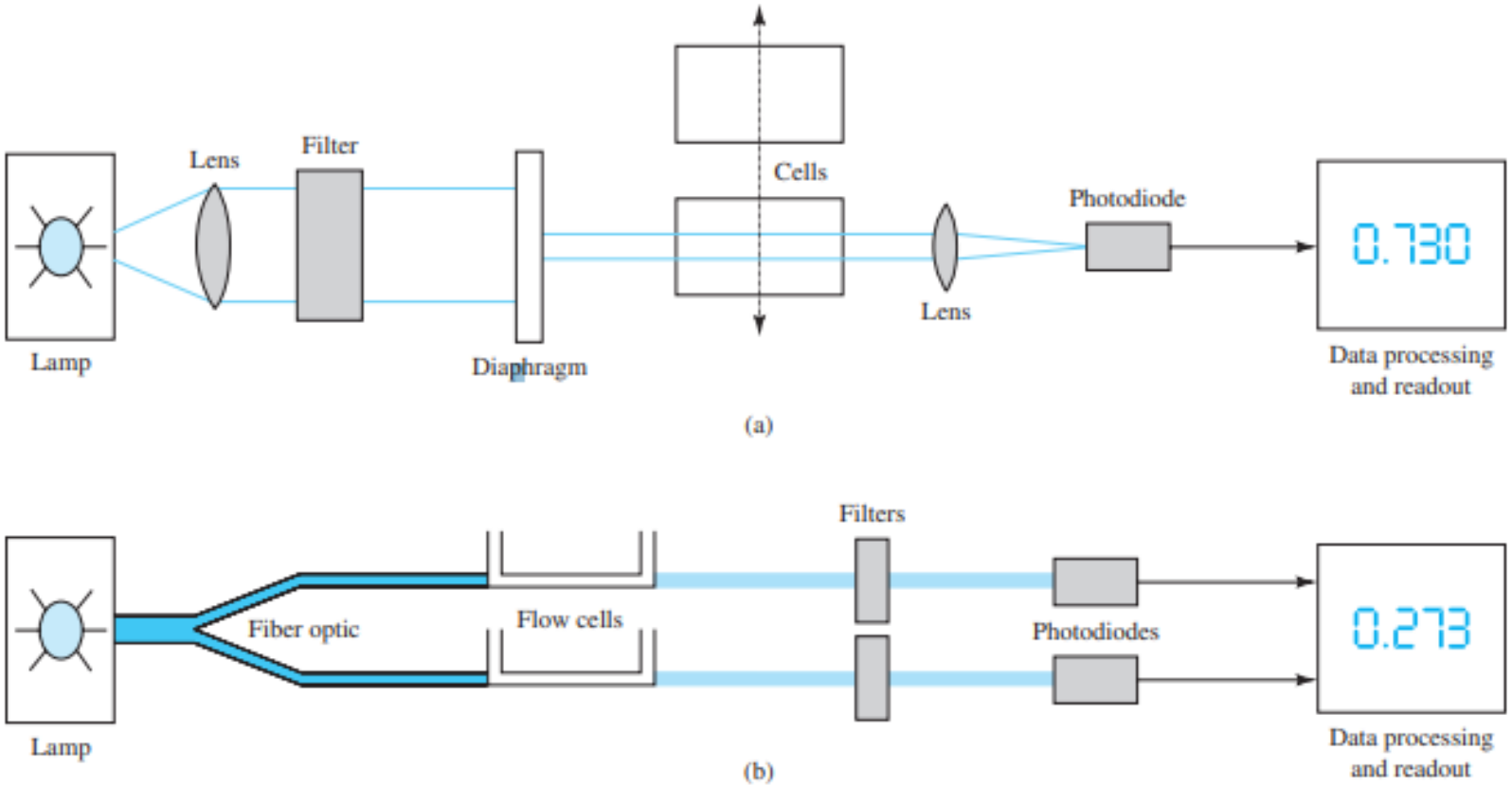
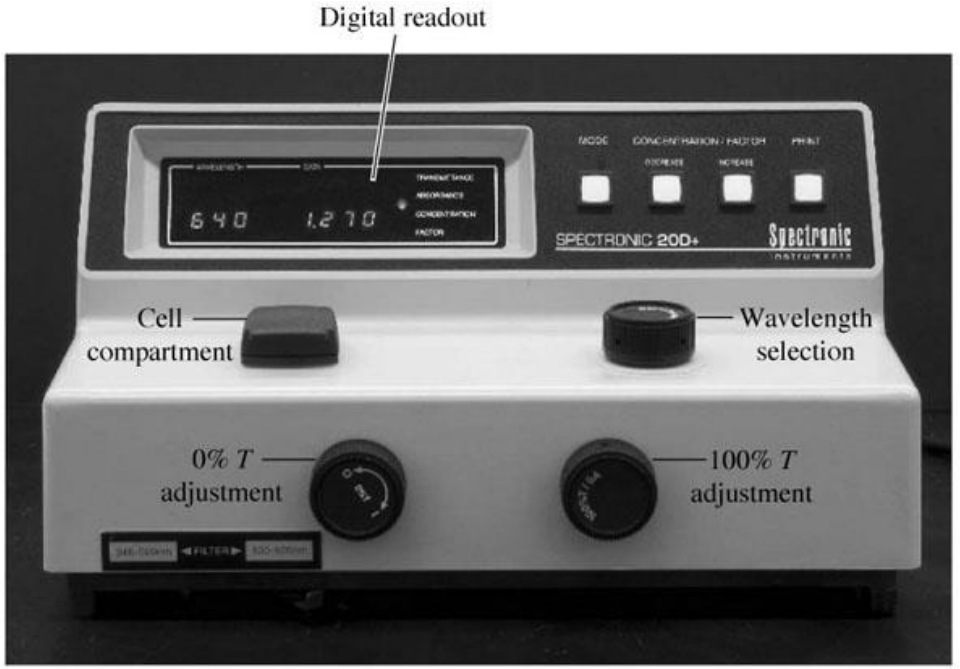
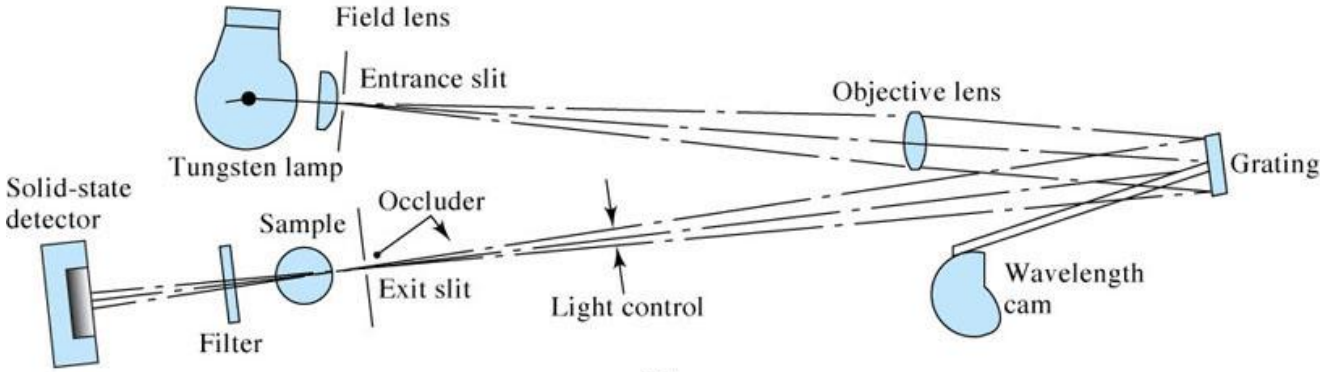


FIGURE 13-16 Single-beam photometer (a) and double-beam photometer for flow analysis (b). In the single-beam system, the reference cell is first placed in the light path and later replaced by the sample cell. In the double-beam system (b), a fiber optic splits the beam into two branches. One passes through the sample cell and the other through the reference cell. Two matched photodiodes are used in this double-beam-in-space arrangement.

Spectrophotometers: MC



(a)



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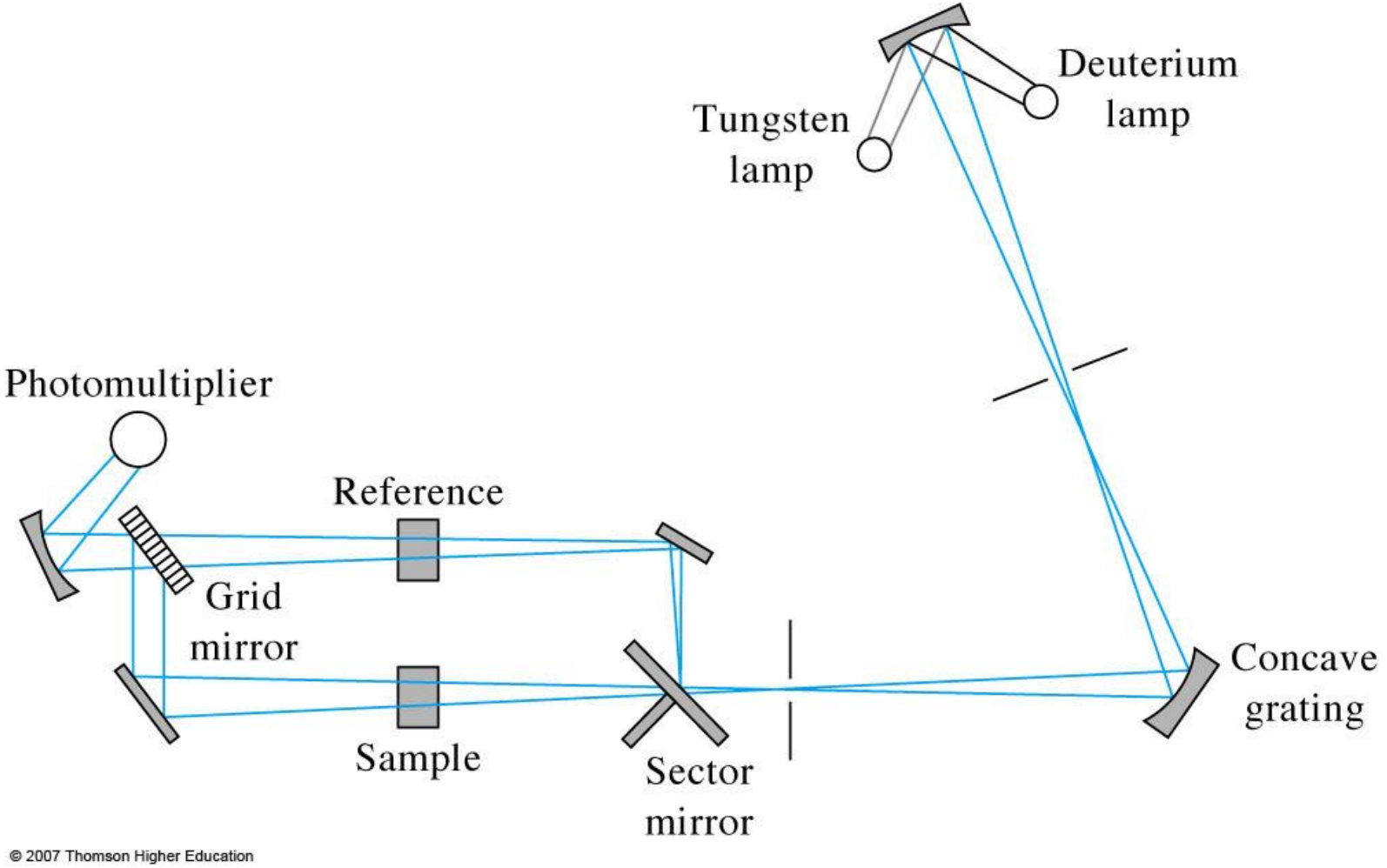


FIGURE 13·21 Schematic of a typical manual double-beam spectrophotometer for the UV-visible region.

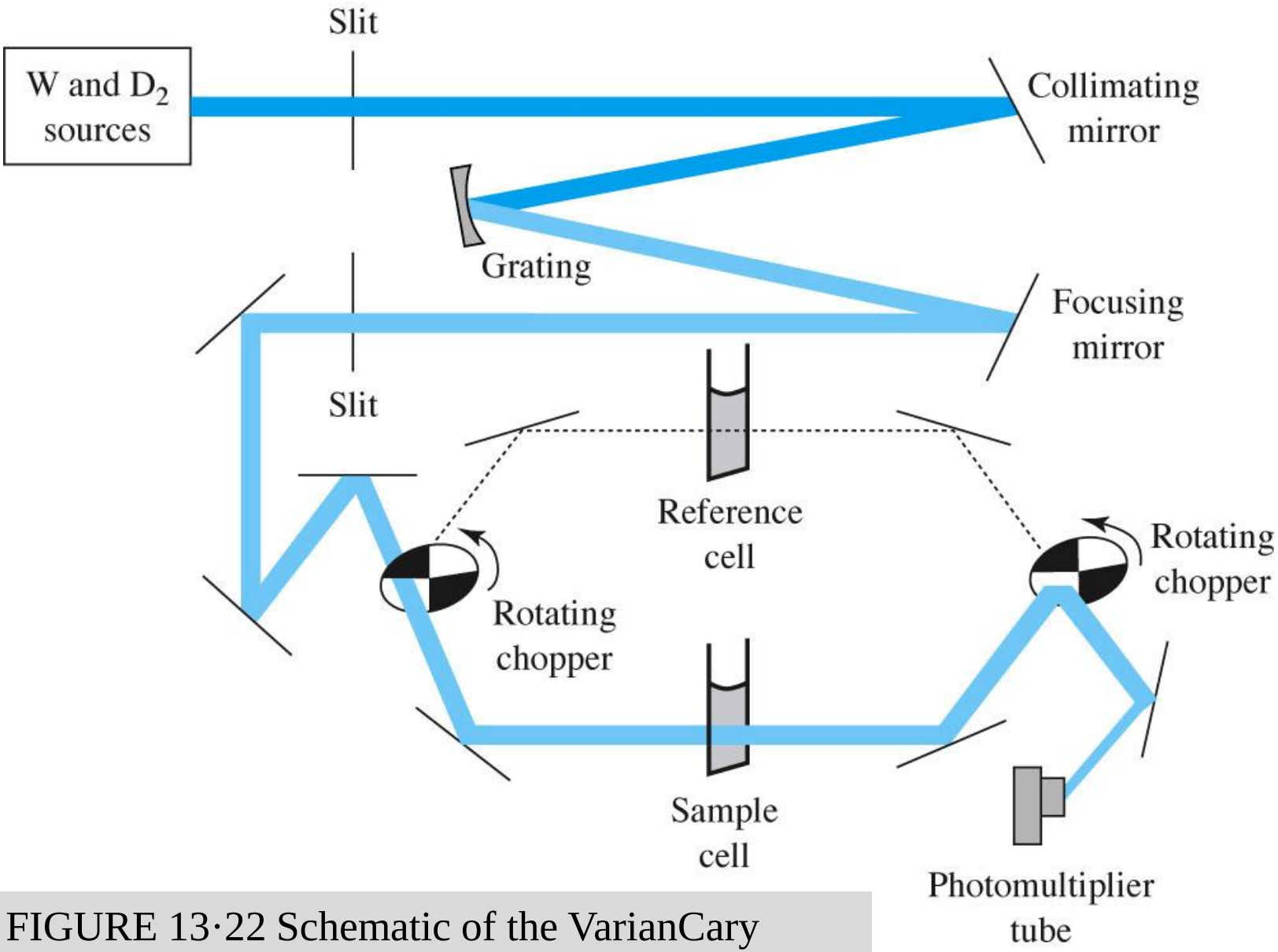


FIGURE 13·22 Schematic of the VarianCary 100 double-beam spectrophotometer for the UV-visible region

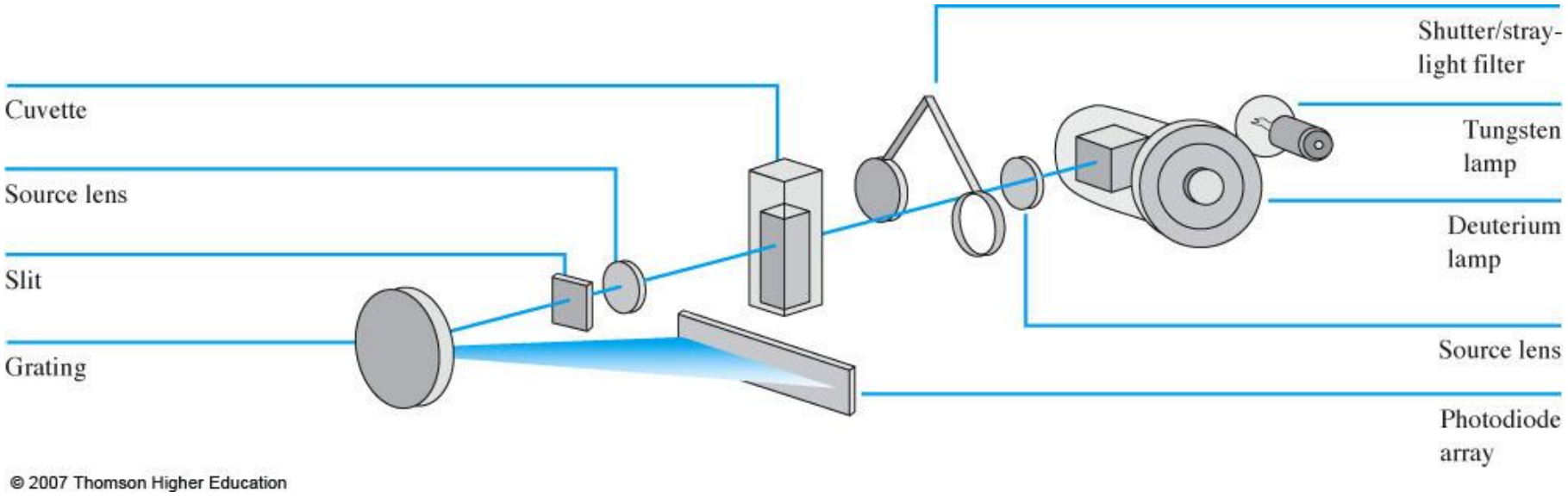


FIGURE 13-25 A multichannel diode-array spectrophotometer, the Agilent Technologies 8453.

Chapter 14: Applications of UV-Vis Molecular Absorption Spectrometry

Characteristics of UV/Vis Methods:

-Wide applicability to organic and inorganic systems

-Sensitivities to 10^{-4} to 10^{-7} *M*

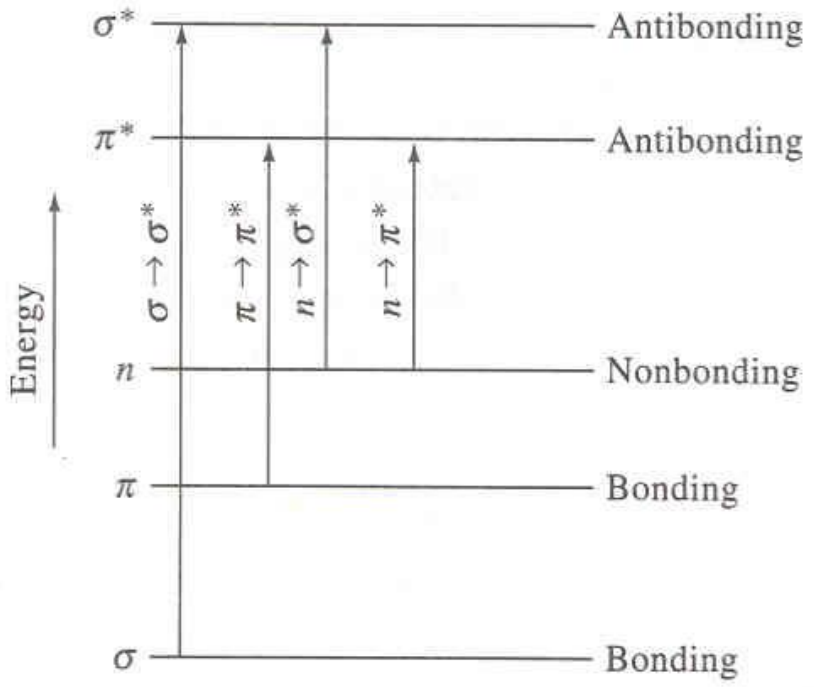
-Moderate to high selectivity

-Good accuracy, about 1-3% relative uncertainty

-Easy and convenient data acquisition

UV/Vis Absorbance:

- Results from excitation of bonding electrons. So, can correlate wavelength of absorption peaks with types of bonds.
- Types of electronic transitions relative to UV/Vis absorbance:
  1) π, σ, and *n* electrons
  2) *d* and *f* electrons
  3) Charge-transfer electrons



Absorbing Electrons:

Electrons that contribute to absorbance in organic molecules are:
1) Those that directly participate in bond formation between atoms and are associated with more than one atom.
2) Nonbonding or unshared outer electrons largely localized about atoms like oxygen, sulfur, nitrogen, halogens.

- The σ to σ* transition requires an absorption of a photon with a wavelength which does not fall in the UV-vis range (see table below).
- Thus, only π to π* and n to π* transitions occur in the UV-vis region are observed.

Compound	λ(nm)	Intensity/ε	transition with lowest energy
CH_4	122	intense	σ-σ*(C-H)
CH_3CH_3	130	intense	σ–σ* (C-C)
CH_3OH	183	200	n–σ* (C-O)
CH_3SH	235	180	n–σ* (C-S)
CH_3NH_2	210	800	n–σ* (C-N)
CH_3Cl	173	200	n–σ* (C-Cl)
CH_3I	258	380	n–σ* (C-I)
$CH_2=CH_2$	165	16000	π–π* (C=C)
CH_3COCH_3	187	950	π-π* (C=O)
	273	14	n–π* (C=O)
CH_3CSCH_3	460	weak	n–π* (C=S)
$CH_3N=NCH_3$	347	15	n–π* (N=N)

TABLE 14-1 Absorption Characteristics of Some Common Chromophores

Chromophore	Example	Solvent	λ_{max}, nm	ε_{max}	Transition Type
Alkene	$C_6H_{13}CH{=}CH_2$	*n*-Heptane	177	13,000	$\pi \rightarrow \pi^*$
Alkyne	$C_5H_{11}CH{\equiv}C{-}CH_3$	*n*-Heptane	178	10,000	$\pi \rightarrow \pi^*$
			196	2000	—
			225	160	—
Carbonyl	CH_3CCH_3 (O)	*n*-Hexane	186	1000	$n \rightarrow \sigma^*$
			280	16	$n \rightarrow \pi^*$
	CH_3CH (O)	*n*-Hexane	180	large	$n \rightarrow \sigma^*$
			293	12	$n \rightarrow \pi^*$
Carboxyl	CH_3COOH	Ethanol	204	41	$n \rightarrow \pi^*$
Amido	CH_3CNH_2 (O)	Water	214	60	$n \rightarrow \pi^*$
Azo	$CH_3N{=}NCH_3$	Ethanol	339	5	$n \rightarrow \pi^*$
Nitro	CH_3NO_2	Isooctane	280	22	$n \rightarrow \pi^*$
Nitroso	C_4H_9NO	Ethyl ether	300	100	—
			665	20	$n \rightarrow \pi^*$
Nitrate	$C_2H_5ONO_2$	Dioxane	270	12	$n \rightarrow \pi^*$

- The excitation energies associated with electrons forming most single bonds are sufficiently high that absorption occurs in the so-called vacuum UV region (λ<185 nm), where components of the atmosphere also absorb radiation strongly. Such transitions involve the excitation of nonbonding n electrons to σ^* orbitals.
The molar absorptivities of $n \rightarrow \sigma^*$ transitions are low to intermediate and usually range between 100 and 3000 L mol^{-1} cm^{-1}. Because of experimental difficulties associated with the vacuum UV region, investigations of organic compounds are performed at λ's longer than 185 nm.

-Most applications of absorption spectroscopy to organic molecules are based on *n* to π^* or π to π^* transitions because the energies required for these processes bring the absorption bands into the UV-visible region (200 to 700 nm).
-Both *n* to π^* and π to π^* transitions require the precence of unsaturated functional groups.
-Molecules containing such functional groups and capable of absorbing UV-visible radiation are called chromophores.

Effect of solvents in reducing fine structure in absorbance spectra

- the visible absorption spectrum for 1,2,4,5-tetrazine vapor shows the fine structure that is due to the numerous rotational and vibrational levels associated with the excited electronic states of this aromatic molecule.
- In the gaseous state, the individual tetrazine molecules are sufficiently separated from one another to vibrate and rotate freely, and the many individual absorption lines appear as a result of the large number of vibrational and rotational energy states.
- In the condensed state or in solution, however, the tetrazine molecules have little freedom to rotate, so lines due to differences in rotational energy levels disappear. Furthermore, when solvent molecules surround the tetrazine molecules, energies of the various vibrational levels are modified in a nonuniform way, and the energy of a given state in a sample of solute molecules appears as a single, broad peak.
- This effect is more pronounced in polar solvents, such as water, than in nonpolar hydrocarbon media.

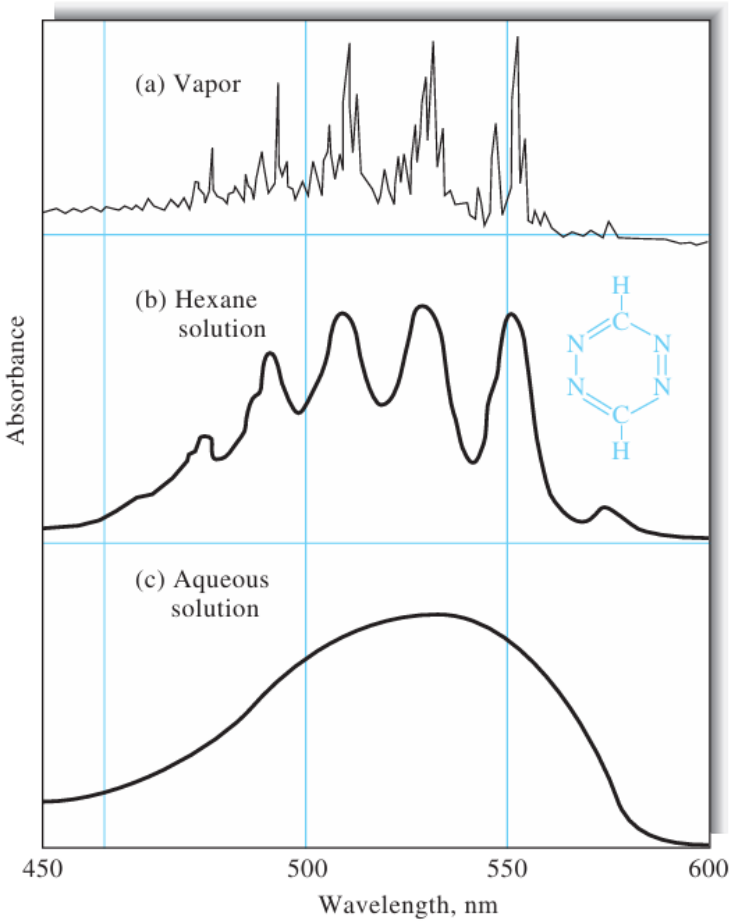


FIGURE 14-1 Ultraviolet absorption spectra for 1,2,4,5-tetrazine. In (a), the spectrum is shown in the gas phase, where many lines due to electronic, vibrational, and rotational transitions can be seen. In a nonpolar solvent (b), the electronic transitions can be observed, but the vibrational and rotational structure has been lost. In a polar solvent (c), the strong intermolecular forces cause the electronic peaks to blend, giving only a single smooth absorption band. (From S. F. Mason, *J. Chem. Soc.*, **1959**, 1265, **DOI**: 10.1039/jr9590001263.)

Absorption of d and f electrons:

- Most transition-metal ions absorb in UV/Vis.
- A number of inorganic anions exhibit UV absorption bands that are a result of exciting nonbonding electrons. e.g. nitrate (313 nm), carbonate (217 nm), nitrite (360 and 280 nm), azido (230 nm), and trithiocarbonate (500 nm) ions.

- Ions or complexes from first and second transition series absorb visible radiation in at least one of their oxidation states and are, as a result, colored (see, for example, Figure 14-3).
- Here, absorption involves transitions between filled and unfilled d-orbitals with energies that depend on the ligands bonded to the metal ions. The energy differences between these d-orbitals (and thus the position of the corresponding absorption maximum) depend on the position of the element in the periodic table, its oxidation state, and the nature of the ligand bonded to it.

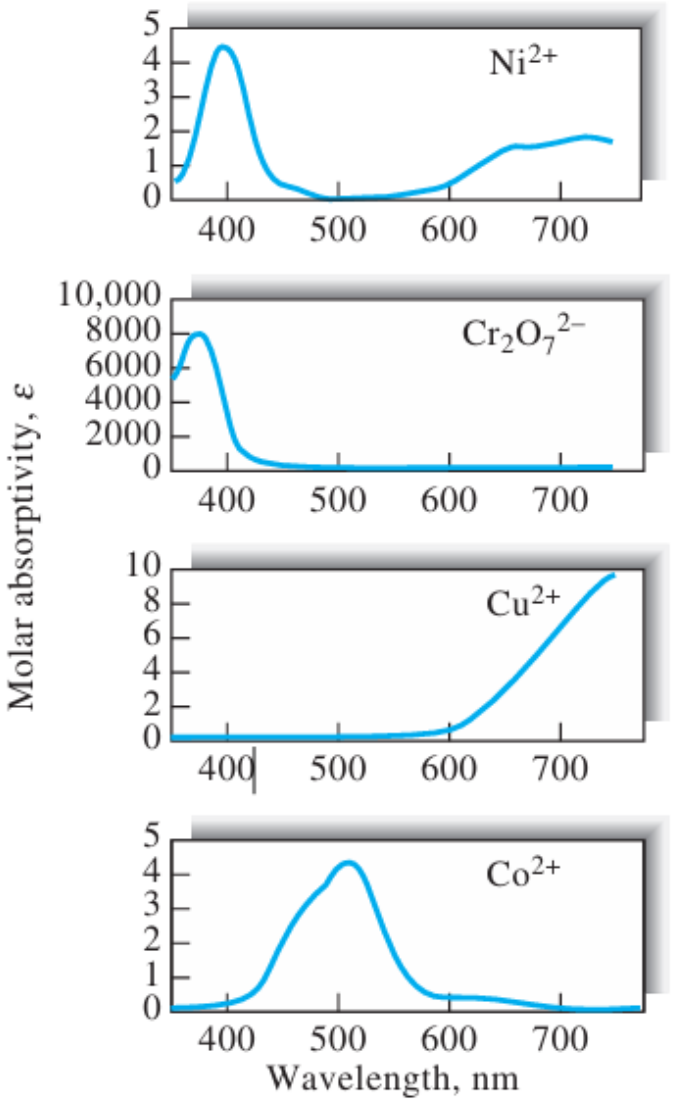
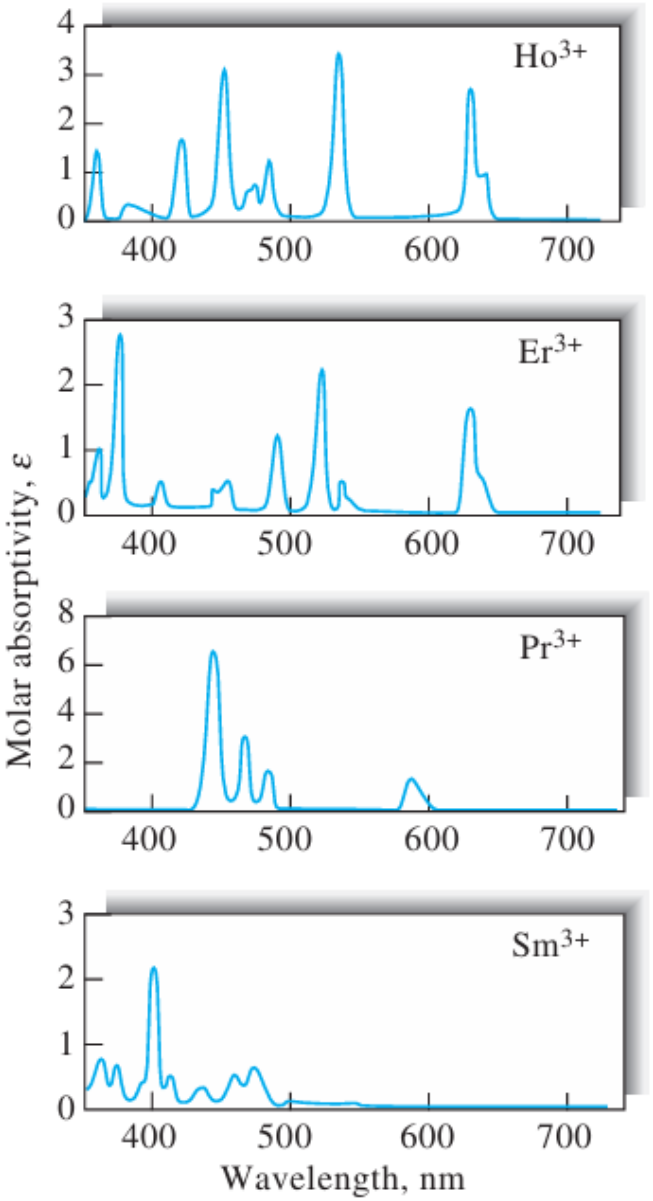


Figure 14-3 absorption spectra of aqueous solutions of transition metal ions.

- *Lanthanides and actinides* have narrow, well-defined, characteristic absorption peaks.
- The electrons responsible for absorption by these elements (4f and 5f, respectively) are shielded from external influences by electrons that occupy orbitals with larger principal quantum numbers. As a result, the bands tend to be narrow and relatively unaffected by the species bonded by the outer electrons



14B-3 Charge-Transfer Absorption

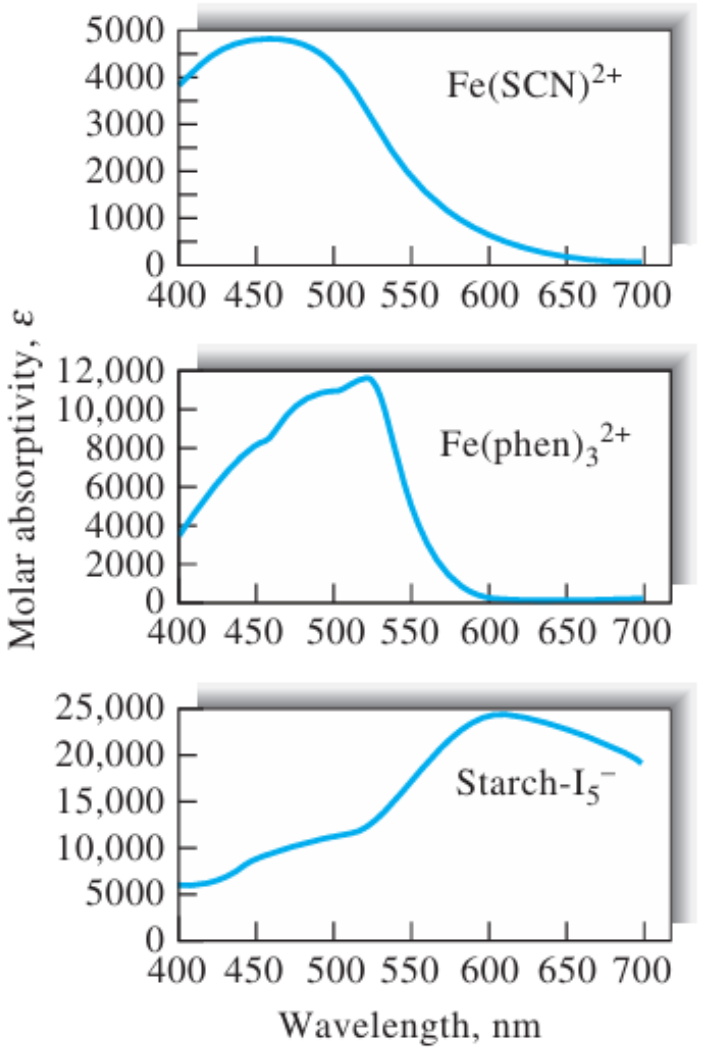
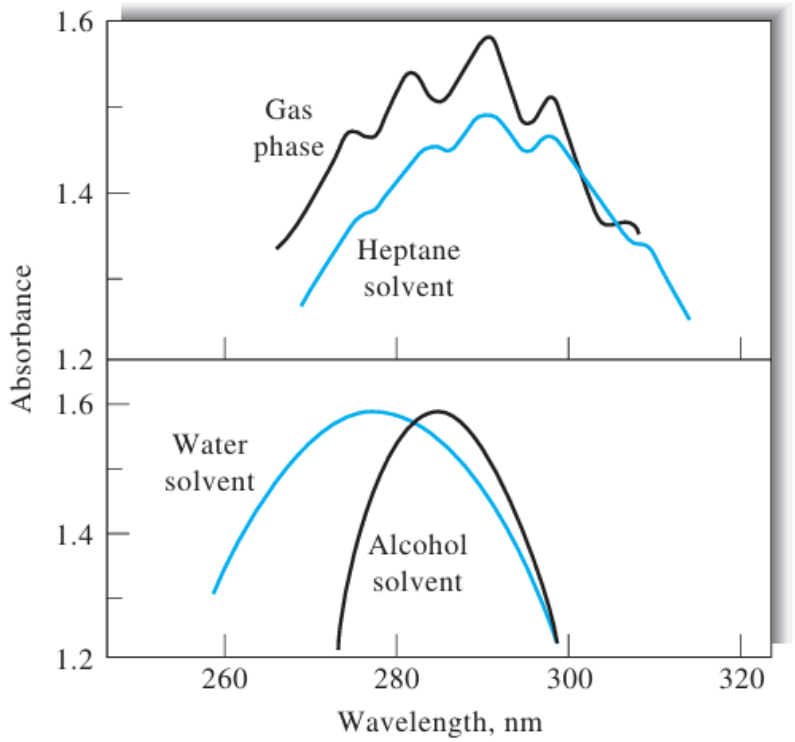


Figure 14-5 absorption spectra of aqueous charge-transfer complexes.

- A charge-transfer complex consists of an electron- donor group bonded to an electron acceptor.
- When this product absorbs radiation, an electron from the donor is transferred to an orbital that is largely associated with the acceptor.
- The excited state is thus the product of a kind of internal oxidation-reduction process. This behavior differs from that of an organic chromophore in which the excited electron is in a molecular orbital shared by two or more atoms.
- Many inorganic and organic complexes exhibit charge-transfer complexes.
  - phenolic complex of iron(III), the 1,10-phenanthroline complex of iron(II), the iodide complex of molecular iodine, and the hexacyanoferrate(II)-hexacyanoferrate(III) complex responsible for the color of Prussian blue. The red color of the iron(III)- thiocyanate complex is a further example of charge-transfer absorption.
- absorption is particularly important because ε are unusually large (>10,000 L mol-1 cm-1), which leads to high sensitivity.

Effect of Solvent on Absorption Spectra

- Polar solvents (such as water, alcohols, esters, and ketones) eliminate fine detail.
- Nonpolar solvent keep spectral details more similar to gas phase measurements.



- the positions of absorption maxima are influenced by the nature of the solvent. As a rule, the same solvent must be used when comparing absorption spectra for identification purposes.

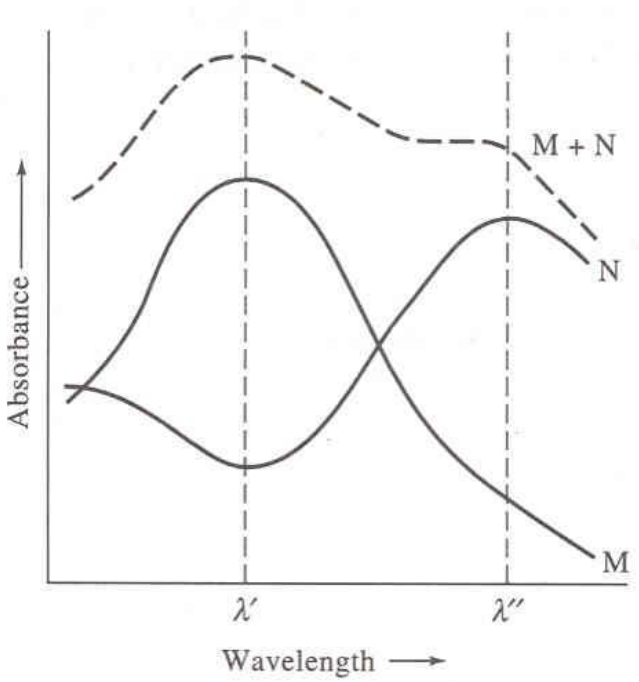
- cutoff wavelengths,

Solvent	Lower Wavelength Limit, nm	Solvent	Lower Wavelength Limit, nm
Water	180	Diethyl ether	210
Ethanol	220	Acetone	330
Hexane	200	Dioxane	320
Cyclohexane	200	Cellosolve	320
Carbon tetrachloride	260		

Absorbance Spectra of Mixtures:

- The absorbance of a solution at a given wavelength is the sum of the absorbances of the individual components.

$$A_1 = \varepsilon_{M_1} b c_M + \varepsilon_{N_1} b c_N$$
$$A_2 = \varepsilon_{M_2} b c_M + \varepsilon_{N_2} b c_N$$

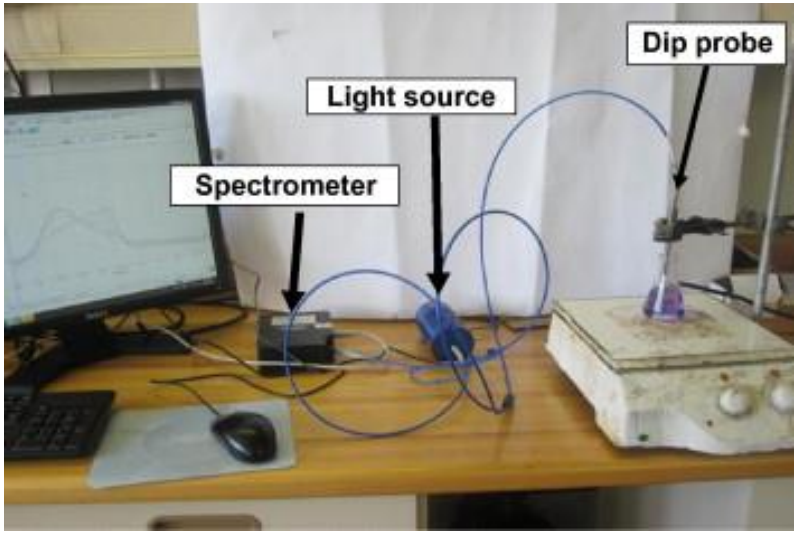


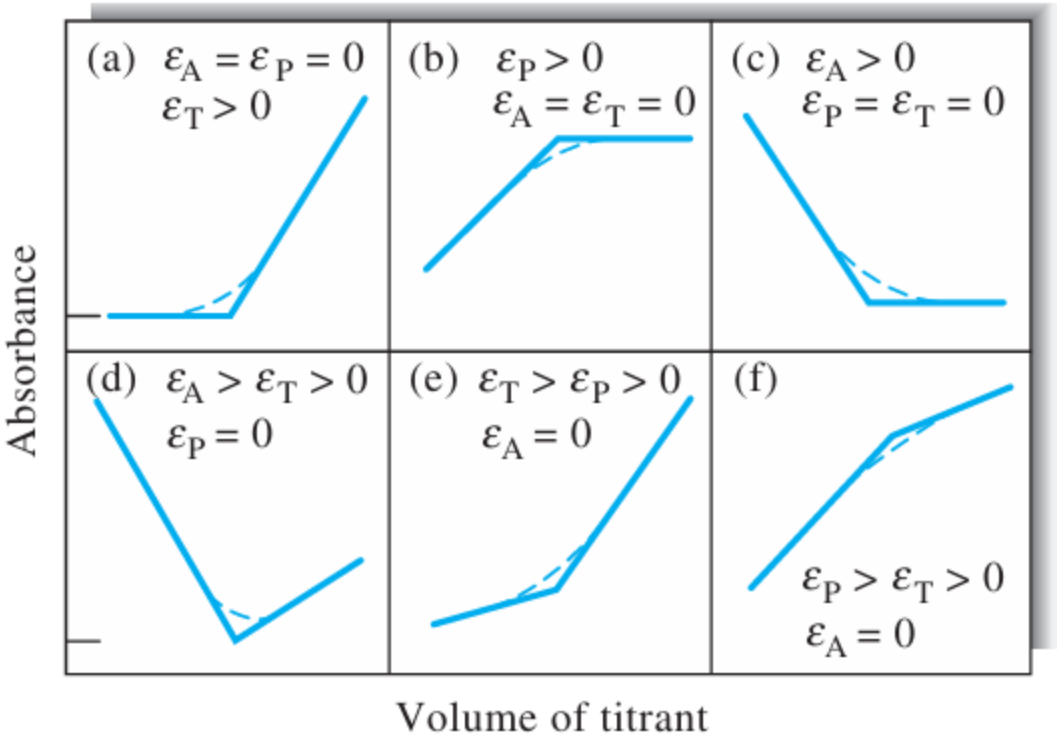
- To analyze the mixture, molar absorptivities for M and N are first determined at wavelengths λ1, and λ2 with sufficient concentrations of the two standard solutions to be sure that Beer's law is obeyed over an absorbance range that encompasses the absorbance of the sample.
- Note that the wavelengths selected are ones at which the molar absorptivities of the two components differ significantly. Thus, at λ1 the molar absorptivity of component M is much larger than that for component N.
- The greatest accuracy is obtained by choosing wavelengths at which the differences in molar absorptivities are large.

Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

14-E Photometric Titration:

- Photometric titrations are ordinarily performed with a spectrophotometer or a photometer that has been modified so that the titration vessel is held stationary in the light path
- Absorbance as a function of volume of titrant is plotted.
- Either analyte, titrant, product, or indicator must absorb.





Source: Skoog, Holler, and Nieman, *Principles of Instrumental Analysis*, 5th edition, Saunders College Publishing.

14-F Spectrophotometric Kinetic Methods

- In kinetic methods, measurements are made under dynamic conditions in which the concentrations of reactants and products are changing as a function of time.
- titrations or procedures using complexing agents to form absorbing products are performed on systems that have come to equilibrium or steady state so that concentrations are static.
- The majority of kinetic methods use spectrophotometry as the reaction monitoring technique.

$A + R \rightarrow P$
A : the analyte,
R : the reagent, and
P : the product.

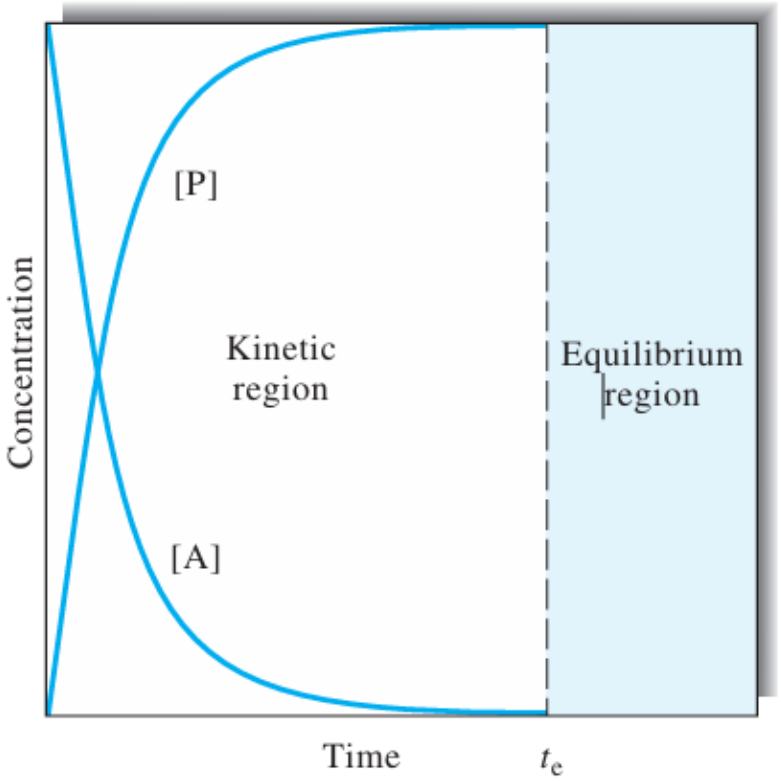


Figure 14-14 Change in concentration of analyte [A] and product [P] as a function of time. Until time t_e the analyte and product concentrations are continuously changing. This is the kinetic regime. In the equilibrium region, after t_e, the analyte and product concentrations are static.

Instrumentation

- Kinetic methods based on reactions with half-lives > about 10 s, can be performed in an ordinary spectrophotometer equipped with a thermostatted cell compartment and provision to introduce and mix samples and reagents.
- Rates of reaction are highly dependent on temperature, and so temperature control to about 0.1°C is necessary for good reproducibility.
- Many commercial spectrophotometers have attachments that allow rates to be obtained. For very slow reactions, sample introduction and mixing can be accomplished prior to placing the reaction mixture in the cell compartment. Usually, however, a stationary cell is used, and all reagents except one are placed in the cell. The reagent needed to start the reaction is then introduced by syringe or pipette and the ensuing reaction is monitored while the mixture is stirred.
- With single-channel spectrophotometers, the reaction is monitored at a single wavelength by measuring the absorbance as a function of time.
- Array-detector-based instruments allow entire spectra to be taken at different time intervals for later analysis.

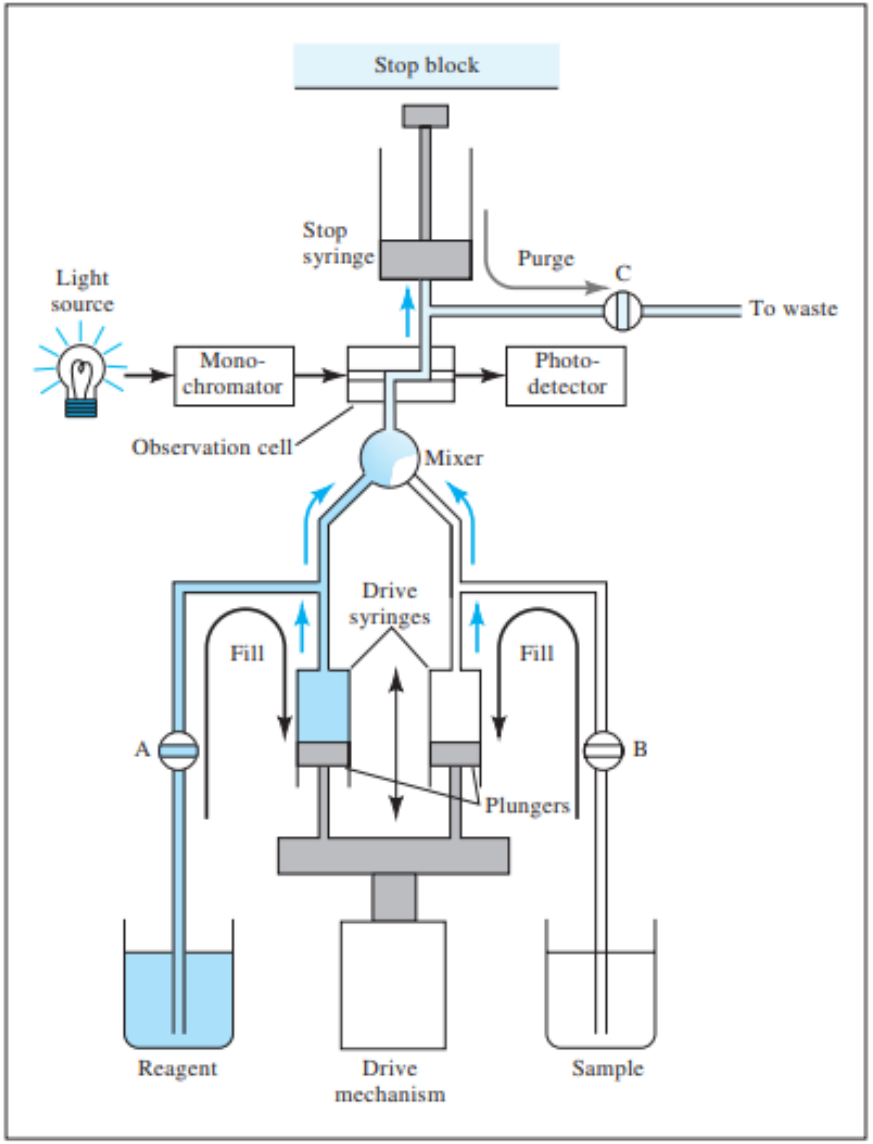


FIGURE 14-16 Stopped-flow mixing apparatus. To begin the experiment, the drive syringes are filled with reagent and sample, and valves A, B, and C are closed. The drive mechanism is then activated to move the drive syringe plungers forward rapidly. The reagent and sample are mixed in the mixer and pass immediately into the observation cell and stop syringe. When the stop syringe fills, the plunger strikes the stop block and the flow ceases almost instantly with a recently mixed plug of solution in the spectrophotometric observation cell. For well-designed systems, the time between mixing and observation can be on the order of 2 to 4 ms.