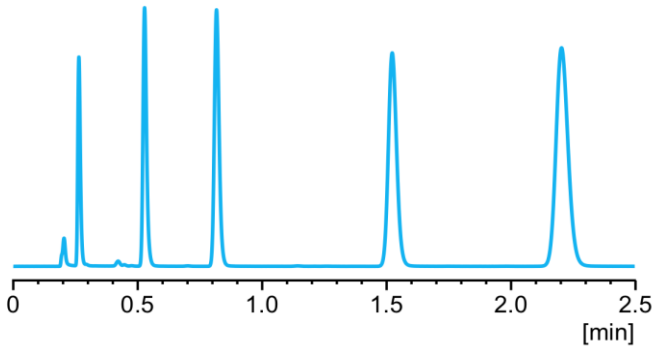
INSTRUMENTAL ANALYSİS LABORATORY MANUAL



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ISTANBUL, 2017

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INSTRUMENTAL ANALYSIS LABORATORY NOTEBOOK

NAME-SURNAME	**NUMBER**	Fotoğraf
E-mail	**Mobile Phone**	
LECTURER		
TERM		

	DENEY ADI	**TARİH**	**İMZA**
1	Determination of Fe^{3+} by UV-VIS Absorption Spectrophotometry		
2	Determination of a Mixture of $KMnO_4$ and $K_2Cr_2O_7$ by UV-VIS Absorption Spectrophotometry		
3	Identification of Organic Compounds using Infrared Spectrometry		
4	Determination of Fluorescein Isothiocyanate (FITC) by Spectrofluorometry		
5	Determination of Heavy Metals by Atomic Absorption Spectrometry		
6	A Mixture Analysis by Thin Layer Chromatography		
7	A Mixture Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)		
8	Determination of Caffeine in Coke by Liquid Chromatography		
9	Determination of the Equivalence Point in Potentiometric Titrations		
10	Voltammetric Determination of Paracetamol		

DECLARATION

NAME-SURNAME:

STUDENT NUMBER:

I have been informed about the safety rules in the Instrumental Analysis Laboratory by instructors. I also have been warned about the possible dangers which I meet in laboratory in case I do not obey the safety rules. I understand the importance of personal safety and know that I should wear **safety glasses, laboratory coat and gloves** for the protection at all times in the Instrumental Analysis Laboratory. I accept the full responsibilities of any possible lab accidents in case of violation of safety rules.

If I do not abide by the safety rules, I will not hold instructors of Instrumental Analysis Laboratory liable for any injuries which result.

Date:

Place: Instrumental Analysis Laboratory

Signature:

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Date:

Place: Instrumental Analysis Laboratory

Signature:

CONTENT

LABORATORY SAFETY RULES AND REGULATIONS

1) DO NOT perform unauthorized experiments or work in a laboratory alone.
2) Approved eye protection must be worn at all times in the laboratory.
3) Long hair and loose clothing must be confined while in a laboratory.
4) Before obtaining any chemicals carefully read the label on the reagent bottles.
5) Eating, smoking, and drinking are not allowed in a chemistry laboratory.
6) Thoroughly wash your hands after leaving the laboratory.
7) Never direct the open end of test tube toward yourself or anyone else.
8) Never pour water into concentrated acid.
9) Always wipe spatulas clean before and after inserting into reagent bottles.
10) Report any accident and/or injury, however minor, to your instructor immediately.
11) Never place anything that is not directly required for the experiment on laboratory desks; other items may interfere with the experiment.
12) Clean up any spill immediately.
13) Your instructor is available for any assistance you may need. Never hesitate to ask questions especially if there is any question concerning proper operating procedure. Be sure that you understand every instruction before proceeding.

1) Determination of Fe (III) by UV-VIS Absorption Spectrophotometry

Objectives: To examine a quantitative analysis with UV-VIS Absorption Spectrophotometer

Introduction

Spectroscopists use the interactions of radiation with matter to obtain information about a sample. Several of the chemical elements were discovered by spectroscopy. The sample is usually stimulated by applying energy in the form of heat, electrical energy, light, particles, or a chemical reaction. Prior to applying the stimulus, the analyte is predominantly in its lowest energy state, or ground state. The stimulus then causes some of the analyte species to undergo a transition to a higher energy, or excited state. We acquire information about the analyte by measuring the electromagnetic radiation emitted as it returns to the ground state or by measuring the amount of electromagnetic radiation absorbed or scattered as a result of excitation.

According to quantum theory, atoms, molecules, and ions have only a limited number of discrete energy levels; for absorption of radiation to occur, the energy of the exciting photon must exactly match the energy difference between the ground state and one of the excited states of the absorbing species. Since these energy differences are unique for each species, a study of the frequencies of absorbed radiation provides a means of characterizing the constituents of a sample of matter. For this purpose, a plot of absorbance as a function of wavelength or frequency is experimentally determined (absorbance, a measure of the decrease in radiant power.

Atomic Absorption

Excitation can occur only by an electronic process in which one or more of the electrons of the atom are raised to a higher energy level.

Molecular Absorption

Absorption spectra for polyatomic molecules, particularly in the condensed state, are considerably more complex than atomic spectra because the number of energy states of molecules is generally enormous when compared with the number of energy states for isolated atoms. The energy E associated with the bands of a molecule is made up of three components. That is,

$$E = E_{electronic} + E_{vibrational} + E_{rotational} \quad (1.1)$$

where $E_{electronic}$ describes the electronic energy of the molecule that arises from the energy states of its several bonding electrons. The second term on the right refers to the total energy associated with the multitude of interatomic vibrations that are present in molecular species. Generally, a molecule has many more quantized vibrational energy levels than it does electronic levels. Finally, $E_{rotational}$ is the energy caused by various rotational motions within a molecule; again the number of rotational states is much larger than the number of vibrational states. Thus, for each electronic energy state of a molecule, there are normally several possible vibrational states. For each of these vibrational states, in turn, numerous rotational states are possible. As a consequence, the number of possible energy levels for a molecule is normally orders of magnitude greater than the number of possible energy levels for an atomic particle. When some of the incident radiation is absorbed, it promotes some of the analyte species to an excited state, as shown in Figure 1.1.

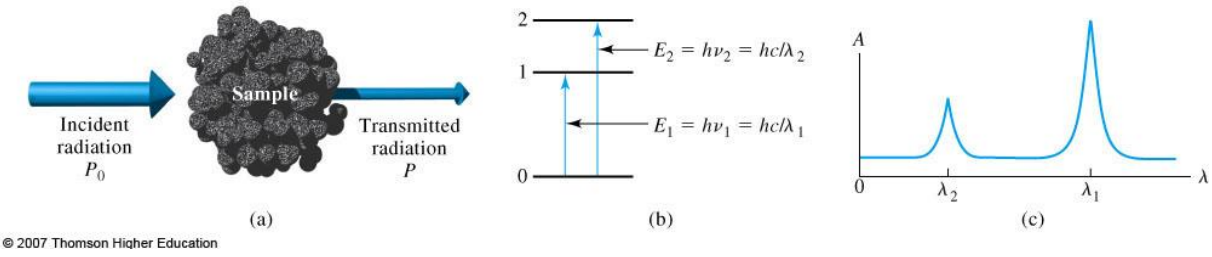


Figure 1.1 Absorption methods. Radiation of incident radiant power P_0 can be absorbed by the analyte, resulting in a transmitted beam of lower radiant power P. For absorption to occur, the energy of the incident beam must correspond to one of the energy differences shown in (b). The resulting absorption spectrum is shown in (c).

Absorbance

The absorbance A of a medium is defined by the equation

$$A = -\log_{10} T = \log \frac{P_0}{P} \tag{1.2}$$

Note that, in contrast to transmittance, the absorbance of a medium increases as attenuation of the beam becomes greater.

Beer's Law

For monochromatic radiation, absorbance is directly proportional to the path length b through the medium and the concentration c of the absorbing species. These relationships are given by

$$A = a.b.c \tag{1.3}$$

where a is *a* proportionality constant called the absorptivity. The magnitude of *a* depends on the units used for band *c*. For solutions of an absorbing species, *b* is often given m centimeters and *c* in grams per liter. Absorptivity then has units of L g^{-1} cm^{-1}.

When the concentration in Equation 6-33 is expressed in moles per liter and the cell length is in centimeters, the absorptivity is called the molar absorptivity and is given the special symbol €. Thus, when b is in centimeters and c is in moles per liter,

$$A = \varepsilon.b.c \tag{1.4}$$

where Ɛ has the units L mol^{-1} cm^{-1}.

Equations 1.3 and 1.4 are expressions of Beer's law, which serves as the basis for quantitative analyses by both atomic and molecular absorption measurements.

Typical spectroscopic instruments contain five components: (1) a stable source of radiant energy: (2) a transparent container for holding the sample; (3) a device that isolates a restricted region of the spectrum for measurement; (4) a radiation detector, which converts radiant energy to a usable electrical signal; and (5) a signal processor and readout, which displays the transduced signal on a meter scale, a computer screen, a digital meter, or another recording device. Figure 1.2 illustrates the components of absorption spectrophotometer.

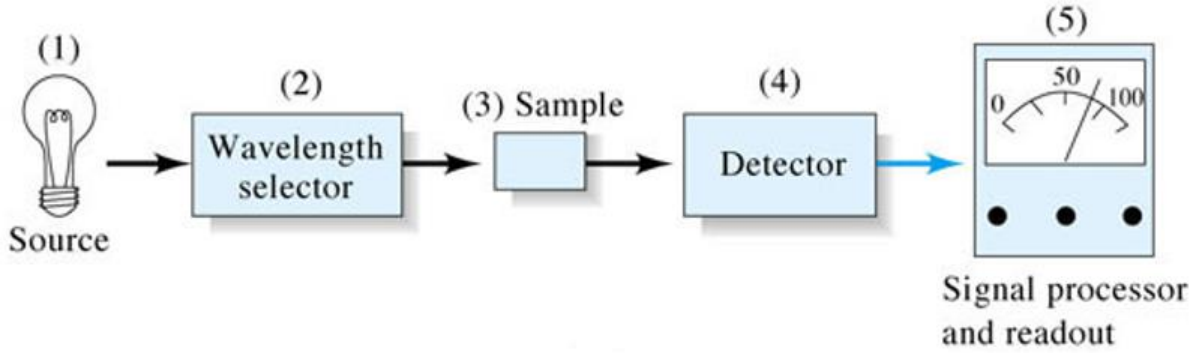


Figure 1.2 Components of absorption spectrophotometer.

For the purpose of molecular absorption measurements, a continuum source is required whose radiant power does not change sharply over a considerable range of wavelengths. A continuum spectrum in the ultraviolet region is produced by electrical excitation of deuterium or hydrogen at low pressure. The most common source of visible and near-infrared radiation is the tungsten

filament lamp. The xenon arc lamp produces intense radiation by the passage of current through an atmosphere of xenon. The tungsten lamp is well into the UV region.

In common with the other optical elements of an absorption instrument, the cells, or cuvettes, that hold the sample and solvent must be constructed of a material that passes radiation in the spectral region of interest. Thus, quartz or fused silica is required for work in the ultraviolet region (below 350 nm). Both of these substances are transparent throughout the visible and near-infrared regions to about 3 f1m.Silicate glasses can be employed in the region between 350 and 2000 nm. Plastic containers are also used in the visible region.

Ultraviolet spectra for qualitative analysis are usually measured using dilute solutions of the analyte. In choosing a solvent, consideration must be given not only to its transparency, but also to its possible effects on the absorbing system. Quite generally, polar solvents such as water, alcohols, esters, and ketones tend to obliterate spectral fine structure arising from vibrational effects. Spectra similar to gas-phase spectra are more likely to be observed in nonpolar solvents such as hydrocarbons. In addition, 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. Common solvents for ultraviolet spectrophotometry include water, 95% ethanol, cyclohexane, and 1,4-dioxane. For the visible region, any colorless solvent is suitable.

Absorption spectroscopy based on ultraviolet and visible radiation is one of the most useful tools available to the scientist for quantitative analysis6 Important characteristics of spectrophotometric and photometric methods include (1) wide applicability to both organic and inorganic systems, (2) typical detection limits of 10^{-4} to 10^{-5} M (in some cases, certain modifications can lead to lower limits of detection), (3) moderate to high selectivity, (4) good accuracy (typically, relative uncertainties are 1% to 3%, although with special precautions. errors can be reduced to a few tenths of a percent), and (5) ease and convenience of data acquisition.

Spectrophotometric determination of any organic compound containing one or more of chromophoric groups is potentially feasible. A number of inorganic species also absorb UV-visible radiation and are thus susceptible to direct determination. We have noted that many ions of the transition metals are colored in solution and can .thus be determined by spectrophotometric measurement. In addition, a number of other species show characteristic

absorption bands, including nitrite, nitrate, and chromate ions, the oxides of nitrogen, the elemental halogens, and ozone.

Numerous reagents react selectively with nonabsorbing species to yield products that absorb strongly in the ultraviolet or visible regions. A host of complexing agents are used to determine inorganic species. Typical inorganic reagents include thiocyanate ion for iron, cobalt, and molyhdenum; hvdrogen peroxide for titanium, vanadium, and chromium; and iodide ion for bismuth, palladium, and tellurium. Of even more importance are organic chelating agents that form stable, colored complexes with cations. Common examples include dlethyldlthiocarbamate for the determination of copper. diphenylthiocarbazone for lead, 1,10-phenanthrolene for iron, and dimethylglyoxime for nickel.

For highest sensitivity, spectrophotometric absorbance measurements are ordinarily made at a wavelength corresponding to an absorption maximum because the change in absorbance per unit of concentration is greatest at this point. In addition, the absorbance is nearly constant with wavelength at an absorption maximum, which leads to close adherence to Beer's law. Finally, small uncertainties that arise from failing to reproduce precisely the wavelength setting of the instrument have less influence at an absorption maximum.

Procedure

1) Reagents

a) 0.1 mg/mL standard solution of Fe^{3+}

b) 0.1 M NH_4SCN

c) 0.1 N HCl

2) Determination of Absorbance

2 mL 0.1 M NH_4SCN and 1 mL 0.1 N HCl are added into 5 mL Fe^{3+} standard solution in a cuvette, after then filled with distilled water and mixed properly. The cuvette is inserted into the cell holder of the spectrophotometer. The wavelength control knob is fixed to 450-530 nm and distilled water is placed into the cell holder. Next, the absorbance is set to zero, which has to be done for each measurement in order to calibrate the instrument. Absorption spectrum plotted between wavelengths and absorbance values can be obtained after recording the absorbance of the samples. λ_{max} which belongs to the highest absorbance has to be determined accurately.

3) Calibration Plot

Five different standard solutions is prepared by the stock solution of 0.1 mg/mL Fe^{3+}. Each volumetric flask has to contain 1, 2, 3, 4 and 5 mL of the stock solution, respectively. After then, 2 mL 0.1 M NH_4SCN and 1 mL 0.1 N HCl have to be added into all volumetric flasks and then filled with distilled water. Each standard solution is mixed and inserted into the cell holder to record their absorbance values at the maximum wavelength of the analyte (λ_{max}). Calibration plot can be drawn by plotting concentration on the axis and absorbances on the ordinate.

4) Sample Analysis

Sample is diluted into 100 mL in a volumetric flask and 1 mL of this solution is placed into a 10 mL volumetric flask and then, 2 ml 0.1 M NH_4SCN and 1 mL 0.1 N HCl are added into this volumetric flask and filled with distilled water. The absorbance of the sample is obtained from the calibration plot after recording its absorbance at the maximum wavelength of the analyte (λ_{max}).

2) Determination of a Mixture of $KMnO_4$ and $K_2Cr_2O_7$ by UV-VIS Absorption Spectrophotometry

Objectives: To analyze a two-component mixture with UV-VIS Absorption Spectrometry

Introduction

The total absorbance of a solution at any given wavelength is equal to the sum of the absorbances of the individual components in the solution. This relationship makes it possible in principle to determine the concentrations of the individual components of a mixture even if their spectra overlap completely. For example, Figure 2.1 shows the spectrum of a solution containing a mixture of species M and species N as well as absorption spectra for the individual components.

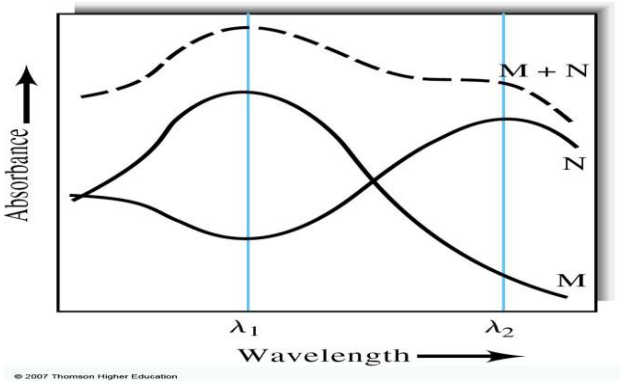


Figure 2.1 Absorption spectrum of a two-component mixture (M+N) with spectra of the individual components.

It is apparent that there is no wavelength where the absorbance is due to just one of these components. To analyze the mixture, molar absorptivities for M and N are first determined at wavelengths λ_1 and λ_2 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 λ_1 the molar absorptivity of component M is much larger than that for component N. The reverse is true for λ_2. To complete the analysis, the absorbance of the mixture is determined at the same two wavelengths. From the known molar absorptivities and path length, the following equations hold:

$$A_1 = \varepsilon_{M_1}.b.c_M + \varepsilon_{N_1}.b.c_N \quad (2.1)$$

$$A_2 = \varepsilon_{M_2}.b.c_M + \varepsilon_{N_2}.b.c_N \quad (2.2)$$

where the subscript 1 indicates measurement at wavelength AI' and the subscript 2 indicates measurement at wavelength A,. With the known values of Ɛ and b, Equations 2.1 and 2.2 represent two equations in two unknowns (c_M and c_N) that can be solved. The relationships are valid only if Beer's law holds at both wavelengths and the two components behave independently of one another. The greatest accuracy is obtained by choosing wavelengths at which the differences in molar ahsorptivities are large.

Mixtures containing more than two absorbing species can be analyzed, in principle at least, if a further absorbance measurement is made for each added component. The uncertainties in the resulting data become greater, however, as the number of measurements increases. Some array-detector spectrophotometers are capable of reducing these uncertainties by overdetermining the system. That is, these instruments use many more data points than unknowns and effectively match the entire spectrum of the unknown as closely' as possible by least-squares techniques using the methods of matrix algebra. The spectra for standard solutions of each component are required for the analysis.

Computer data-processing methods based on factor analysis or principal components analysis have been developed to determine the number of components and their concentrations or absorptivities in mixtures. These methods are usually applied to data obtained from array-detector-based spectrometers.

Procedure

1) Reagents
   a) $4x10^{-4}$ and $20x10^{-4}$ M $KMnO_4$
   b) $1.7x10^{-3}$ and $6.8x10^{-3}$ M $K_2Cr_2O_7$
   c) 0.5 M H_2SO_4

2) Calibration Plot

   Five different standard solutions are prepared from $4x10^{-4}$ and $20x10^{-4}$ M $KMnO_4$ and $1.7x10^{-3}$ and $6.8x10^{-3}$ M $K_2Cr_2O_7$ for each one. These five dilutions of the stock solutions are prepared using 2, 4, 6, 8, and 10 mL diluted to 10 mL with distilled water. The UV absorption spectrums of diluted solutions prepared with $4x10^{-4}$ M $KMnO_4$ and $6.8x10^{-3}$ M $K_2Cr_2O_7$ are measured at λ = 525 nm, while those of diluted solutions prepared with $1,7.10^{-3}$ M $K_2Cr_2O_7$ and 20.10^{-4} M

$KMnO_4$ are measured at λ = 440 nm. Blank solutions for the standard solutions of $KMnO_4$ and $K_2Cr_2O_7$, which have to be used for the calibration of the instrument, are distilled water and 0.5 M H_2SO_4, respectively. After recording the absorbance values, calibration plots are plotted absorbance versus molar concentrations. Molar absorptivities ($Є_{440}$ ($KMnO_4$), $Є_{525}$ ($KMnO_4$), $Є_{440}$ ($K_2Cr_2O_7$) and $Є_{440}$ ($K_2Cr_2O_7$)) are obtained from the slopes of each calibration plot (slope = Є.b) for each wavelengths.

3) Simultaneously Determination of $KMnO_4$ and $K_2Cr_2O_7$

The mixture consisting of $KMnO_4$ and $K_2Cr_2O_7$ is measured at the wavelength of 440 nm and 525 nm.

$$A^{440} = Є_1^{440}.b.c_1 + Є_2^{440}.b.c_2 \quad (2.3)$$

$$A^{525} = Є_1^{525}.b.c_1 + Є_2^{525}.b.c_2 \quad (2.4)$$

where the subscript 1 shows $KMnO_4$ and the subscript 2 shows $K_2Cr_2O_7$. From the above equations, the unknown concentrations can be calculated after placing A, Є and b values.

3) Identification of Organic Compounds using Infrared Spectrometry

Objectivities: To determine some compounds' structures with Infrared Spectrometer

Introduction

The infrared (IR) region of the spectrum encompasses radiation with wavenumbers ranging from about 12,800 to 10 cm^{-1} or wavelengths from 0.78 to 1000 μm. Because of similar applications and instrumentation, the IR spectrum is usually subdivided into three regions, the near-IR, the mid-IR, and the far-IR. Table 3.1 gives the rough limits of each of the three regions.

Table 3.1 IR Spectral Regions

Region	Wavelengths (λ), μm	Wavenumbers ($\bar{\nu}$), cm^{-1}	Frequencies (ν), Hz
Near	0.78 to 2.5	12800 to 4000	3.8×10^{14} to 1.2×10^{14}
Middle	2.5 to 50	4000 to 200	1.2×10^{14} to 6.0×10^{12}
Far	50 to 1000	200 to 10	6.0×10^{12} to 3.0×10^{11}
Most used	2.5 to 15	4000 to 670	1.2×10^{14} to 2.0×10^{13}

IR radiation is not energetic enough to bring about the kinds of electronic transitions that we have encountered in our discussions of ultraviolet and visible radiation. Absorption of IR radiation is thus confined largely to molecular species that have small energy differences between various vibrational and rotational states.

To absorb IR radiation. a molecule must undergo a net change in dipole moment as it vibrates or rotates. Only under these circumstances can the alternating electric field of the radiation interact with the molecule and cause changes in the amplitude of one of its motions. For example the charge distribution around a molecule such as hydrogen chloride is not symmetric because the chlorine has a higher electron density than the hydrogen. Thus hydrogen chloride has a significant dipole moment and is said to be polar. The dipole moment is determined by the magnitude of the charge difference and the distance between the two centers of charge. As a hydrogen chloride molecule vibrates, a regular fluctuation in its dipole moment occurs, and a field is established that can interact with the electric field associated with radiation. If the frequency of the radiation exactly matches a natural vibrational frequency of the molecule, absorption of the radiation takes place that produces a change in the amplitude of the molecular vibration. Similarly, the rotation of asymmetric molecules around their centers of mass results in periodic dipole moment fluctuations that allow interaction with the radiation field.

Vibrational energy levels are also quantized and for most molecules the energy differences between quantum states correspond to the mid-IR region. The IR spectrum of a gas usually consists of a series of closely spaced lines, because there are several rotational energy levels for each vibrational level. On the other hand, rotation is highly restricted in liquids and solids; in such samples, discrete vibrational-rotational lines disappear, leaving only somewhat broadened vibrational bands.

Vibrations fall into the basic categories of stretching and bending. A stretching vibration involves a continuous change in the interatomic distance along the axis of the bond between two atoms. Bending vibrations arc characterized by a change in the angle between two bonds and are of four types: scissoring, rocking, wagging and twisting. These are shown schematically in Figure 3.1.

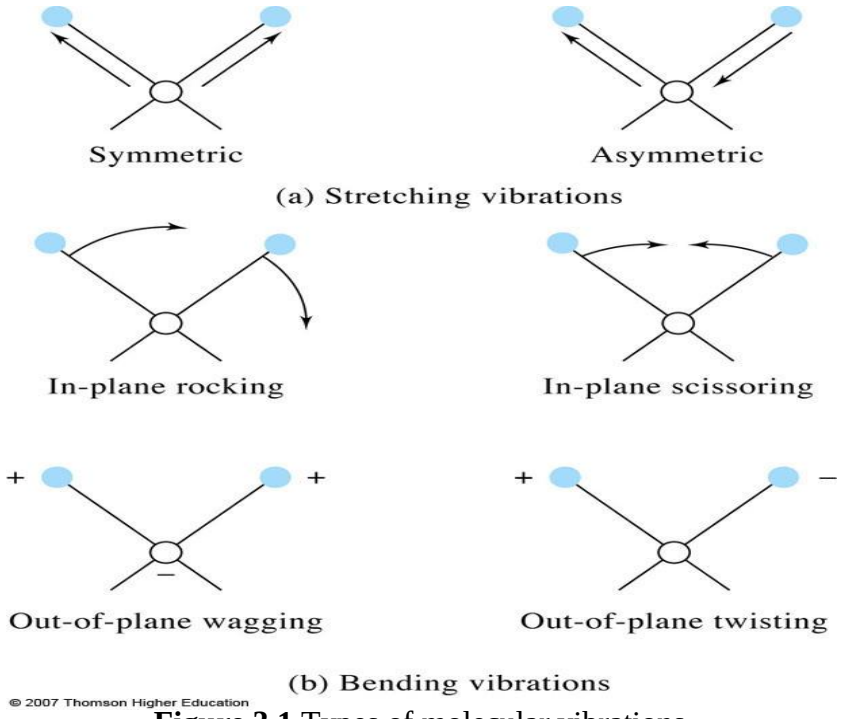


Figure 3.1 Types of molecular vibrations.

Mid-IR absorption and reflection spectrometry are major tools for determining the structure of organic and biochemical species. IR spectroscopy is used for the characterization of solid, liquid and gaseous samples.

The spectrum of a low-boiling-point liquid or gas can be obtained by permitting the sample to expand into an evacuated cylindrical cell equipped with suitable windows. For this purpose, a variety of cylindrical cells are available with path lengths that range from a few centimeters to 10 m or more.

When feasible, a convenient way of obtaining IR spectra ison solutions prepared to contain a known concentration of sample, as is generally done in ultraviolet visible spectrometry. This technique is somewhat limited in its applications, however, by the availability of solvents transparent over significant regions in the IR.

Figure 3.2 lists several common solvents employed for IR studies of organic compounds. This figure illustrates that no single solvent is transparent throughout the entire mid-IR region. Water and the alcohols are difficult to use as solvents in IR spectrometry. Because of the tendency for solvents to absorb IR radiation, IR liquid cells are ordinarily much narrower (0.01 to 1mm) than those employed in the ultraviolet and visible regions. Often, relatively high sample concentrations (from 0.1% to 10%) are required because of the short path lengths and the low molar absorptivities of IR bands.

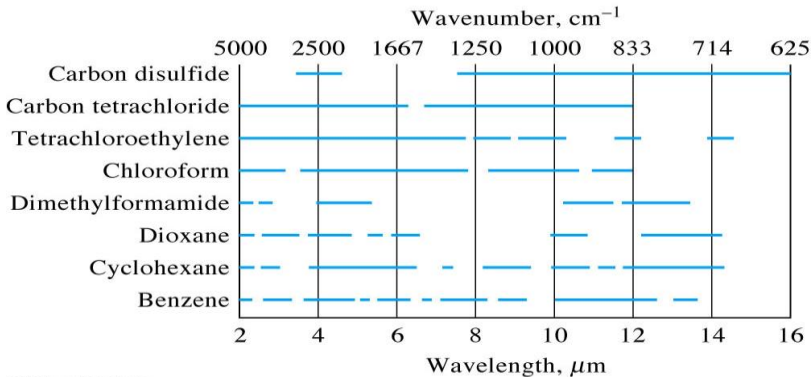


Figure 3.2 Solvents for IR spectroscopy.

When the amount of a liquid sample is small or when a suitable solvent is unavailable, it is common practice to obtain spectra on the pure (neat) liquid. Here, only a very thin film has a sufficiently short path length to produce satisfactory spectra. Commonly, a drop of the neat liquid is squeezed between two rock-salt plates to give a layer 0.015 mm thick or less. The two plates, held together by capillary action, are then placed in the beam path. This technique does not give particularly reproducible transmittance data, but the resulting spectra are usually satisfactory for qualitative investigations.

Most organic compounds exhibit numerous absorption bands throughout the mid-IR region, and finding a solvent that does not have overlapping peaks is often impossible. Because of this spectra are often obtained on dispersions of the solid in a liquid or solid matrix. Generally in these techniques the solid sample must be ground until its particle size is less than the wavelength of the radiation to avoid the effects of scattered radiation. One of the most popular

techniques for handling solid samples has been KBr pelleting (other alkali-metal halides have also been used). Halide salts have the property of cold flow, in which they have glasslike transparent or translucent properties when sufficient pressure is applied to the finely powdered materials. In using this technique, a milligram or less of the finely ground sample is intimately mixed with about 100 mg of dried potassium bromide powder. Mixing can be carried out with a mortar and pestle or, better in a small ball mill. The mixture is then pressed in a special die at 10.000 to 15,000 pounds per square inch to yield a transparent disk. Best results are obtained if the disk is formed in a vacuum to eliminate occluded air. The disk is then held in the instrument beam for spectroscopic examination.

Identification of an organic compound from a spectrum of this kind is a two-step process. The first step involves determining what functional groups are most likely present by examining the group frequency region, which encompasses radiation from about 3600 cm^{-1} to approximately 1250 cm^{-1}. The second step involves a detailed comparison of the spectrum of the unknown with the spectra of pure compounds that contain all of the functional groups found in the first step, Here, the fingerprint region, from 1200 to 600 cm^{-1} is particularly useful because small differences in the structure and constitution of a molecule result in significant changes in the appearance and distribution of absorption bands in this region. Consequently, a close match between two spectra in the fingerprint region (as well as others) constitutes almost certain evidence that the two compounds are identical. Table 3.2 gives some group frequencies for organic functional groups and an example shown in Figure 3.3.

Table 3.2 Abbreviated table of group frequencies for organic functional groups

Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C—H	Alkanes	2850–2970	Strong
		1340–1470	Strong
C—H	Alkenes ($>C=C<^{H}$)	3010–3095	Medium
		675–995	Strong
C—H	Alkynes (—C≡C—H)	3300	Strong
C—H	Aromatic rings	3010–3100	Medium
		690–900	Strong
O—H	Monomeric alcohols, phenols	3590–3650	Variable
	Hydrogen-bonded alcohols, phenols	3200–3600	Variable, sometimes broad
	Monomeric carboxylic acids	3500–3650	Medium
	Hydrogen-bonded carboxylic acids	2500–2700	Broad
N—H	Amines, amides	3300–3500	Medium
C=C	Alkenes	1610–1680	Variable
C=C	Aromatic rings	1500–1600	Variable
C≡C	Alkynes	2100–2260	Variable
C—N	Amines, amides	1180–1360	Strong
C≡N	Nitriles	2210–2280	Strong
C—O	Alcohols, ethers, carboxylic acids, esters	1050–1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690–1760	Strong
NO_2	Nitro compounds	1500–1570	Strong
		1300–1370	Strong

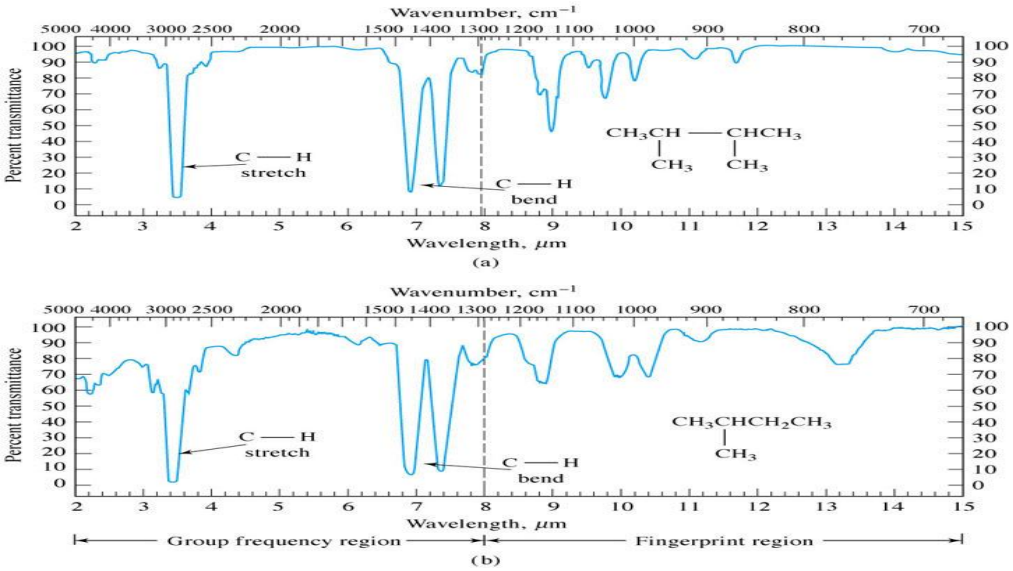


Figure 3.3 An example for IR spectrum

Internal-reflection spectroscopy is a technique for obtaining IR spectra of samples that are difficult to deal with, such as solids of limited solubility, films, threads, pastes, adhesives, and powders.

When a beam of radiation passes from a more dense to a less dense medium, reflection occurs. The fraction of the incident beam reflected increases as the angle of incidence becomes larger; beyond a certain critical angle, reflection is complete. It has been shown both theoretically and experimentally that during the reflection process the beam penetrates a small distance into the less dense medium before reflection occurs. The depth of penetration, which varies from a fraction of a wavelength up to several wavelengths, depends on the wavelength, the index of refraction of the two materials, and the angle of the beam with respect to the interface. The penetrating radiation is called the evanescent wave. At wavelengths where the less dense medium absorbs the evanescent radiation, attenuation of the beam occurs, which is known as attenuated total reflectance, or ATR. The resulting ATR spectrum resembles that of a conventional IR spectrum with some differences. ATR spectra are similar but not identical to ordinary absorption spectra. In general, although the same bands are observed, their relative intensities differ. With ATR spectra, the absorbances, although dependent on the angle of incidence, are independent of sample thickness, because the radiation penetrates only a few micrometers into the sample.

There are two types of spectrometer for IR spectrometers:

a) Dispersive spectrometer
b) Fourier transform infrared spectrometer (FTIR)

The use of Fourier transform instruments has several major advantages. The first is the throughput, or Jaquinot advantage, which is realized because Fourier transform instruments have few optical elements and no slits to attenuate radiation. As a result, the radiant power that reaches the detector is much greater than in dispersive instruments and much greater signal-to noise ratios are observed.

A second advantage of Fourier transform instruments is their extremely high resolving power and wavelength reproducibility that make possible the analysis of complex spectra in which the sheer number of lines and spectral overlap make the determination of individual spectral features difficult.

A third advantage arises because all elements of the source reach the detector simultaneously. This characteristic makes it possible to obtain data for an entire spectrum in 1 second or less. Figure 3.3 illustrates single-beam FTIR spectrometers and Figure 3.4 shows double-beam FTIR spectrometers.

An interferometer in an FTIR instrument does not separate energy into individual frequencies for measurement of the infrared spectrum. Each point in the interferogram contains information from each wavelength of light being measured. In contrast, every wavelength across the spectrum must be measured individually in a dispersive spectrometer. This is a slow process, and typically only one measurement scan of the sample is made in a dispersive instrument. The FTIR advantage is that many scans can be completed and combined on an FTIR in a shorter time than one scan on a dispersive instrument. An FTIR instrument does not use a slit to limit the individual frequency reaching the sample and detector as a dispersive instrument does. There are also fewer mirror surfaces in an FTIR spectrometer, so there are less reflection losses than in a dispersive spectrometer. Overall, more energy reaches the sample and hence the detector in an FTIR spectrometer than in a dispersive spectrometer. This means that the signal-to-noise ratio of an infrared spectrum measured on an FTIR is higher than the signal-to-noise ratio attained on a dispersive instrument. Higher signal-to-noise means that the sensitivity of small peaks will be greater, and details in a sample spectrum will be clearer and more distinguishable in the FTIR spectrum than the dispersive spectrum of the same sample.

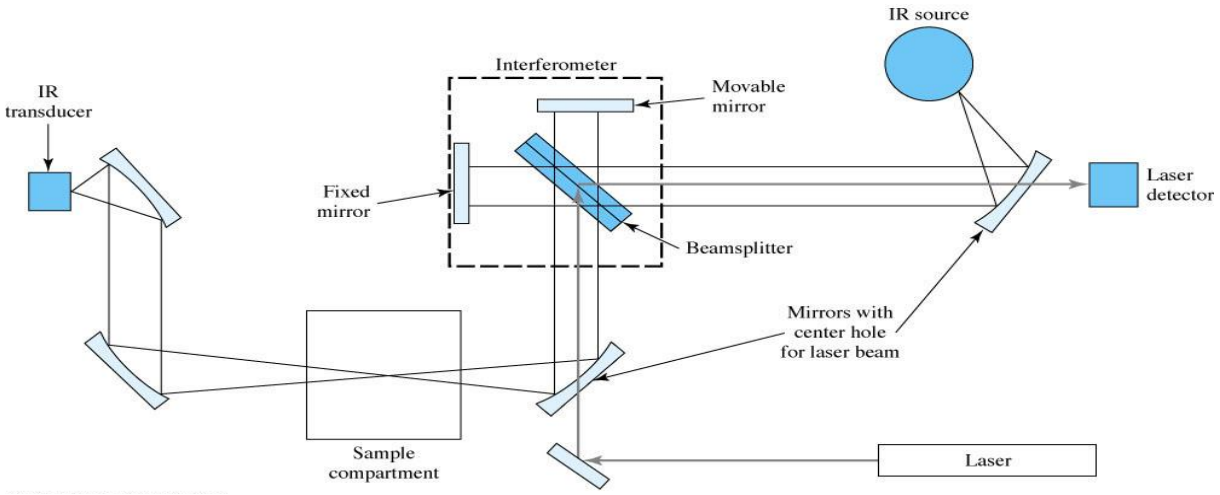


Figure 3.4 Single-beam FTIR spectrometer.

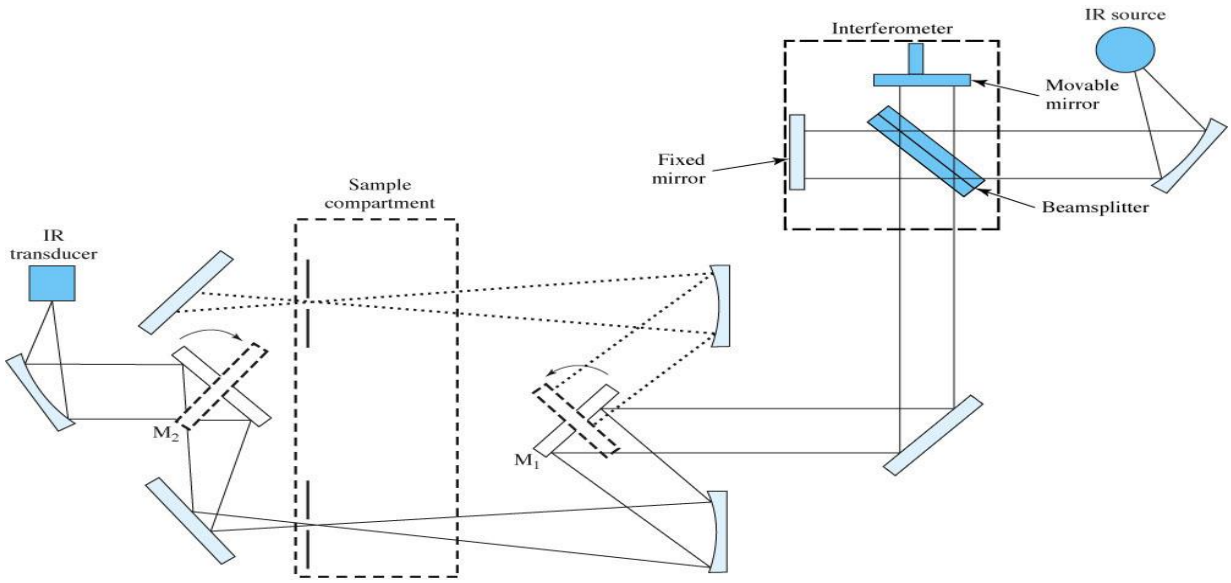


Figure 3.5 Double-beam FTIR spectrometer.

Procedure

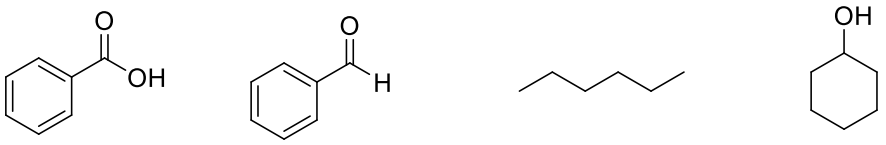
4) Reagents

a) Benzoic acid

b) Benzaldehyde

c) n-hexane

d) Cyclohexanol



(a) (b) (c) (d)

5) Obtaining the IR Spectrum

First step is to record the background against the air with 'collect background' part in the instrument's software. The reason why the spectrometer is set to zero is to eliminate peaks belonging to the constituents of the air like CO_2, O_2, and N_2. Note that the background is recorded with a clean and empty diamond. It is not necessary to fix the background every time for each measurement unless the spectrometer is turned off, but only once when the instrument is turned on.

The sample is applied onto the diamond and the knob of ATR is tightened and also this process is not necessary for liquid samples. In this way, the sample is placed into the light path. IR spectrums can be taken by 'collect sample' part in the software. In order to point the peak frequencies, 'find peaks' part has to be employed.

Before applying another sample, the diamond should be cleaned with ethanol.

These above processes are repeated for FTIR spectrum of the other organic molecules.

6) Structure Determination

Structures of these four organic molecules can be determined with the help of frequencies specified in their FTIR spectrums and Table 3.2.

4) Determination of Fluorescein Isothiocyanate (FITC) by Spectrofluorometry

Objectives: To detect fluorescent substances quantitatively with Spectrofluorometer

Introduction

Fluorescence and phosphorescence are analytically important emission processes in which species are excited by absorption of a beam of electromagnetic radiation: radiant emission then occurs as the excited species return to the ground state.

The Pauli exclusion principle states that no two electrons in an atom can have the same set of four quantum numbers. This restriction requires that no more than two electrons occupy an orbital and furthermore the two have opposed spin states. Under this circumstance, the spins are said to be paired. Because of spin pairing, most molecules exhibit no net magnetic field and are thus said to be diamagnetic - that is they are neither attracted nor repelled by static magnetic fields. In contrast, free radicals which contain unpaired electrons, have a magnetic moment and consequently are attracted by a magnetic field. Free radicals are thus said to be paramagnetic.

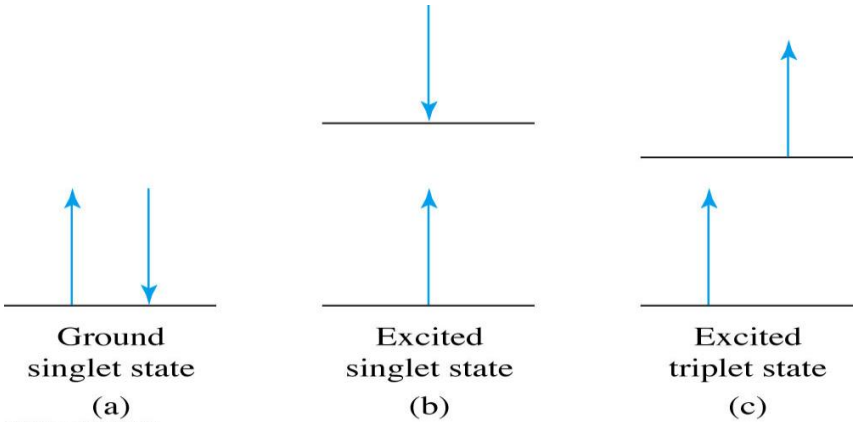


Figure 4.1 Electronic spin states of molecules.

The properties of a molecule in the excited triplet state differ significantly from those of the excited singlet state. For example a molecule is paramagnetic in the triplet state and diamagnetic in the singlet. More important however is that a singlet-to-triplet transition (or the reverse), which also involves a change in electronic state, is a significantly less probable event than the corresponding singlet-to-singlet transition. As a consequence, the average lifetime of an excited triplet state may range from 10-' to several seconds, compared with an average lifetime of -~10^{-8} s for an excited singlet state. Furthermore, radiation-induced

excitation of a ground-state molecule to an excited triplet state has a low probability of occurring and absorption bands due to this process are several orders of magnitude less intense than the analogous singlet-singlet absorption. We shall see, however, that an excited triplet state can be populated from an excited singlet state of certain molecules. Phosphorescence emission is often a result of such a process. Fluorescence occurs more rapidly than phosphorescence and is generally complete after about 10^{-5} s from the time of excitation. Phosphorescence emission takes place over periods longer than 10^{-5} s and may indeed continue for minutes or even hours after irradiation has ceased.

Figure 4.2 is a partial energy-level diagram, called a *Jablonski diagram* for a typical photoluminescent molecule. The lowest heavy horizontal line represents the ground-state energy of the molecule which is normally a singlet state, and is labeled S_0. At room temperature this state represents the energies of most of the molecules in a solution. The upper heavy lines arc energy levels for the ground vibrational states of three excited electronic states. The two lines on the left represent the first (S_1) and second (S_2) electronic singlet states. The one on the right (T_1) represents the energy of the first electronic triplet state. As is normally the case, the energy of the first excited triplet state is lower than the energy of the corresponding singlet state. Numerous vibrational energy levels are associated with each of the four electronic states. as suggested by the lighter horizontal lines. As shown in Figure 4.2 absorption transitions can occur from the ground singlet electronic state (S_0) to various vibrational levels of the excited singlet electronic states (S_1 and S_2). Note that direct excitation to the triplet state is not shown. Because this transition involves a change in multiplicity, it has a very low probability of occurrence. A low probability transition of this type is called a forbidden transition. Molecules excited to electronic states 51 and 5, rapidly lose any excess vibrational energy and relax to the ground vibrational level of that electronic state. This non radiational process is termed vibrational relaxation.

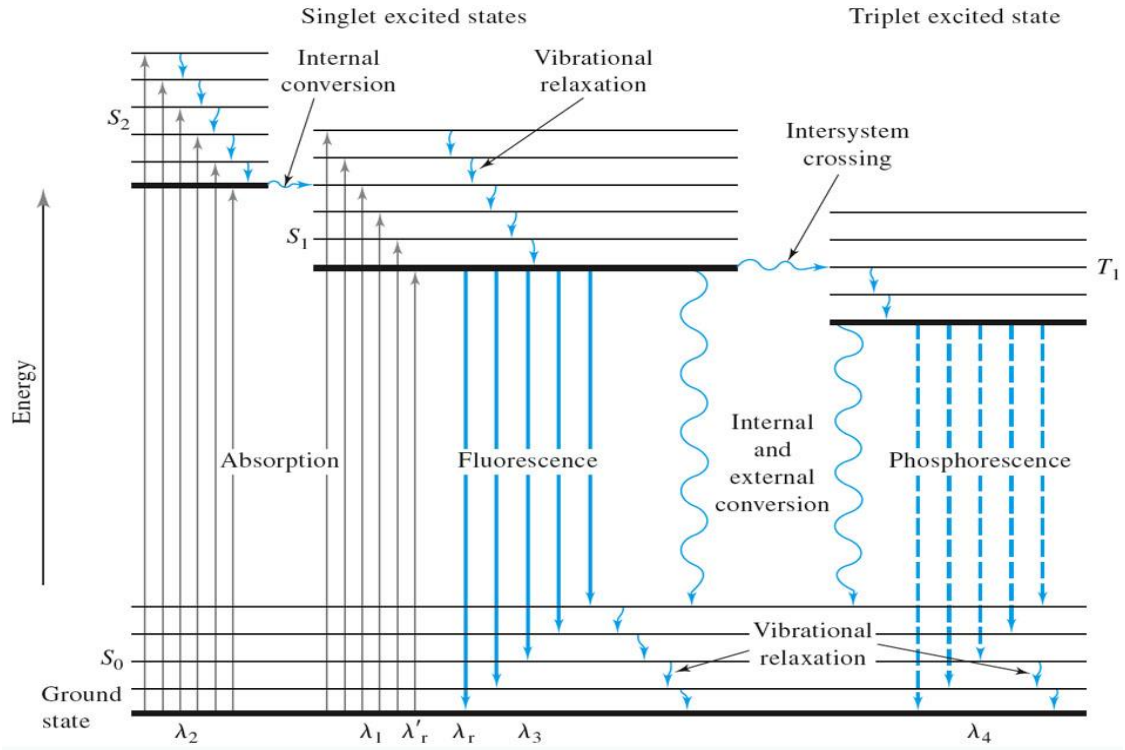


Figure 4.2 Partial energy-level diagram for a photoluminescent system.

An excited molecule can return to its ground state by a combination of several mechanistic steps. As shown by the straight, downward pointing, vertical arrows in Figure 4.2, two of these steps, fluorescence and phosphorescence involve the emission of a photon of radiation. The other deactivation steps, indicated by wavy arrows, are radiationless processes. The favored route to the ground state is the one that minimizes the lifetime of the excited state. Thus, if deactivation by fluorescence is rapid with respect to the radiationless processes such emission is observed. On the other hand, if a radiationless path has a more favorable rate constant, fluorescence is either absent or less intense.

A molecule may be promoted to any of several vibrational levels during the electronic excitation process. Collisions between molecules of the excited species and those of the solvent lead to rapid energy transfer with a minuscule increase in temperature of the solvent. Vibrational relaxation is so efficient that the average lifetime of a vibrationally excited molecule is 10^{-12} s or less, a period significantly shorter than the average lifetime of an electronically excited state. As a consequence fluorescence from solution always involves a transition from the lowest vibrational level of an excited electronic state. Several closely spaced emission lines are produced, however, and the transition can terminate in any of the vibrational levels of the ground state (see Figure 4.2). A consequence of the efficiency of

vibrational relaxation is that the fluorescence band for a given electronic transition is displaced toward lower frequencies or longer wavelengths from the absorption band (the Stokes shift). Overlap occurs only for the resonance peak involving transitions between the lowest vihrational level of the ground state and the corresponding level of an excited state. In Figure 4.2, the wavelength of absorbed radiation that produces the resonance peak λ_r, is labeled λ_r'.

The term internal conversion describes intermolecular processes by which a molecule passes to a lower energy electronic state without emission of radiation. These processes are neither well defined nor well understood, but they are often highly efficient. Internal conversion is a crossover between two states of the same multiplicity (singlet-singlet or triplet-triplet). It is particularly efficient when two electronic energy levels are sufficiently close for there to be an overlap in vibrational energy levels. This situation is illustrated in Figure 4.2 for the two excited singlet states S_2 and S_1 .At the overlaps shown the potential energies of the two excited states arc essentially equal which permits an efficient crossover from S_2 to S_1 .Internal conversion can also occur between state 5, and the ground electronic state S_0. Internal conversion through overlapping vibrational levels is usually more probable than the loss of energy by fluorescence from a higher excited state. Thus referring again to Figure 4.2, excitation by the band of radiation labeled λ_2 usually produces a fluorescence band centered at wavelength λ_3 to the exclusion of a band that would result from a transition between S_2 and S_0. Here, the excited molecule proceeds from the higher electronic state to the lowest vibrational state of the lower electronic excited state via a series of vibrational relaxations, an internal conversion and then further relaxations. Under these circumstances, the fluorescence occurs at λ_3 only, regardless of whether radiation of wavelength λ_1 or λ_2 was responsible for the excitation.

Deactivation of an excited electronic state may involve interaction and energy transfer between the excited molecule and the solvent or other solutes. This process is called *external conversion*. Evidence for external conversion includes a marked solvent effect on the fluorescence intensity of most species. Furthermore those conditions that tend to reduce the number of collisions between particles (low temperature and high viscosity) generally lead to enhanced fluorescence.

Intersystem crossing is a process in which there is a crossover between electronic states of different multiplicity. The most common process is from the singlet state to the triplet state ($S_1 \rightarrow T_1$) as shown in Figure 4.2. As with internal conversion, the probability of intersystem crossing is enhanced if the vibrational levels of the two states overlap. Note that in the singlet triplet crossover shown in Figure 4.2 the lowest singlet vibrational level overlaps one of the upper triplet vibrational levels and a change in spin state is thus more probable.

Deactivation of electronic excited states may also involve phosphorescence, After intersystem crossing to the triplet state, further deactivation can occur either by internal or external conversion or by phosphorescence, A triplet→ singlet transition is much less probable than a singlet-singlet conversion, Transition probability and excited-state lifetime are inversely related. Thus, the average lifetime of the excited triplet state with respect to emission is large and ranges from 10^{-4} to 10 s or more. Emission from such a transition may persist for some time after irradiation has ceased.

External and internal conversion compete so successfully with phosphorescence that this kind of emission is ordinarily observed only at low temperatures in highly viscous media or by using special techniques to protect the triplet state.

It is observed empirically that fluorescence is more commonly found in compounds in which the lowest energy transition is of a $\pi \rightarrow \pi^*$ type (π, π *excited singlet state) than in compounds in which the lowest energy transition is of the $n \rightarrow \pi^*$ type (n, π^* excited state): that is, the quantum efficiency is greater for $\pi^* \rightarrow \pi$ transitions.

The most intense and the most useful fluorescence is found in compounds containing aromatic functional groups with low-energy $\pi \rightarrow \pi^*$ transitions. Compounds containing aliphatic and alicyclic carbonyl structures or highly conjugated double-bond structures may also exhibit fluorescence, but the number of these is smaller than the number in the aromatic systems. Most unsubstituted aromatic hydrocarbons fluoresce in solution, the quantum efficiency usually increasing with the number of rings and their degree of condensation.

It is found empirically that fluorescence is particularly favored in molecules with rigid structures. The quantum efficiency of fluorescence in most molecules decreases with increasing temperature because the increased frequency of collisions at elevated temperatures improves the probability for deactivation by external conversion. A decrease in solvent viscosity also increases the likelihood of external conversion and leads to the

same result. The fluorescence of a molecule is decreased by solvents containing heavy atoms or other solutes with such atoms in their structure: carbon tetrabromide and ethyl iodide are examples. The fluorescence of an aromatic compound with acidic or basic ring substituents is usually pH dependent. Both the wavelength and the emission intensity arc likely to be different for the protonated and unprotonated forms of the compound.

The power of fluorescence emission F is proportional to the radiant power of the excitation beam that is absorbed by the system. A plot of the fluorescence radiant power of a solution versus concentration of the emitting species should be linear at low concentrations.

$$F = 2.303K'\varepsilon bcP_0 \tag{4.1}$$

or, at constant P_0

$$F = K.c \tag{4.2}$$

Components of a fluorometer are shown in Figure 4.3. The most common source for filter fluorometers is a low-pressure mercury vapor lamp equipped with a fused silica window. Interference and absorption filters have been used in fluorometers for wavelength selection of both the excitation beam and the resulting fluorescence radiation. Excitation and emission wavelength selectors are placed a 90° angle to minimize effects of excitation beam.

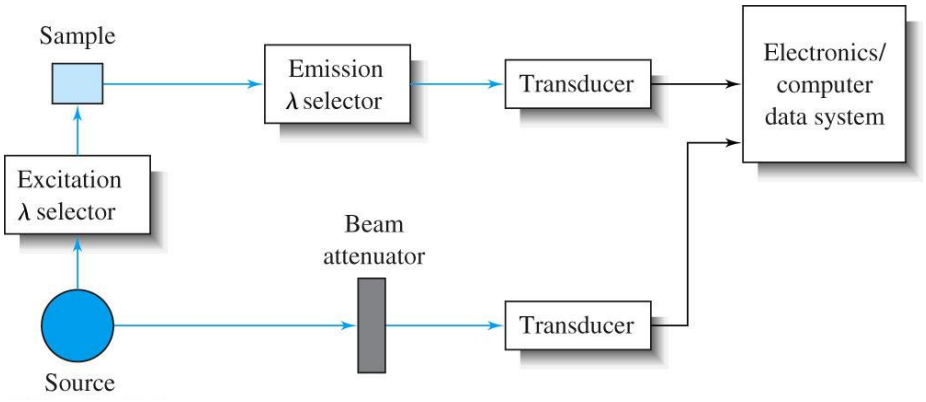


Figure 4.3 Components of a fluorometer or spectrofluorometer.

Procedure

1) Reagents

   $1x10^{-3}$M Fluorescein isothiocyanate (FITC)

   Instrument: PTI_(Photon Technology International)_Time Master C-71

2) Selection of the wavelengths

FITC standard solution is put into a plastic cuvette and after then the cuvette is inserted into the cell holder of the fluorometer. After FeliX32 is employed the analysis is completed. The maximum emission ($\lambda_{emission}$) and excitation ($\lambda_{excitation}$) wavelengths of the analyte are chosen with the wavelengths of nitrogen emission spectrum in the FeliX32.

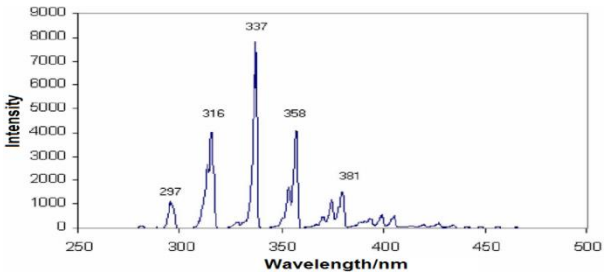
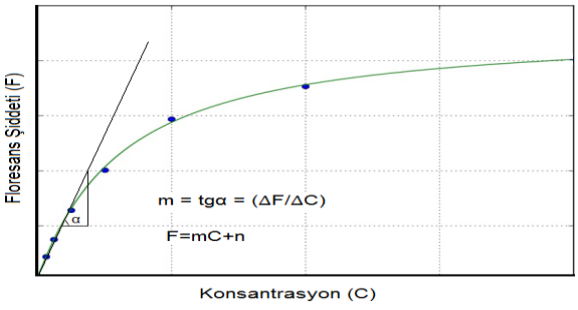


Figure 4.4 Nitrogen emission spectrum

3) Preparation of the standard solution and the measurement of the fluorescence

$5x10^{-6}$, $8x10^{-5}$, $1x10^{-5}$, $3x10^{-5}$, $6x10^{-5}$ and $1x10^{-4}$ M FITC standard solutions are prepared by the stock solution FITC of $1x10^{-3}$M. For this purpose, 25, 40, 50, 150, 300 and 500 µl of the stock solution are diluted to 5 mL with distilled water. In order to draw calibration plot which is fluorescence intensity versus molar concentration of the analyte, each standard solution is measured at the maximum emission wavelength ($\lambda_{emission}$) and fluorescence intensities are recorded. Next, calibration plot can be drawn after eliminating a point of influence which has a disproportionate effect on the position of the regression line. Hence, the slope of calibration plot can be calculated.



4) Sample analysis

A sample with the unknown concentration of FITC is measured at the maximum emission wavelength and its fluorescence intensity is written in the equation of F= mC+n to calculate the FITC concentration of the sample.

5) Determination of Heavy Metals by Atomic Absorption Spectrometry

Objectivities: To determine heavy metals with Atomic Absorption Spectrometer

Introduction

Atomic absorption is based on the absorption of electromagnetic beam by atomic particles. In a flame atomizer, a solution of the sample is nebulized by a flow of gaseous oxidant, mixed with a gaseous fuel, and carried into aflame where atomization occurs. As shown in Figure 5-1, a complex set of interconnected processes then occur in the flame. The first is desolvation, in which the solvent evaporates to produce a finely divided solid molecular aerosol. The aerosol is then volatilized to form gaseous molecules. Dissociation of most of these molecules produces an atomic gas. Some of the atoms in the gas ionize to form cations and electrons. Other molecules and atoms are produced in the flame as a result of interactions of the fuel with the oxidant and with the various species in the sample. As indicated in Figure 5.1, a fraction of the molecules, atoms, and ions are also excited by the heat of the flame to yield atomic, ionic, and molecular emission spectra. With so many complex processes occurring, it is not surprising that atomization is the most critical step in flame spectroscopy and the one that limits the precision of such methods. Because of the critical nature of the atomization step, it is important to understand the characteristics of flames and the variables that affect these characteristics.

As shown in Figure 5.2 important regions of a flame include the primary combustion zone, the interzonal region, and the secondary combustion zone. The appearance and relative size of these regions vary considerably with the fuel-to-oxidant ratio as well as with the type of fuel and oxidant.

Table 5.1 lists the common fuels and oxidants used in flame spectroscopy and the approximate range of temperatures realized with each of these mixtures.

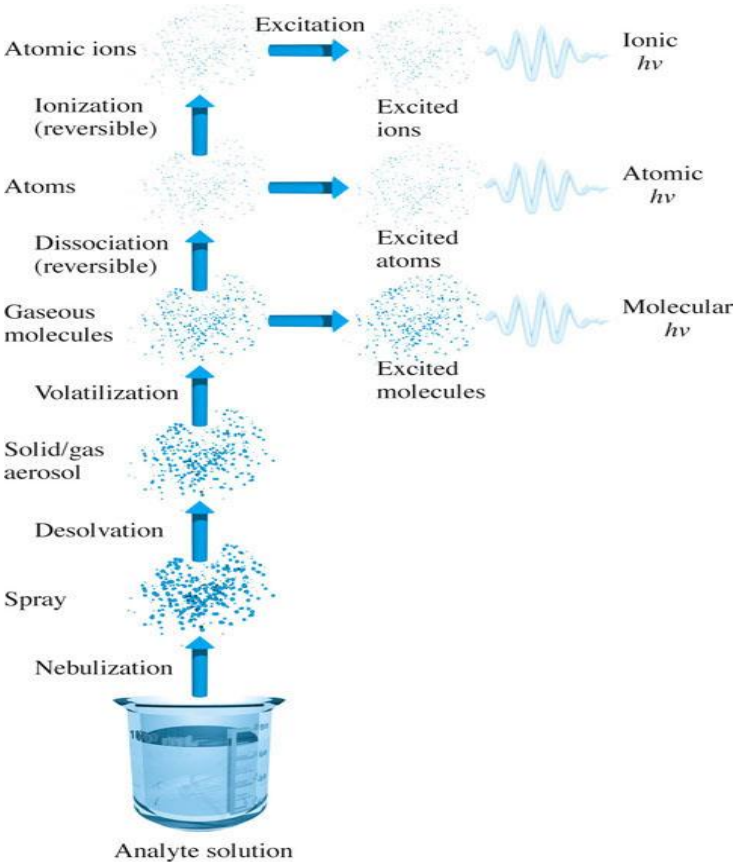


Figure 5.1 Processes occurring during atomization.

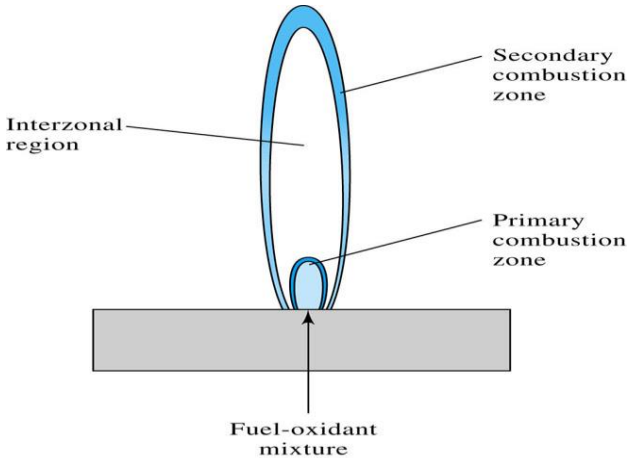


Figure 5.2 Regions in a flame

Table 5.1 Properties of flames

Fuel	Oxidant	Temperature, °C	Maximum Burning Velocity, cm s^{-1}
Natural gas	Air	1700–1900	39–43
Natural gas	Oxygen	2700–2800	370–390
Hydrogen	Air	2000–2100	300–440
Hydrogen	Oxygen	2550–2700	900–1400
Acetylene	Air	2100–2400	158–266
Acetylene	Oxygen	3050–3150	1100–2480
Acetylene	Nitrous oxide	2600–2800	285

Instruments for AAS are similar in general design to that shown in Figure 5.3 a and consist of a radiation source, a sample holder a wavelength selector a detector, and a signal processor and readout.

Atomic absorption methods are potentially highly specific; because atomic absorption lines arc remarkably narrow (0.002 to 0.005 nm) and because electronic transition energies are unique for each element. On the other hand, narrow line widths create a problem that does not normally occur in molecular absorption spectroscopy, a linear relationship between the analytical signal (absorbance) and concentration requires a narrow source bandwidth relative to the width of an absorption line or band. Even good-quality monochromators, however, have effective bandwidths significantly greater than the width of atomic absorption lines. The problem created by the limited width of atomic absorption lines has been solved by the use of line sources with bandwidths even narrower than the absorption line width. The most common source for atomic absorption measurements is the hollow-cathode lamp, such as the one shown in Figure 5.4. This type of lamp consists of a tungsten anode and a cylindrical cathode sealed in a glass tube filled with neon or argon at a pressure of 1 to 5 torr. The cathode is constructed of the metal whose spectrum is desired or serves to support a layer of that metal.

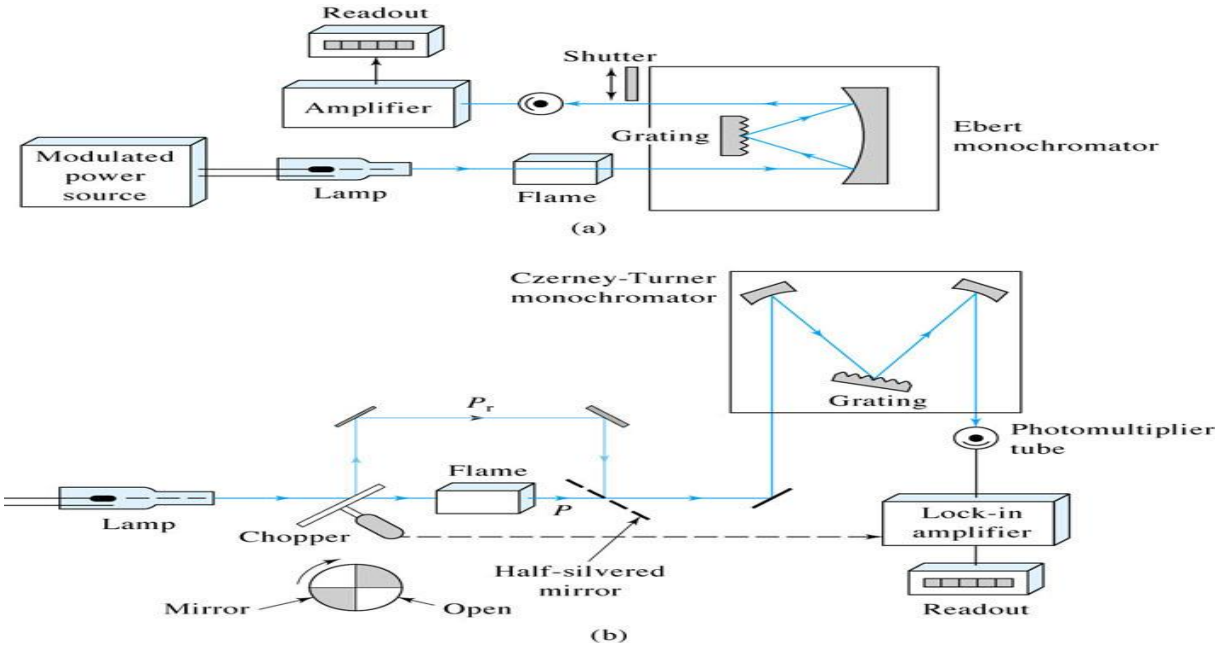


Figure 5.3 Typical flame spectrophotometers: (a) single-beam design and (b) double-beam design.

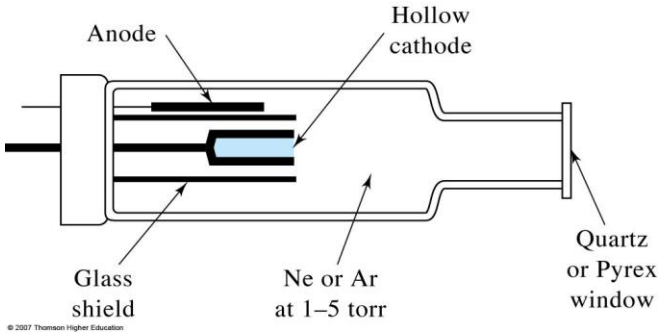


Figure 5.4 Schematic cross section of a hollow-cathode lamp.

Procedure

1) Reagents

a) 100 µg/mL standard solution of Cu^{2+}

b) Acetylene-air mixture for the instrument

2) Calibration plot

Different standard solutions are used to calculate the copper concentration in the sample; therefore, 2, 4, 6, 8, and 10 mL Cu^{2+} stock solutions are poured into different 100 mL volumetric flasks and then filled with distilled water. In other words, their final concentrations are 2, 4, 6,

8, and 10 mg/L Cu^{2+}, respectively. Note that the highest concentration for a metal is different from the other ones and there are some deviations from the linearity at the high concentrations of the analyte. Each standard solution is introduced as an aerosol into the flame by the sample-introduction system consisting of a nebulizer and spray chamber. The appropriate wavelength for copper is 324.7 nm in this analysis. The following table is filled and a calibration plot is plotted absorbance values versus concentrations.

Cu (mg/L)	Absorbance
2	
4	
6	
8	
10	
Sample	

3) Sample Analysis

A sample is dissolved into distilled water and filled up to 100 mL. After recording its absorbance, its copper concentration can be found with the help of the calibration plot.

6) A Mixture Analysis by Thin Layer Chromatography

Objectivities: A mixture analysis with Thin Layer Chromatography

Introduction

Chromatography encompasses a diverse and important group of methods that allow the separation, identification, and determination of closely related components of complex mixtures; many of these separations are impossible by other means.' In all chromatographic separations the sample is dissolved in a mobile phase, which may be a gas, a liquid, or a supercritical fluid. This mobile phase is then forced through an immiscible stationary phase, which is fixed in place in a column or on a solid surface. The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phases to varying degrees. Those components strongly retained by the stationary phase move only slowly with the flow of mobile phase. In contrast, components that are weakly held by the stationary phase travel rapidly. As a consequence of these differences in migration rates, sample components separate into discrete bands, or zones, that can be analyzed qualitatively and quantitatively.

Chromatographic methods can be categorized in two ways. The first classification is based on the physical means by which the stationary and mobile phases are brought into contact. In column chromatography, the stationary phase is held in a narrow tube through which the mobile phase is forced under pressure. In planar chromatography, the stationary phase is supported on a flat plate or in the interstices of paper; here, the mobile phase moves through the stationary phase by capillary action or under the influence of gravity. It is important to point out here, however, that the equilibria on which the two types of chromatography are based are identical and that the theory developed for column chromatography is readily adapted to planar chromatography. A more fundamental classification of chromatographic methods is one based on the types of mobile and stationary phases and the kinds of equilibria involved in the transfer of solutes between phases. Table 6.1 lists three general categories of chromatography: gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SFC). As the names imply, the mobile phases in the three techniques are gases, liquids, and supercritical fluids, respectively. As shown in column 2 of the table, several specific chromatographic methods fall into each of the first two general categories, Note that only LC can be performed either in columns or on planar surfaces; GC and SFC, on the other hand, are restricted to column procedures so that the column walls contain the mobile phase.

Table 6.1 Classification of column chromatographic methods.

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
1. Gas chromatography (GC)	a. Gas-liquid chromatography (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	b. Gas-solid	Solid	Adsorption
2. Liquid chromatography (LC)	a. Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	b. Liquid-solid, or adsorption	Solid	Adsorption
	c. Ion exchange	Ion-exchange resin	Ion exchange
	d. Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	e. Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
3. Supercritical fluid chromatography (SFC; mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Planar chromatographic methods include thin-layer chromatography (TLC) and paper chromatography (PC). Each makes use of a flat, relatively thin layer of material that is either self-supporting or is coated on a glass, plastic, or metal surface. The mobile phase moves through the stationary phase by capillary action, sometimes assisted by gravity or an electrical potential. Currently, most planar chromatography is based on the thin-layer technique, which is faster, has better resolution, and is more sensitive than its paper chromatography equivalent. Typical thin-layer separations are performed on a glass plate coated with a thin and adherent layer of finely divided particles; this layer constitutes the stationary phase. A thin-layer plate is prepared by spreading an aqueous slurry of the finely ground solid on the clean surface of a glass or plastic plate or microscope slide. Cellulose, silica gel, polyamide and aluminum oxide are used as thin plates commercially.

Usually, the sample, as a 0.01% to 0.1% solution, is applied as a spot 1 to 2 cm from the edge of the plate. For best separation efficiency, the spot should have a minimal diameter - about 5mm for qualitative work and smaller for quantitative analysis.

Plate development is the process in which a sample is carried through the stationary phase by a mobile phase; it is analogous to elution in LC. The most common way of developing a plate is to place a drop of the sample near one edge of the plate and mark its position with a pencil. After the sample solvent has evaporated, the plate is placed in a closed container saturated with vapors of the developing solvent One end of the plate is immersed in the developing solvent, with care being taken to avoid direct contact between the sample and the developer. After the developer has traversed one half or two thirds of the length of the plate, the plate is removed from the container and dried.

Several methods can be used to locate sample components after separation.

1) One of these methods involves spraying with a solution of sulfuric acid or placing the plate in a chamber containing a few crystals of iodine. Both of these reagents react with organic compounds on the plate to yield dark products. Several specific reagents (such as ninhydrin) are also useful for locating separated species.
2) Another method of detection is based on incorporating a fluorescent material into the stationary phase. After development, the plate is examined under ultraviolet light. The sample components quench the fluorescence of the material so that all of the plate fluoresces except where the nonfluorescing sample components are located.

Procedure

1) Reagents
    a) Methyl orange
    b) Bromothymol blue
    c) Methyl red
    d) Stationary phase: Silica gel G (0.50 mm)
    e) Mobile phase: Dichloromethane/ ethyl acetate/ methanol (56/ 22/ 22)
2) Spotting

    First, using a pencil gently draw a line across the bottom edge of the silica gel plate (20x20) cm, 1.5-2 cm up from the bottom. Standard solutions and the unknown substance are applied onto the plate with capillary tubes. The plate is placed carefully into a jar containing the mobile phase and let the solvent level get above the line at the bottom of the plate. After then the plate is removed and dried when the solvent level reaches 1 - 2 cm from the top of the plate.
3) Visualization and calculation of Rf values

    The distance that a compound travels in a specific chromatography system, compared to how far the solvent has traveled, is a constant. The relationship between the distance traveled by the solvent front and the substance is usually expressed as the Rf value:

$$Rf = \frac{distance\ traveled\ by\ substance\ (cm)}{distance\ traveled\ by\ solvent\ front\ (cm)}$$

    The unknown substance can be specified with the comparison of its Rf value and color to the standard values.

7) A Mixture Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

Objectivities: To separate a mixture's components and determine their structures with Gas Chromatography- Mass Spectrometer

Introduction

In gas chromatography, the components of a vaporized sample are separated as a consequence of being partitioned between a mobile gaseous phase and a liquid or a solid stationary phase held in a column. In performing a gas chromatographic separation, the sample is vaporized and injected onto the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase. In contrast to most other types of chromatography, the mobile phase does not interact with molecules of the analyte; its only function is to transport the analyte through the column.

There are two types of gas chromatography: gas-liquid chromatography (GLC) and gas-solid chromatographv (GSC). GLC finds widespread use in all fields of science; its name is usually shortened to gas chromatography (OC). GSC is based on a solid stationary phase in which retention of analytes occurs because of physical adsorption. The application of OSC is limited because of semipermanent retention of active or polar molecules and severe tailing of elution peaks. Tailing is a result of the nonlinear nature of the adsorption process. Thus, this technique is not widely used except for the separation of certain low-molecular-mass gaseous species. In GLC the analyte is partitioned between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid packing or on the walls of a capillary tubing.

The retention time for an analyte on a column depends on its distribution constant, which in turn is related to the chemical nature of the liquid stationary phase. To separate various sample components, their distribution constants must be sufficiently different to accomplish a clean separation. At the same time, these constants must not be extremely large or extremely small because large distribution constants lead to prohibitively long retention times and small constants produce such short retention times that separations are incomplete. To have a reasonable residence time in the column, an analyte must show some degree of compatibility (solubility) with the stationary phase. Here, the principle of "like dissolves like" applies, where "like" refers to the polarities of the analyte and the immobilized liquid. Generally, the polarity of the stationary phase should match that of the sample components. When the match is good, the order of elution is determined by the boiling point of the eluents.

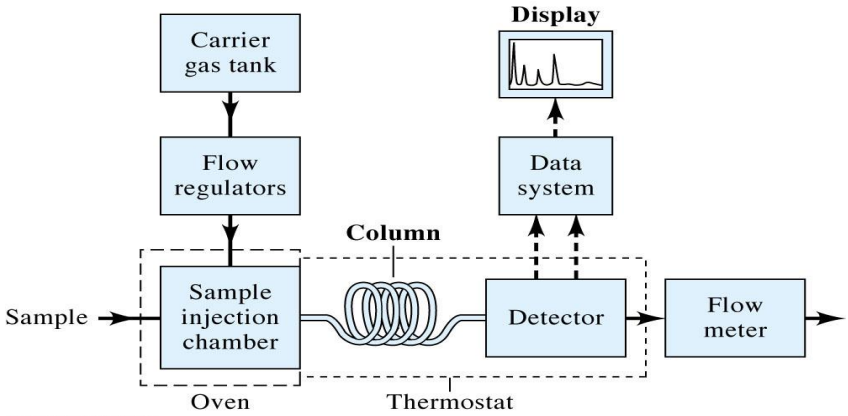


Figure 7.1 Block diagram of a typical gas chromatography.

The mobile-phase gas in GC is called the carrier gas and must be chemically inert. Helium is the most common mobile-phase gas used, although argon, nitrogen, and hydrogen are also used. To achieve high column efficiency, the sample must be of a suitable size and introduced as a "plug" of vapor; slow injection or oversize samples cause band spreading and poor resolution. Calibrated microsyringes are used to inject liquid samples through a rubber or silicone diaphragm, or septum, into a heated sample port located at the head of the column. Two general types of columns are used in GC, packed and open tubular, also called capillary. In the past, the vast majority of gas chromatographic analyses used packed columns. For most current applications, packed columns have been replaced by the more efficient open tubular columns. Packed chromatographic columns vary in length from 1 m to 5 m, and capillary columns can range from 3 m to 100 m. Column temperature is an important variable that must be controlled to a few tenths of a degree for precise work. Thus, the column is ordinarily housed in a thermostatted oven. The optimal column temperature depends on the boiling point of the sample and the degree of separation required. Roughly, a temperature equal to or slightly above the average boiling point of a sample results in a reasonable elution time (2 to 30 min). For samples with a broad boiling range, it is often desirable to employ temperature programming, in which the column temperature is increased either continuously or in steps as the separation proceeds. Dozens of detectors have been investigated and used with gas chromatographic separations, shown in Table 7.1.

Table 7.1 Typical gas chromatographic detectors.

Type	Applicable Samples	Typical Detection Limit
Flame ionization	Hydrocarbons	1 pg/s
Thermal conductivity	Universal detector	500 pg/mL
Electron capture	Halogenated compounds	5 fg/s
Mass spectrometer (MS)	Tunable for any species	0.25 to 100 pg
Thermionic	Nitrogen and phosphorous compounds	0.1 pg/s (P), 1 pg/s (N)
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s, 2 pg S/s, 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier transform IR (FTIR)	Organic compounds	0.2 to 40 ng

While the instrument runs, the computer generates a graph from the signal. This graph is called a chromatogram. Each of the peaks in the chromatogram represents the signal created when a compound elutes from the GC column into the detector. The x-axis shows the retention time, and the y-axis shows the intensity (abundance) of the signal.

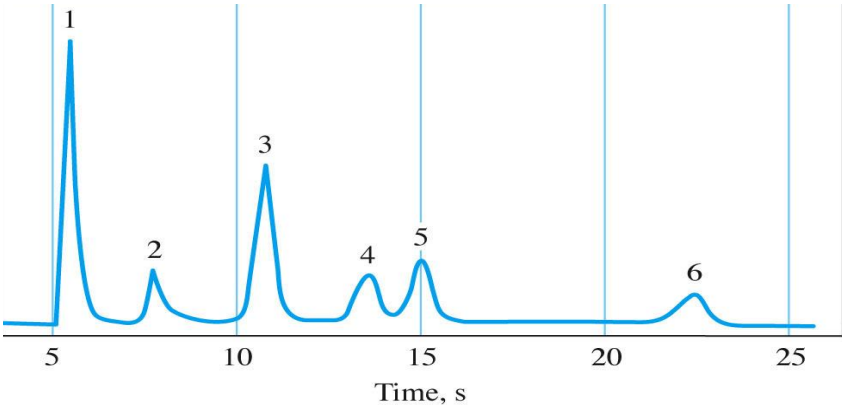


Figure 7.2 A chromatogram consisting of six different compounds.

First, GC is a tool for performing separations. In this role, GC methods are unsurpassed when applied to complex organic, metal-organic, and biochemical systems made up of volatile species or species that can be derivatized to yield volatile substances. The second role that GC plays is in the completion of an analysis. In this role, retention times or volumes are used for qualitative identification, and peak heights or peak areas provide quantitative information.

One of the most powerful detectors for GC is the mass spectrometer. The flow rate from capillary columns is generally low enough that the column output can be fed directly into the ionization chamber of the mass spectrometer. A schematic of a typical system is shown in Figure 7.3.

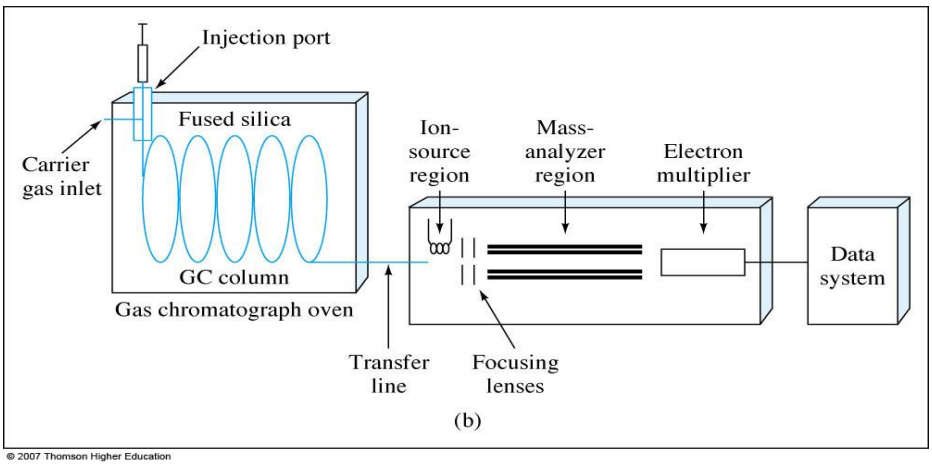


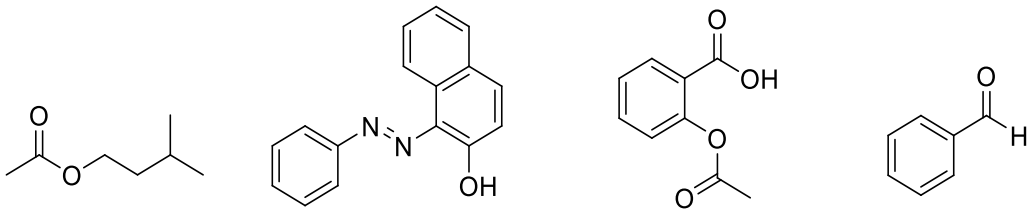
Figure 7.3 Schematic of a typical capillary GC/MS system.

The most common ion sources used in GC/MS are electron-impact ionization and chemical ionization. The most common mass analyzers are quadrupole and ion-trap analyzers. Time-of-flight mass analyzers are also used, but not as frequently as quadrupoles and ion traps.

In GC/MS, the mass spectrometer scans the masses repetitively during a chromatographic experiment. If the chromatographic run is 10 minutes, for example, and a scan is taken each second, 600 mass spectra are recorded. The data can be analyzed by the data system in several different ways. First, the ion abundances in each spectrum can be summed and plotted as a function of time to give a total-ion chromatogram. This plot is similar to a conventional chromatogram. One can also display the mass spectrum at a particular time during the chromatogram to identify the species eluting at that time. Finally, a single mass-to-charge (m/z) value can be selected and monitored throughout the chromatographic experiment, a technique known as selected-inn monitoring. Mass spectra of selected ions obtained during a chromatography experiment are known as mass chromatograms. Molecular weights and molecule structures can be determined with mass spectrums.

Procedure

1) Reagents
   a) Isoamyl acetate
   b) Phenyl azo β naphthol
   c) Aspirin
   d) Benzaldehyde



(a) (b) (c) (d)

2) Sample preparation

   All samples are dissolved in a volatile solvent. Nonvolatile solvents like DMSO and DMF should not be used for dissolution of any kind of substances. An analyte which is injected into GC-MS should have low melting point and dissolve in any volatile solvent. If the analyte do not dissolve completely in a solvent, the solution should be filtered before the injection.

3) Injection and data analysis

1 μL sample solution is injected into GC-MS column from its septum with an injector. After the sample analyses, samples' molecular weights and fragment ions can be determined via their spectrums.

8) Determination of Caffeine in Coke by Liquid Chromatography

Objectivities: To separate a mixture's components and analyze the mixture quantitatively.

Introduction

In several basic types of chromatography, the mobile phase is a liquid. The stationary phase can be a liquid, a solid, an ion exchange or a polymer gel. Major increases in column efficiency could be achieved by decreasing the particle size of packings. It was not until the late 1960s, however, that the technology for producing and using packings with particle diameters as small 3 to 10 μm was developed. This technology required sophisticated instruments operating at high pressures, which contrasted markedly with the simple glass columns of classic gravity-flow liquid chromatography. The name high-performance liquid chromatography (HPLC) was originally used to distinguish these newer procedures from the original gravity-flow methods. Today, virtually all LC is done using pressurized flow and we use the abbreviations LC and HPLC interchangeably.

A pump aspirates the mobile phase from the solvent resorvoir and forces it through the system's column and detecter. An injector for an HPLC system should provide injection of the liquid sample within the range of 0.1-100 mL of volume with high reproducibility and under high pressure (up to 4000 psi). The HPLC detector, located at the end of the column detect the analytes as they elute from the chromatographic column. Separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

Figure 8.1 is a diagram showing the important components of a typical LC instrument. A modern LC apparatus is equipped with one or more glass reservoirs, each of which contains 500 mL or more of a solvent. Provisions are often included to remove dissolved gases and dust from the liquids. Dissolved gases can lead to irreproducible flow rates and band spreading; in addition, both bubbles and dust interfere with the performance of most detectors. Degassers may consist of a vacuum pumping system, a distillation system, a device for heating and stirring, or as shown in Figure 28-3, a system for sparging, in which the dissolved gases are swept out of solution by fine bubbles of an inert gas that is not soluble in the mobile phase. Often the systems also contain a means of filtering dust and particulate matter from the solvents to prevent these particles from damaging the pumping or injection systems or clogging the column. It is not necessary that the degassers and filters be integral parts of the HPLC system

as shown in Figure 8.1. For example, a convenient way of treating solvents before introduction into the reservoir is to filter them through a millipore filter under vacuum. This treatment removes gases as well as suspended matter.

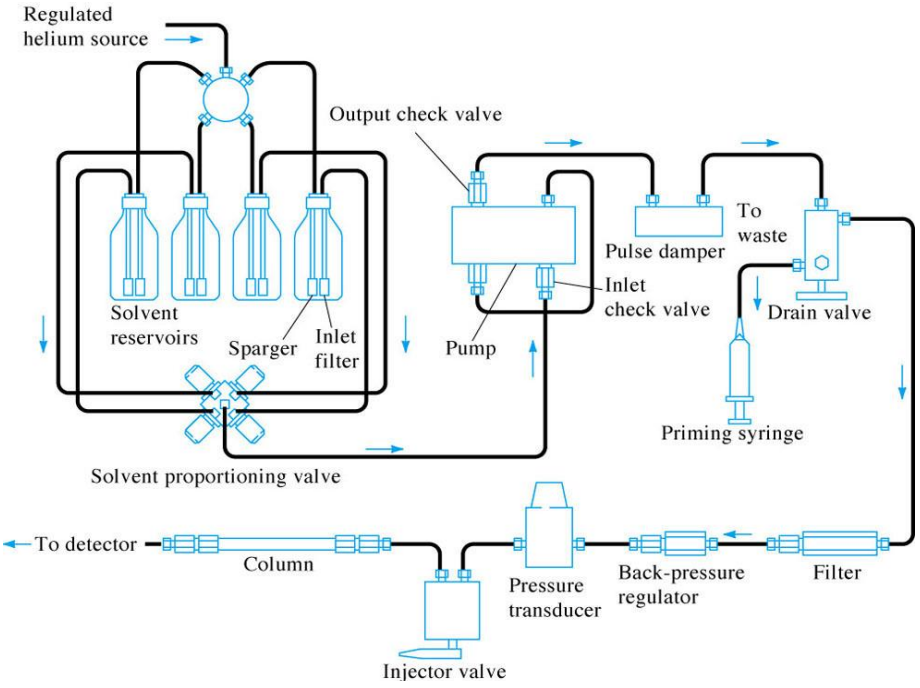


Figure 8.1 Block diagram showing components of a typical apparatus for HPLC.

An elution with a single solvent or solvent mixture of constant composition is termed an isocratic elution. In gradient elution, two (and sometimes more) solvent systems that differ significantly in polarity are used and varied in composition during the separation. Note that gradient elution shortened the time of separation significantly without sacrificing the resolution of the early peaks.

The requirements for liquid chromatographic pumps include (1) the generation of pressures of up to 6000 psi (lb/in.^{2}), or 414 bar, (2) pulse-free output, (3) flow rates ranging from 0.1 to 10 mL/min, (4) flow reproducibilities of 0.5% relative or better, and (5) resistance to corrosion by a variety of solvents. Two major types of pumps are used in LC: the screw-driven syringe type and the reciprocating pump.

The most widely used method of sample introduction in LC is based on sampling loops. These devices are often an integral part of liquid-chromatographic equipment and have interchangeable loops providing a choice of sample sizes from 1µL to 100 µL or more. Most chromatographs today are sold with autoinjectors. Such units are capable of injecting samples into the LC from vials on a sample carousel or from microtiter plates. They usually contain

sampling loops and a syringe pump for injection volumes from less than 1 μL to more than 1 mL. Most are programmable to allow for unattended injections into the LC system.

Liquid-chromatographic columns' are usually constructed from smooth-bore stainless steel tubing. Several columns like analytical and guard column are available for different purposes in HPLC. Usually, a short guard column is introduced before the analytical column to increase the life of the analytical column by removing not only particulate matter and contaminants from the solvents but also sample components that bind irreversibly to the stationary phase. The composition of the guard-column packing should be similar to that of the analytical column; the particle size is usually larger, however, to minimize pressure drop. Two basic types of packings have been used in LC, pellicular and porous particle. A thin, porous layer of silica, alumina, a polystyrene-divinylbenzene synthetic resin, or an ion-exchange resin was deposited on the surface of these beads. Small porous microparticles have completely replaced these large pellicular particles. In recent years, small pellicular packings have been reintroduced for separation of proteins and large biomolecules.

Analytical columns range from 5 to 25 cm long with inside diameter of 3 to 5 mm, and particle size of 3 or 5 μm. Liquid chromatographic detectors are of two basic types. Bulk-property detectors respond to a mobile phase bulk property, such as refractive index, dielectric constant, or density that is modulated by the presence of solutes. In contrast, solute-property detectors respond to some property of solutes, such as UV absorbance, fluorescence, or diffusion current, that is not possessed by the mobile phase. Table 8.1 lists the most common detectors for HPLC.

Table 8.1 Performance of HPLC detectors.

HPLC Detector	Commercially Available	Mass LOD* (typical)	Linear Range† (decades)
Absorbance	Yes	10 pg	3–4
Fluorescence	Yes	10 fg	5
Electrochemical	Yes	100 pg	4–5
Refractive index	Yes	1 ng	3
Conductivity	Yes	100 pg–1 ng	5
Mass spectrometry	Yes	<1 pg	5
FTIR	Yes	1 μg	3
Light scattering	Yes	1 μg	5
Optical activity	No	1 ng	4
Element selective	No	1 ng	4–5
Photoionization	No	<1 pg	4

The most widely used detectors for LC are based on absorption of ultraviolet or visible radiation. Fluorescence, refractive-index, and electrochemical detectors are also widely used.

Mass spectrometry (MS) detectors are currently quite popular. Which detector can be used is depend on the structure of analyte.

The combination of LC and mass spectrometry would seem to be an ideal merger of separation and detection" Just as in GC, a mass spectrometer can greatly aid in identifying species as they elute from the chromatographic column. There are major problems, however, in the coupling of these two techniques. A gas-phase sample is needed for mass spectrometry, and the output of the LC column is a solute dissolved in a solvent. As a first step, the solvent must be vaporized. There have been several devices developed to solve the problems of solvent removal and LC column interfacing. Today, the most popular approaches use a low-flow-rate atmospheric pressure ionization technique.

The combination of HPLC and mass spectrometry gives high selectivity because unresolved peaks can be isolated by monitoring only a selected mass. The LC/MS technique can provide fingerprinting of a particular eluate instead of relying on retention time as in conventional HPLC. The combination also can give molecular mass, structural information, and accurate quantitative analysis. Figure 8.2 gives a general diagram of LC/MS. For some complex mixtures, the combination of LC and MS does not provide enough resolution. In recent years it has become feasible to couple two or more mass analyzers to form tandem mass spectrometers. When combined with LC, the tandem mass spectrometry system is called an LC/MS/MS instrument.

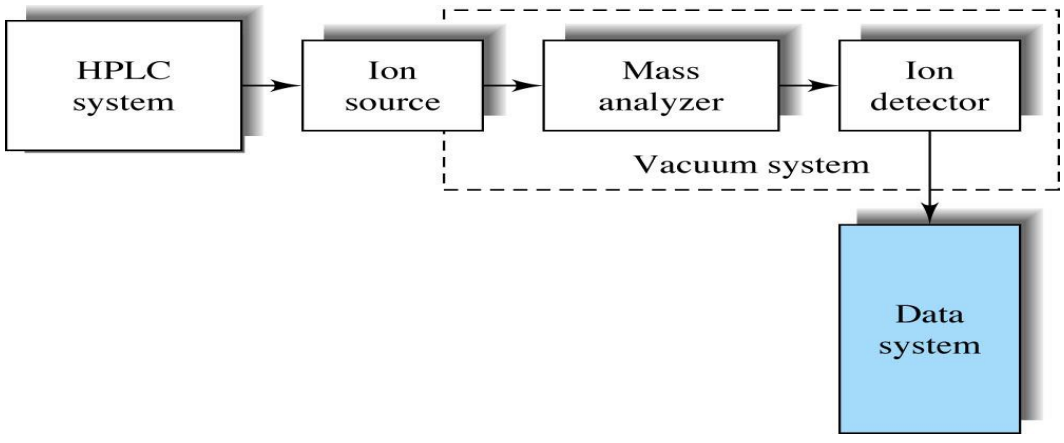


Figure 8.2 Block diagram of an LC/MS system.

The most widely used type of HPLC is partition chromatography, in which the stationary phase isa second liquid that is immiscible with the liquid mobile phase. In the past, most of the applications have been to nonionic, polar compounds of low to moderate molecular mass. The

early forms of partition chromatography used liquid-liquid columns. These have been replaced in modern LC systems by liquid-bonded-phase columns. In liquid-liquid chromatography, the liquid was held in place by physical adsorption. In bonded-phase chromatography, on the other hand, it is attached by chemical bonding, resulting in highly stable packings insoluble in the mobile phase.

Two types of partition chromatography are distinguishable based on the relative polarities of the mobile and stationary phases. Early work in LC was based on highly polar stationary phases such as triethylene glycol or water; a relatively nonpolar solvent such as hexane or i-propyl ether then served as the mobile phase. For historic reasons, this type of chromatography is now called normal-phase chromatography. In reversed-phase chromatography, the stationary phase is nonpolar, often a hydrocarbon, and the mobile phase is a relatively polar solvent (such as water, methanol, acetonitrile, or tetrahydrofuran). Bonded-phase packings are classified as reversed phase when the bonded coating is nonpolar in character and as normal phase when the coating contains polar functional groups. It has been estimated that more than three quarters of all HPLC separations are currently performed in columns with reversed-phase packings. The major advantage of reversed-phase separations is that water can be used as the mobile phase. Water is an inexpensive, nontoxic, UV-transparent solvent compatible with biological solutes. Also, mass transfer is rapid with nonpolar stationary phases, as is solvent equilibration after gradient elution. Most commonly, the R group of the siloxane in these coatings is a C_8 chain (n-octyl) or a C_{18} chain (n-octyldecyl). In commercial normal-phase bonded packings, the R in the siloxane structure is a polar functional group such as cyano, (-C_2H_4CN); diol, (-$C_3H_6OCH_2CHOHCH_2OH$); amino, (-$C_3H_6NH_2$); and dimethylamino, (- $C_3H_6N(CH_3)_2$).

Successful chromatography with interactive mobile phases requires a proper balance of intermolecular forces among the three active participants in the separation process - the solute, the mobile phase, and the stationary phase. These intermolecular forces are described qualitatively in terms of the relative polarity of each of the three reactants. Often, in choosing a column for a partttton chromatographic separation, the polarity of the stationary phase is matched roughly with that of the analytes; a mobile phase of considerably different polarity is then used for elution. This procedure is generally more successful than one in which the polarities of the solute and mobile phase are matched but different from that of the stationary phase. Unfortunately, theories of mobile-phase and stationary-phase interactions with any given set of sample components are Imperfect, and at best, the user can only narrow the choice of

stationary phase to a general type. Having made this choice, some trial-and-error experiments must be performed in which chromatograms are obtained with various mobile phases until a satisfactory separation is realized.

For reversed-phase chromatography, the three solvent modifiers are methanol, acetonitrile, and tetrahydrofuran. Water is then used to adjust the solvent strength of the mixtures and yield a suitable value of k (retention factor). For normal-phase separations, n-hexane, ethyl ether, methylene chloride, and chloroform can be used. It means that the analyte and mobile phase have to be compatible with each other in terms of their solubilities.

In some instances, it is useful to convert the components of a sample to a derivative before, or sometimes after, chromatographic separation. Such treatment may be desirable (1) to reduce the polarity of the species so that partition rather than adsorption or ion exchange columns can be used; (2) to increase the detector response, and thus sensitivity, for all of the sample components; and (3) to selectively enhance the detector response to certain components of the sample.

Procedure

1) Reagents
   a) Caffeine
   b) Chromatographic condition: Reverse phase liquid chromatography
   c) Column: Octadecyl silane (C_{18})
   d) Mobile phase: Metanol:Water (70:30, v/v)
   e) Flow rate: 1.0 mL/dk
   f) Detector: Absorbance detector, λ: 272 nm
2) Sample and mobile phase preparation

   Standard solution: 0.01 g pure caffeine is dissolved with some distilled water in a 10 mL volumetric flask and diluted to 10 mL. (1000 μg/mL stock solution) 100 μg/mL caffeine standard solution is prepared by using 1 mL of this stock solution diluted to 10 mL.

   Sample solution: Caffeine in coke is analyzed by HPLC.

   Mobile phase: 70 ml methanol and 30 ml ultrapure water are mixed and then the solution is filtered with a millipore filter and gases in the solution are removed by degassers.

3) Data analysis

   100 μg/mL standard solution and the sample solution are filtered with 0.45 μm filters and after percolation of the solution, they should be degassed and injected 20 μL from each of them into the instrument.

4) Sample analysis

   Sample's and standard's peak areas are compared with each other and the amount of caffeine is calculated according to this direct comparison.

9) Determination of the Equivalence Point in Potentiometric Titrations

Objectivities: To titrate an acid sample and calculate its equivalence point with potentiometric titration.

Introduction

Electroanalytical chemistry encompasses a group of qualitative and quantitative analytical methods based on the electrical properties of a solution of the anaylte when it is made part of an electrochemical cell. Electroanalytical techniques are capable of producing low detection limits and a wealth of characterization information describing electrochemically accessible systems. Such information includes the stoichiometry and rate of interfacial charge transfer, the rate of mass transfer, the extent of adsorption or chemisorption, and the rates and equilibrium constants for chemical reactions.

Electroanalytical methods have certain general advantages over other types of procedures discussed in this book. First, electrochemical measurements are often specific for a particular oxidation state of an element. For example, in electrochemical methods it is possible to determine the concentration of each of the species in a mixture of cerium (III) and cerium (IV), whereas most other analytical methods can reveal only the total cerium concentration. A second important advantage of electrochemical methods is that the instrumentation is relatively inexpensive. A third feature of certain electrochemical methods, which may be an advantage or a disadvantage, is that they provide information about activities rather than concentrations of chemical species. Generally, in physiological studies, for example, activities of ions such as calcium and potassium are more important than concentrations.

Many types of electroanalytical methods have been developed. Many of the methods that are generally useful, and arc discussed in this book, are shown in Figure 9.1. These methods are divided into interfacial methods and bulk methods. Interfacial methods, which are more widely used than bulk methods, arc based on phenomena that occur at the interface between electrode surfaces and the thin layer of solution just adjacent to these surfaces. Bulk methods, in contrast, arc based on phenomena that occur in the bulk of the solution; every effort is made to avoid interfacial effects.

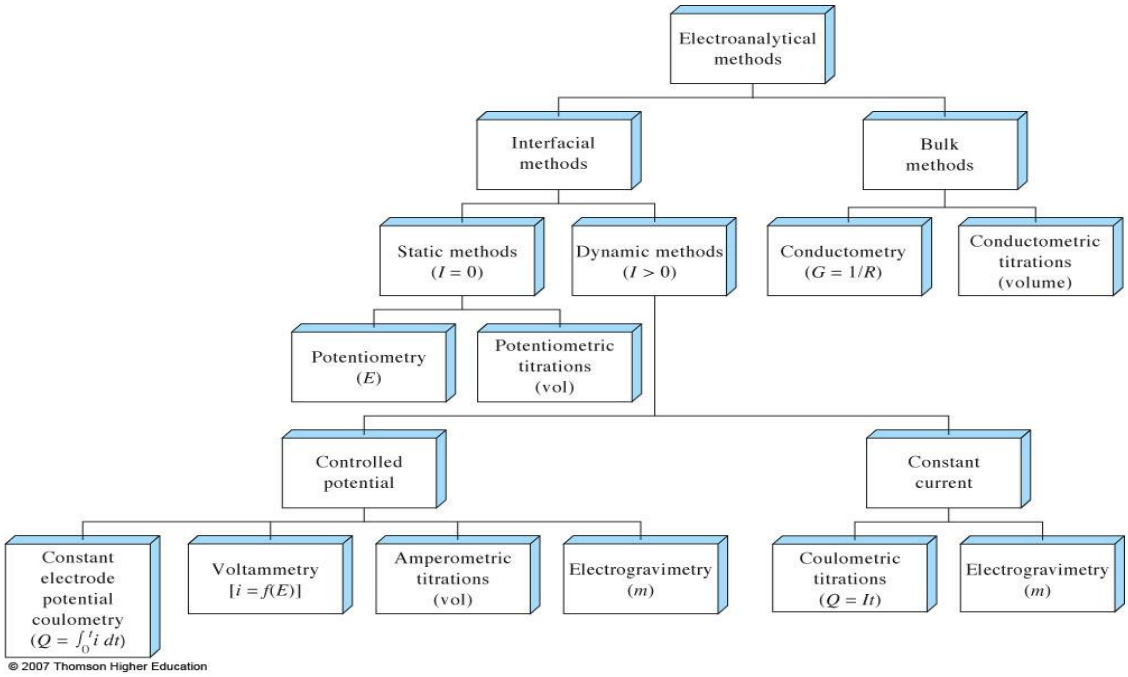


Figure 9.1 Summary of common electroanalytical methods.

Interfacial methods can be divided into two major categories, static methods and dynamic methods, depending on whether there is a current in the electrochemical cells. The static methods, which require potentiometric measurements, are extremely important because of their speed and selectivity. Dynamic interfacial methods, in which currents in electrochemical cells play a vital part, are of several types. In three of the methods shown on the left in Figure 9.1, the potential of the cell is controlled while measurements of other variables are made. Generally, these methods are sensitive and have relatively wide dynamic ranges (typically, 10^{-3} to 10^{-8} M). Furthermore, many of these procedures can be carried out with microliter or even nanoliter volumes of sample. Thus, these methods may achieve detection limits in the picomole range. In constant-current dynamic methods, the current in the cell is held constant while data are collected.

Potentiometric methods of analysis are based on measuring the potential of electrochemical cells without drawing appreciable current. For nearly a century, potentiometric techniques have been used for the location of end points in titrations. More recently, ion concentrations have been measured directly from the potential of an ion-selective membrane electrode. Such electrodes are relatively free from interference and provide a rapid and convenient means for quantitative estimations of numerous important anions and cations.

The equipment required for potentiometric methods is simple and inexpensive and includes an indicator electrode, a reference electrode, and a potential measuring device.

The reference electrode in this diagram is a half-cell with an accurately known electrode potential, E_{ref} , that is independent of the concentration of the analyte or any other ions in the solution under study. It can be a standard hydrogen electrode but seldom is because a standard hydrogen electrode is somewhat troublesome to maintain and use. The indicator electrode which is immersed in a solution of the analyte, develop a potential, E_{ind}, that depends on the activity of the analyte. The ideal reference electrode has a potential that is known constant, and completely insensitive to the composition of the solution under study. In addition, this electrode should be rugged and easy to assemble and should maintain a constant potential even when there is a net current in the cell.

Calomel reference electrodes consist of mercury in contact with a solution that is saturated with mercury (l) chloride (calomel) and that also contains a known concentration of potassium chloride. The saturated calomel electrode (SCE) is widely used because of the case with which it can be prepared. Compared with the other calomel electrodes, however, its temperature coefficient is significantly larger. A further disadvantage is that when the temperature is changed, the potential comes to a new value only slowly because of the time required for solubility equilibrium for the potassium chloride and for the calomel to be reestablished. The potential of the SCE at 25"C is 0.2444 V. The most widely marketed reference electrode system consists of a silver electrode immersed in a solution of potassium chloride that has been saturated with silver chloride. Normally, this electrode is prepared with either a saturated or a 3.5M potassium chloride solution. Figure 9.2 shows a commercial model of this electrode. Silver-silver chloride electrodes have the advantage that they can be used at temperatures greater than 60^{o}C, whereas calomel electrodes cannot. On the other hand, mercury (II) ions react with fewer sample components than do silver ions (which can react with proteins, for example); such reactions can lead to plugging of the junction between the electrode and the analyte solution.

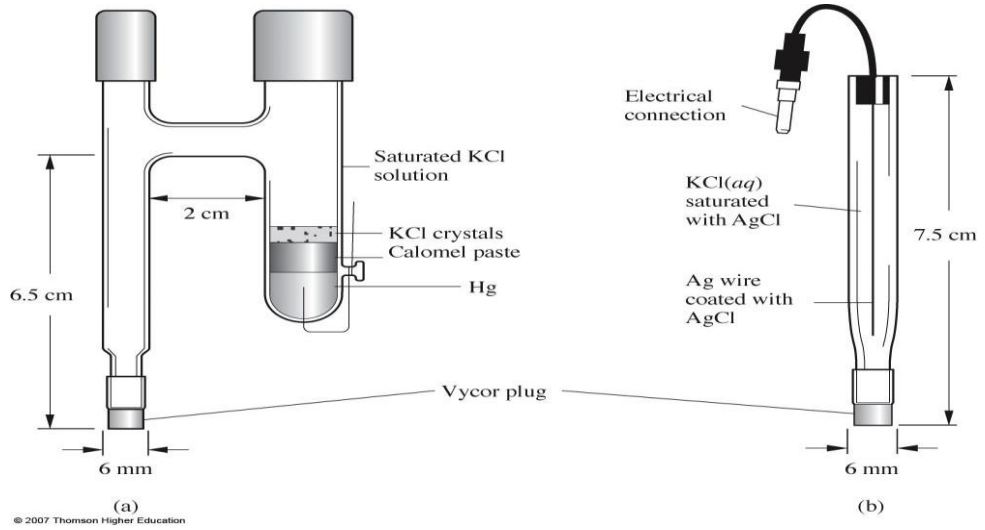


Figure 9.2 Typical commercial reference electrodes. (a)An SCE. (b)A silver-silver chloride electrode.

An ideal indicator electrode responds rapidly and reproducibly to changes in activity of the analyte ion. Although no indicator electrode is absolutely specific in its response, a few are now available that are remarkably selective. There are two types of indicator electrodes: metallic and membrane. It is convenient to classify metallic indicator electrodes as electrodes of the first kind, electrodes of the second kind, electrodes of the third kind, and inert redox electrodes.

Metallic indicator electrodes;

1) Electrodes of the First Kind
2) Electrodes of the Second Kind
3) Electrodes of the Third Kind
4) Metallic Redox Indicators

A wide variety of membrane electrodes are available from commercial sources that permit the rapid and selective determination of numerous cations and anions by direct potentiometric measurements." Often, membrane electrodes are called ion-selective electrodes because of the high selectivity of most of these devices. They are also referred to as pIon electrodes because their output is usually recorded as a p-function, such as pH, pCa, or pNO_3.

Membrane indicator electrodes;

1) Glass Electrodes
2) Liquid Membrane Electrodes

3) Solid State Electrodes
4) Gas-sensing Membrane Electrodes

The potential of a suitable indicator electrode is convenient for determining the equivalence point for a titration (a potentiometric titration). A potentiometric titration provides different information than does a direct potentiometric measurement. For example, the direct measurement of 0.100 M acetic and 0.100 M hydrochloric acid solutions with a pH-sensitive electrode yield widely different pH values because acetic acid is only partially dissociated. On the other hand, potentiometric titrations of equal volumes of the two acids require the same amount of standard base for neutralization. The potentiometric end point is widely applicable and provides inherently more accurate data than the corresponding method with indicators. It is particularly useful for titration of colored or turbid solutions and for detecting the presence of unsuspected species in a solution.

Procedure

1) Reagents
   a) Sample
   b) 0.1 M NaOH
2) Treatment of potentiometric data

   25-50 mL sample solution with the unknown concentration of the analyte is diluted to 100 mL with distilled water. 0.1 M NaOH solution is poured into a burette. pH electrode is immersed into the solution in order to record the pH before adding the titrant. PH values are read after each 0.5 mL NaOH addition. In order to locate the equivalence point exactly, ph values are recorded after 0.1 ml titrant addition near the equivalence point. Added titrant volumes and potentials are recorded in the following table.

Volume (mL)	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	5.0	5.5	6.0	6.5	7.0
pH														

3) Data analysis

The end point of the titration can be specified with these three methods:

1) E-V Graph: The titrant volume (mL) and the potential (Volt) are plotted on the axis and ordinate, respectively. The end point volume can be found with the volume half-way up the endpoint break.
2) $\Delta E/\Delta V$-V Graph: The titrant volume (mL) and the $\Delta E/\Delta V$ values are plotted on the axis and ordinate, respectively, and the maximum point of the graph should be determined.
3) $\Delta^2 E/\Delta V^2$-V Graph: The titrant volume (mL) and the $\Delta E/\Delta V$ values are plotted on the axis and ordinate, respectively, and the point where the ordinate of the second derivative is zero should be calculated.

   The end point volume and the concentration of the analyte (ppm) in the sample can be calculated with the help of these graphical methods.

10) Voltammetric Determination of Paracetamol

Objectivities: To determine the amount of paracetamol in a drug sample with carbon-based electrodes by voltammetry.

Introduction

Voltammetry comprises a group of electroanalytical methods in which information about the analyte is obtained by measuring current as a function of applied potential under conditions that promote polarization of an indicator, or working, electrode. When current proportional to analyte concentration is monitored at fixed potential the technique is called amperometry. Generally, to enhance polarization, working electrodes in voltammetry and amperometry have surface areas of a few square millimeters at the most and, in some applications, a few square micrometers or less.

Voltammetry is based on the measurement of the current that develops in an electrochemical cell under conditions where concentration polarization exists. In contrast, potentiometric measurements are made at currents that approach zero and where polarization is absent. Voltammetry differs from coulometry in that, with coulometry, measures are taken to minimize or compensate for the effects of concentration polarization. Furthermore, in voltammetry there is minimal consumption of analyte, whereas in coulometry essentially all of the analyte is converted to another state.

Voltammetry is widely used by inorganic, physical, and biological chemists for nonanalytic purposes, including fundamental studies of oxidation and reduction processes in various media, adsorption processes on surfaces, and electron-transfer mechanisms at chemically modified electrode surfaces.

In voltammetry, a variable potential excitation signal is impressed on a working electrode in an electrochemical cell. This excitation signal produces a characteristic current response, which is the measurable quantity in this method. The waveforms of four of the most common excitation signals used in voltammetry are shown in Figure 10.1.

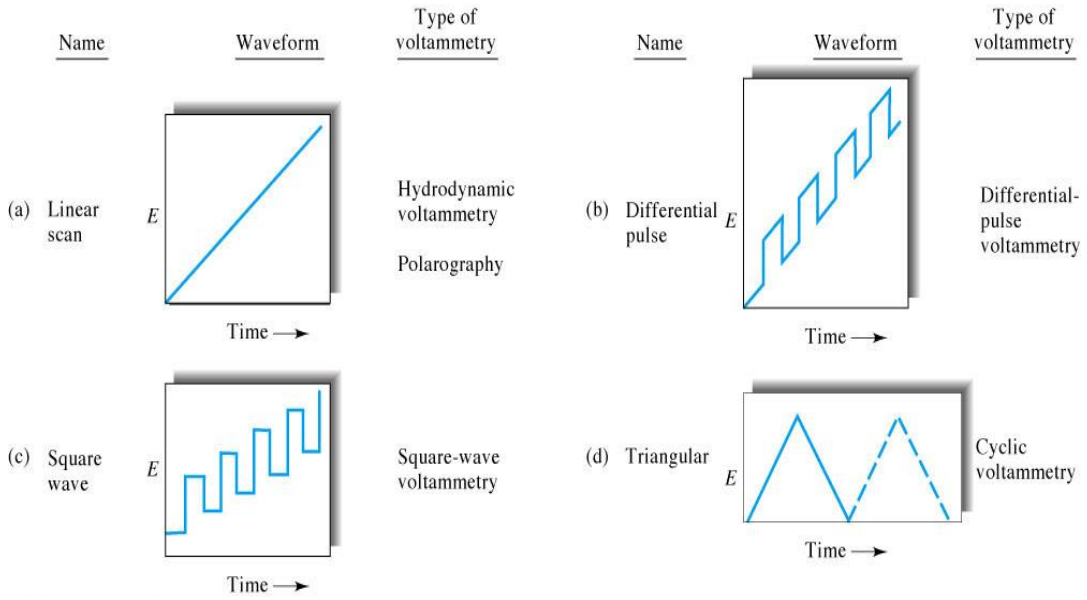


Figure 10.1 Voltage versus time excitation signals used in voltammetry.

The classical voltammetric excitation signal is the linear scan shown in Figure 10.1a, in which the voltage applied to the cell increases linearly (usually over a 2-to 3-V range) as a function of time. The current in the cell is then recorded as a function of time, and thus as a function of the applied voltage. In amperometry, current is recorded at fixed applied voltage. Two pulse excitation signals are shown in Figure 10.1b and c. Currents are measured at various times during the lifetime of these pulses. With the triangular waveform shown in Figure 10.1d, the potential is cycled between two values, first increasing linearly to a maximum and then decreasing linearly with the same slope to its original value. This process may be repeated numerous times as the current is recorded as a function of time. A complete cycle may take 100 or more seconds or be completed in less than 1 second. To the right of each of the waveforms of Figure 10.1 is listed the types of voltammetry that use the various excitation signals.

The working electrodes used in voltammetry take a variety of shapes and forms. Often, they are small flat disks of a conductor that are press filled into a rod of an inert material, such as Teflon or Kel-F, that has embedded in it a wire contact (see Figure 10.2a). The conductor may be a noble metal, such as platinum or gold; a carbon material, such as carbon paste, carbon fiber, pyrolytic graphite, glassy carbon, diamond, or carbon nanotubes; a semiconductor, such as tin or indium oxide; or a metal coated with a film of mercury. Generally, the positive potential limitations arc caused by the large currents that develop because of oxidation of the water to give molecular oxygen. The negative limits arise from the reduction of water to produce hydrogen.

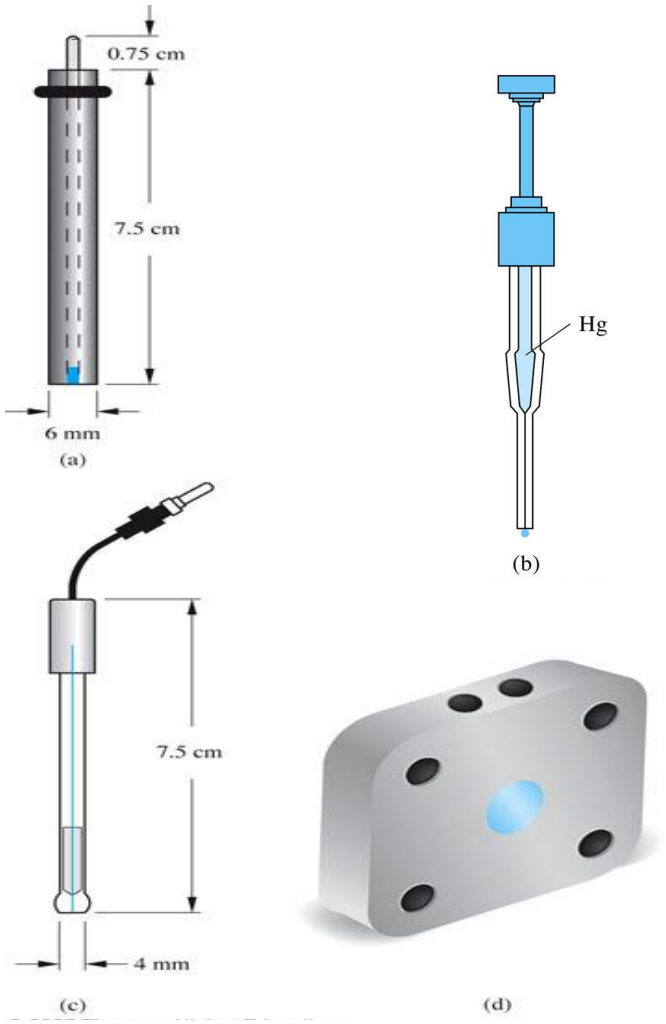


Figure 10.2 Some common types of commercial voltammetric electrodes: (a) a disk electrode; (b) a hanging mercury drop electrode (HMOE); (c)a microelectrode; (d) a sandwich-type flow electrode.

Mercury working electrodes have been widely used in voltammetry for several reasons. One is the relatively large negative potential range just described. Furthermore, a fresh metallic surface is readily formed by simply producing a new drop. The ability to obtain a fresh surface readily is important because the currents measured in voltammetry are quite sensitive to cleanliness and freedom from irregularities. An additional advantage of mercury electrodes is that many metal ions are reversibly reduced to amalgams at the surface of a mercury electrode, which simplifies the chemistry.

An active area of research in electrochemistry is the development of electrodes produced by chemical modification of various conductive substrates. Such electrodes have been tailored to accomplish a broad range of functions. Modifications include applying irreversibly adsorbing

substances with desired functionalities covalent bonding of components to the surface, and coating the electrode with polymer films or films of other substances.

First, the surface of the electrode is oxidized to create functional groups tin the surface as shown in Figure 10.3a and b. Then linking agents such as organosilanes (Figure 10.3c) or amines (Figure 10.3d) are attached to the surface prior to attaching the target group. Polymer films can be prepared from dissolved polymers by dip coating spin coating, e1ectrodeposition, or covalent attachment. They can also be produced from the monomer by thermal, plasma, photochemical, or electrochemical polymerization methods.

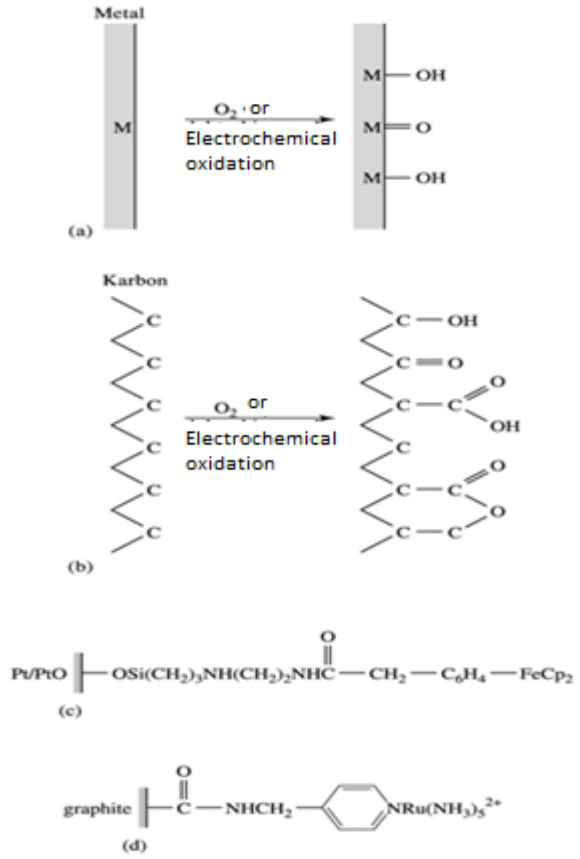


Figure 10.3 Functional groups formed on (a)a metal or (b)a carbon surface by oxidation. (c)A linking agent such as the organosilane shown is often bonded to the functionalized surface. Reactive components, such as ferrocenes, viologens, and metal bipyridine complexes, are then attached to form the modified surfaces. A Pt electrode is shown with a ferrocene attached.

In cyclic voltammetry (CV) the current response of a small stationary electrode in an unstirred solution is excited by a triangular voltage waveform, such as that shown in Figure 10.4. In this

example, the potential is first varied linearly from +0.8 V to -0.15 V versus an SCE. When the extreme of -0.15 V is reached, the scan direction is reversed, and the potential is returned to its original value of +0.8 V. This excitation cycle is often repeated several times. The voltage extrema at which reversal takes place are called switching potentials.

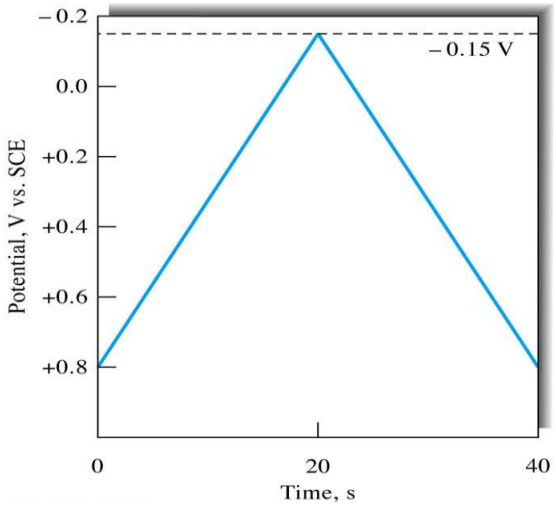


Figure 10.4 Cyclic voltammetry excitation signals.

Figure 10.5b shows the current response when a solution that is 6 mM in $K_3Fe(CN)_6$ and 1M in KNO_3, is subjected to the cyclic excitation signal shown in Figures 10.4 and 10.5a. The working electrode was a carefully polished stationary platinum electrode and the reference electrode was an SCE. At the initial potential of +0.8 V a tiny anodic current is observed, which immediately decreases to zero as the scan is continued. This initial negative current arises from the oxidation of water to give oxygen (at more positive potentials, this current rapidly increases and becomes quite large at about +0.9 V). No current is observed between a potential of +0.7 and +0.4 V because no reducible or oxidizable species is present in this potential range. When the potential becomes less positive than approximately +0.4 V a cathodic current begins to develop (point B) because of the reduction of the hexacyanoferrate(III) ion to hexacyanoferrate(II) ion. The reaction at the cathode is then

$$Fe(CN)_6^{3-} + e^- \longleftrightarrow Fe(CN)_6^{4-}$$

Important variables in a cyclic voltammogram are the cathodic peak potential E_{pc}, the anodic peak potential E_{pa} the cathodic peak current i_{pc} and the anodic peak current i_{pa}. The definitions and measurements of these parameters are illustrated in Figure 10.5.

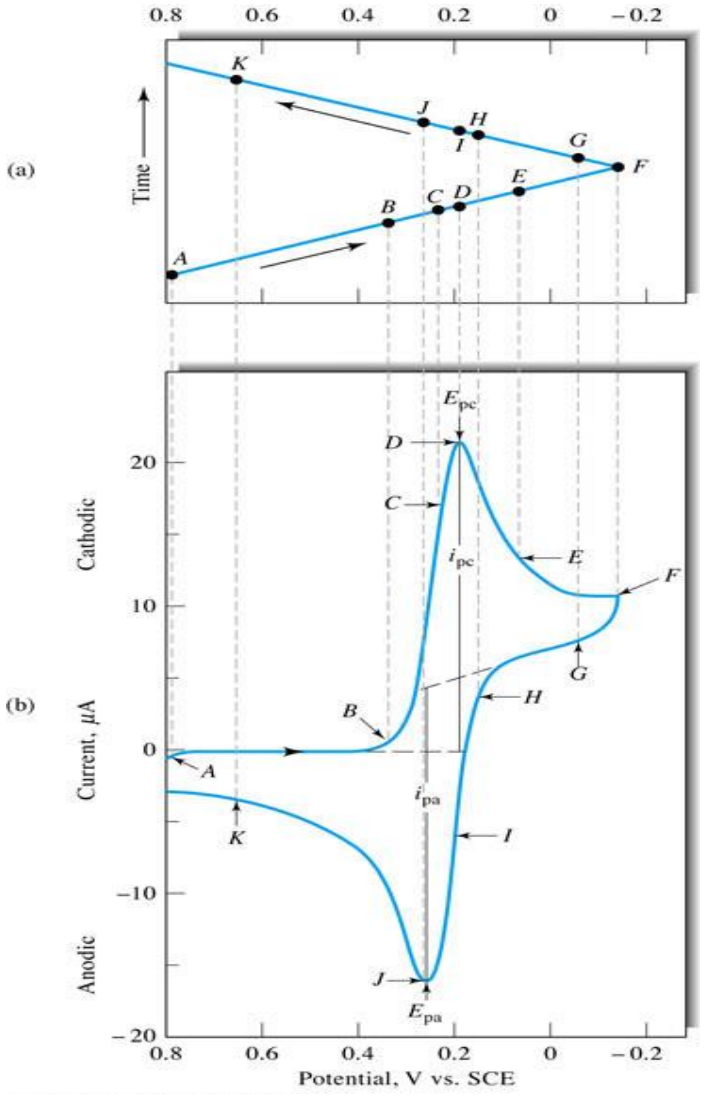


Figure 10.5 (a) Potential versus time wave form and (b) cyclic voltammogram for a solution that is 6.0 mM in $K_3Fe(CN)_6$ and 1.0 M in KNO_3.

For a reversible electrode reaction, anodic and cathodic peak currents are approximately equal in absolute value but opposite in sign. For a reversible electrode reaction at 25°C the difference in peak potentials, ΔE_p is expected to be

$$\Delta E_p = E_{pa} - E_{pc} = 0.0593/n \qquad (10.1)$$

where n is the number of electrons involved in the half-reaction. Irreversibility because of slow electron transfer kinetics results in ΔE_p exceeding the expected value. Although an electron-transfer reaction may appear reversible at a slow sweep rate, increasing the sweep rate may lead to increasing values of ΔE_p, a sure sign of irreversibility. Hence, to detect slow electron-transfer kinetics and to obtain rate constants, ΔE_p is measured for different sweep rates.

Quantitative information is obtained from the Randles-Sevcik equation, which at 25°C is

$$i_p = 2.686 \times 10^5 n^{3/2} A c D^{1/2} v^{1/2} \quad (10.2)$$

where *ip* is the peak current (A), A is the electrode area (cm^2), D is the diffusion coefficient (cm^2/s), c is the concentration (mol/cm^3), and v is the scan rate (V/s). CV offers a way of determining diffusion coefficients if the concentration, electrode area, and scan rate are known.

Equation 10.2 shows that peak currents in CV are directly proportional to analyte concentration. Although it is not common to use CV peak currents in routine analytical work, occasionally such applications do appear in the literature, and they are appearing with increasing frequency.

Many of the limitations of traditional linear-scan voltammetry were overcome by the development of pulse methods. We will discuss the two most important pulse techniques, differential-pulse voltammetry and square-wave voltammetry. The idea behind all pulse voltammetric methods is to measure the current at a time when the difference between the desired faradaic curve and the interfering charging current is large.

Figure 10.6 shows the two most common excitation signals used in commercial instruments for differential pulse voltammetry. The first (Figure 10.6a), which is usually used in analog instruments, is obtained by superimposing a periodic pulse on a linear scan. The second waveform (Figure 10.6b), which is typically used in digital instruments, is the sum of a pulse and a staircase signal. In either case, a small pulse, typically 50-mV, is applied during the last 50 ms of the period of the excitation signal.

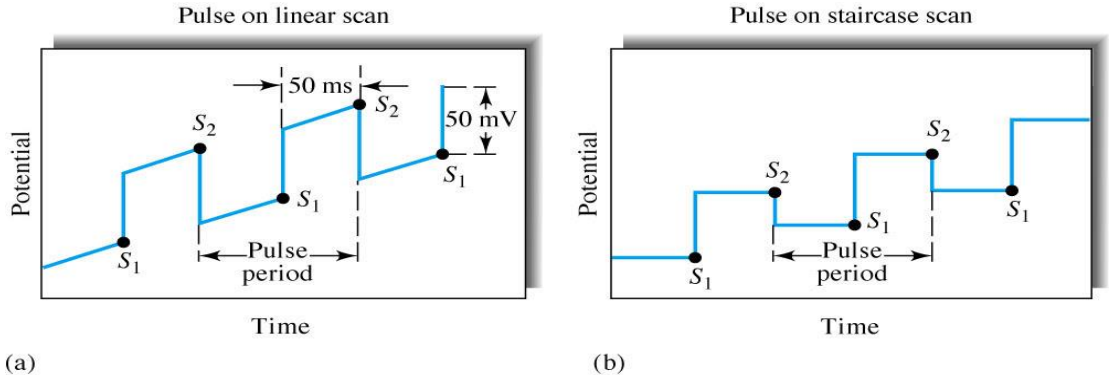


Figure 10.6 Excitation signals for differential-pulse voltammetry.

Generally, detection limits with differential-pulse voltammetry are two to three orders of magnitude lower than those for classical voltammetry and lie in the range of 10^{-7} to 10^{-8} M. The greater sensitivity of differential-pulse voltammetry can be attributed to two sources. The first is an enhancement of the faradaic current, and the second is a decrease in the non-faradaic charging current. Reliable instruments for differential-pulse voltammetry are now available

commercially at reasonable cost. The method has thus become one of the most widely used analytical voltammetric procedures and is especially useful for determining trace concentrations of heavy metal ions.

Procedure

1) Reagents
   a) Paracetamol
   b) A drug sample
2) Voltammetric analysis

   Paracetamol (acetaminophen) is a pain reliever and a fever reducer. (Figure 10.7) There are several voltammetric method to analyze drug active substances quantitatively. Paracetamol can be analyzed with electrochemical methods thanks to being an electroactive substance. It is oxidized by accepting two electrons on the surface of solid electrodes and this reaction is half-reversible. Some of this reaction's products react with water and produce benzokinonimin molecules. The mechanism of this oxidation reaction is illustrated in Figure 10.8. In recent years, electrochemical researches on the determination of paracetamol show that low detection limits can be obtained from these techniques.

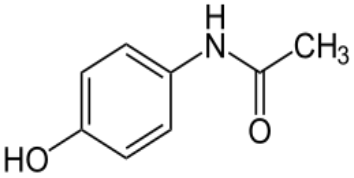


Figure 10.7 Paracetamol molecule structure

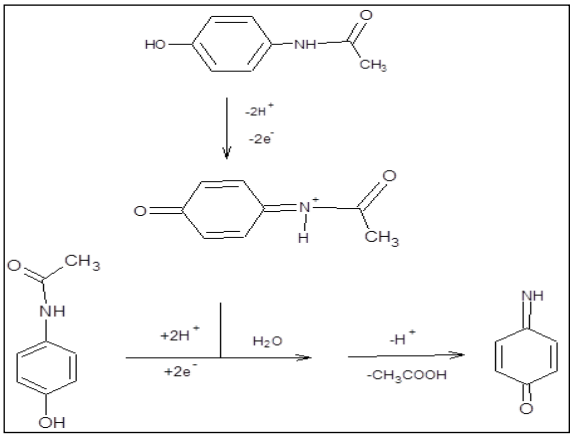


Figure 10.8 The mechanism of the half-reversible oxidation reaction of paracetamol.

Three electrode system is used in electrochemical studies and carbon-based electrodes, Ag/AgCl, and Pt electrodes are used as working, reference and indicator electrodes, respectively. An electrolyte solution should be selected in accordance with the potential range in which paracetamol is oxidized and reduced and the proper pH value during the analysis. After preparing all solution being used in the analysis, cyclic voltammetry method is performed to investigate the electrochemical behavior of the analyte and analyze it qualitatively. Differential-puls voltammograms are recorded with a series standard solutions of paracetamol in order to find the concentration of paracetamol in a drug sample which is previously diluted to a suitable concentration. The amount of paracetamol is calculated in mg with the current-concentration graph; therefore, its qualitative and quantitative analyses can be performed with the electrochemical techniques.

REFERENCES

1) 1.Holler,F.J., Skoog,A.D.,Crouch,S.R. (2013) Principles of Instrumental Analysis

PERIODIC TABLE