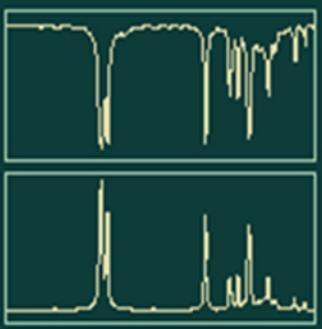
HANDBOOK OF ORGANIC COMPOUNDS

NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants

1

Methods and Interpretations



JERRY WORKMAN, JR.

Handbook of Organic Compounds

NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants (a 3-volume set)

Volume 1
Methods and Interpretations

Handbook of Organic Compounds

NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants (a 3-volume set)

Volume 1
Methods and Interpretations

Jerry Workman, Jr.
Kimberly-Clark Corporation
Neenah, WI

ACADEMIC PRESS

A Harcourt Science and Technology Company

San Diego San Francisco New York Boston
London Sydney Tokyo

This book is printed on acid-free paper. ♾

ACADEMIC PRESS
A Harcourt Science and Technology Company
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA
http://www.academicpress.com

ACADEMIC PRESS
Harcourt Place, 32 Jamestown Road, London, NW1 7BY, UK

Library of Congress Catalog Card Number: 00-105503
International Standard Book Number: 0-12-763561-0

Printed in the United States of America
00 01 02 03 04 IP 9 8 7 6 5 4 3 2 1

I am forever grateful to:

Norman Colthup, Clara Craver, Bill Fateley, Connie Paralez, Jeanette Grasselli, Robert Hannah, Dana Mayo, and Foil Miller, who have tirelessly and successfully taught the complexities of infrared interpretation to so many scientists, both young and old, at the Fisk and Bowdoin infrared interpretive courses

Professor Larry Dieterman, who passed along his truly contagious love for chemistry to so many students

Bertie, the best proofreader, and friend, on the planet

My parents, for their loving care and for engendering my intellectual drive

CONTENTS

PREFACE

This *Handbook of Organic Compounds: NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants* is a compendium of practical spectroscopic methodology, comprehensive reviews, and basic information for organic materials, surfactants, and polymer spectra covering the ultraviolet, visible, near-infrared, infrared, Raman, and dielectric measurement techniques. It represents the first comprehensive multivolume handbook to provide basic coverage for UV-Vis, 4th-overtone NIR, 3rd-overtone NIR, NIR, infrared, and Raman spectra and dielectric data for organic compounds, polymers, surfactants, contaminants and inorganic materials commonly encountered in the laboratory. The text includes a description and reviews of interpretive and chemometric techniques used for spectral data analysis. The spectra found within the atlas are useful for identification purposes as well as for instruction in the various interpretive and data-processing methods discussed. This work is designed to be of help to students and vibrational spectroscopists in their daily efforts at spectral interpretation and data processing of organic spectra, polymers, and surfactants. All spectra are presented in terms of wavenumber and transmittance; ultraviolet, visible, 4th-overtone NIR, 3rd-overtone NIR, and NIR spectra are also presented in terms of nanometers and absorbance space. In addition, horizontal ATR spectra are presented in terms of wavenumber and absorbance space. All spectra are shown with essential peaks labeled in their respective units. Several individuals contributed to the material in this handbook, and comments were received from a variety of workers in the field of molecular spectroscopy. This handbook can provide a valuable reference for the daily activities of students and professionals working in modern molecular spectroscopy laboratories.

ACKNOWLEDGMENTS

The editor is grateful to the following individuals from the Analytical Science & Technology Group at Kimberly-Clark Corporation for their valuable assistance in compiling spectral data included in this work:

Tom Last, Mike King, Maurice Pheil, Mike Faley (student coop from the University of Wisconsin—Oshkosh), **Maria Delgado** (student summer intern from the University of Wisconsin—Madison), **Rick Beal, Jerry Kochanny, Tom Eby,** and **Wade Thompson.**

The editor also acknowledges **Paul R. Mobley** and **Bruce R. Kowalski** of the Laboratory for Chemometrics, Dept. of Chemistry, The University of Washington, Seattle, WA 98105, and **Rasmus Bro** of the Food Technology Chemometrics Group, Dept. of Dairy and Food Science, Rolighedsvej 30, 1958 Frederiksberg, Copenhagen, Denmark, who worked with me to write a three-part review of chemometrics in spectroscopy for *Applied Spectroscopy Reviews*.

MEASUREMENT CONDITIONS
FOR SPECTRAL CHARTS (VOLS. 2 AND 3)

VOLUME 2

Ultraviolet-Visible Region

Liquids

Spectral Region: 174 nm to 900 nm
1456 data points
Source: deuterium (to 350 nm), quartz-tungsten-halogen (to 900 nm)
Detector: R-928 photomultiplier (red sensitive)
Scan rate: 200 nm/min.
Integration time: 0.30
Slit height: 1/3 full
2 nm resolution (slit bandwidth)
Varian Cary 5G in transmittance mode with 1 cm pathlength cells for liquids. The measurements for liquids were made using a dual channel optical geometry with dry air as the initial and second channel background reference.

Solids

Spectral Region: 174 nm to 900 nm
1456 data points
Source: deuterium (to 350 nm), quartz-tungsten-halogen (to 900 nm)
Detector: R-928 photomultiplier (red sensitive)
Scan rate: 200 nm/min.
Integration time: 0.30
Slit height: 1/3 full
2 nm resolution (slit bandwidth)
Varian Cary 5G in reflectance mode for solids using Labsphere DRA CA-50, 150 mm (inner diameter) integrating sphere with photomultiplier and PbS detectors (useful range 250 – 2500 nm). The measurements for solids were made using a Spectralon® coated sphere with a background reference of Spectralon® SRS-99 (99% reflectance).

Short wave-Near Infrared Region

Liquids only

Spectral Region: 800 nm to 1080 nm
799 data points
Source: tungsten-halogen (Vis-NIR)
Detector: 1024-element silicon DA
3.3 nm resolution
Default measurement values
Integration time per spectrum: 128 ms, 20 second data collection time.
Perkin-Elmer PIONIR 1024 Diode-Array transmittance with 10 cm pathlength for liquids; 1024 element silicon linear diode array detector. Self-referencing dual-path liquid cell was used. Dry air was used as initial background reference.

Long wave-Near Infrared Region

Liquids

Spectral Range A: 12000 cm^{-1} to 3500.17 cm^{-1}
6377 data points
Source: tungsten-halogen
Detector: NIR-PE
Beamsplitter: KBr
Phase resolution: 128
Phase correction: Power spectrum
Apodization: Blackman-Harris 4-term
Zero filling factor: 4
8 cm^{-1} resolution
1.0 mm aperture
1 cm pathlength quartz cell for liquids
Bruker Model FTS-66 FT-NIR, 3 minute data collection (215 co-added scans per measurement). Dry air was used as background reference.

Solids

Spectral Range B: 12000 cm^{-1} to 3498 cm^{-1}
3498 data points
Source: tungsten-halogen
Detector: NIR-PE
Beamsplitter: KBr
Phase resolution: 128
Phase correction: Power spectrum
Apodization: Blackman-Harris 4-term
Zero filling factor: 4
16 cm^{-1} resolution
Bruker Model FTS-66 FT-NIR
Specular Reflectance Accessory (30° incidence and reflectance angle)
3 minute data collection (215 co-added scans per measurement)
Polymer pellets and powders were measured “as received” using a cylindrical sample cell with silica windows. A gold-coated reflectance mirror was used for the background reference.

VOLUME 3

Mid-Infrared Spectral Region

Organic Compounds and Polymers

Spectral Range: 4000 cm^{-1} to 500 cm^{-1}
1816 data points
Source: Globar
Detector: DTGS KBr
Beamsplitter: KBr
Autogain velocity: 1.5825
Apodization: Happ-Genzel
Zero filling factor: 1 level
Aperture: 25
4 cm^{-1} resolution
Nicolet Model 510 FT-IR Spectrometer
32 and 64 co-added scans per measurement
KBr pellet – typical sampling for transmittance mode
Blank KBr was sued as the typical background reference material

Surfactants

Spectral Range: 3750 cm^{-1} to 650 cm^{-1}
1551 data points
Source: Globar
Detector: DTGS KBr
Beamsplitter: KBr
Autogain velocity: 1.5825
Apodization: Happ-Genzel
Zero filling factor: 1 level
Aperture: 25
2 cm^{-1} resolution
Nicolet Model 710 and Model SX FT-IR Spectrometers
64 co-added scans per measurement
Capillary films, cast films, or KBr disks for transmittance mode using blank transmittance plate materials for the background reference

HATR (Horizontal Attenuated Total Reflectance) Measurements

Liquids

Spectral Range: 4000 cm^{-1} to 650 cm^{-1}
1738 data points
Source: Globar (Everglo™ Mid-IR source)
Detector: DTGS KBr
Beamsplitter: KBr
Autogain velocity: 0.6329
Apodization: Happ-Genzel
Zero filling factor: None
Phase correction: Mertz
4 cm^{-1} resolution
Nicolet Avatar Model 360 FT-IR Spectrometer
32 co-added scans per measurement

zinc selenide (ZnSe) 45° - 10 bounce horizontal ATR crystal
No sample present for background reference measurements

Raman Spectral Measurements

Liquids and Solids

Spectral Range: 3800 cm^{-1} to 200 cm^{-1}
1801 data points
Source: Nd:YAG laser at 1064 nm (0-300 mW power)
Detector: Raman - germanium (Ge)
Beamsplitter: calcium fluoride (CaF2)
Apodization: Blackman-Harris 4 term
Zero filling factor: 4
Aperture: 6 mm
Phase correction: Power spectrum
2 cm^{-1} resolution
Bruker FTS-66 FT-NIR Spectrometer
Neodymium: Yttrium Aluminum Garnet (Nd:YAG) excitation laser at 1064 nm
1000 co-added scans per measurement, focused beam diameter approximately 2 mm.
Samples for measurement were contained in silica NMR tubes with 11 mm (outer diameter) by 9 mm (inner diameter); approximately 30 mm height.

PERMISSIONS AND CREDITS

Permission to reprint copyrighted material has been granted by the individual copyright owners.

The following articles have been adapted with permission from Marcel Dekker, Inc., New York:

J. Workman. Interpretive spectroscopy for near-infrared. *Applied Spectroscopy Reviews* 31(3), 1996.

J. Workman, P. Mobley, B. Kowalski, R. Bro. Review of chemometrics applied to spectroscopy, part 1. *Applied Spectroscopy Reviews* 31 (1 & 2), 73–124, 1996.

P. Mobley, B. Kowalski, J. Workman, R. Bro. Review of chemometrics applied to spectroscopy, part 2. *Applied Spectroscopy Reviews* 31(4), 1996.

Rasmus Bro, J. Workman, Paul Mobley, Bruce Kowalski. Review of chemometrics applied to spectroscopy: 1985–1995, part 3. *Applied Spectroscopy Reviews* 32(3), 1997.

J. Workman. Review of process and non-invasive near-infrared and infrared spectroscopy: 1993–1999. *Applied Spectroscopy Reviews* 34 (1 & 2): 1–91, 1999.

The following was adapted with Permission from Academic Press, Boston:

J. Workman, A. Springsteen, eds. *Applied Spectroscopy: A Compact Reference for Practitioners.* Boston: Academic Press, 1998.

Chapter 1. Optical Spectrometers (J. Workman)

Chapter 2. Ultraviolet, Visible, and Near-Infrared Spectrometry (J. Workman)

Appendix A: Sources, Detectors, and Window Materials for UV-Vis, NIR, and IR Spectroscopy (J. Workman)

Appendix B: Practices of Data Preprocessing for Optical Spectrophotometry (J. Workman)

Appendix C: Infrared Microspectroscopy (J. Workman)

The practical guide for evaluating ultraviolet spectra is reprinted with permission from D. Pavia, G. Lampman, and G. Kriz, *Introduction to Spectroscopy: A Guide for Students of Organic Chemistry* (Philadelphia: Saunders College, 1979).

I.

OPTICAL SPECTROSCOPY

1.

OPTICAL SPECTROMETERS

A common assumption in spectroscopic measurements is that Beer's law relationship applies between a change in spectrometer response and the concentration of analyte material present in a sample specimen. The *Bouguer, Lambert, and Beer relationship* assumes that the transmission of a sample within an incident beam is equivalent to 10 exponent the negative product of the molar extinction coefficient (in mol^{-1} cm^{-1}), times the concentration of a molecule in solution (in mol^{-1}) times the pathlength (in cm) of the sample in solution. There are some obvious (and not-so-obvious) problems with this assumption. The main difficulties in the assumed relationship are that the molecules often interact, and the extinction coefficient may vary due to changes in the molecular configuration of the sample. The obvious temperature, pressure, and interference issues also create a less-than-ideal situation for the analyst. However, for many (if not most) analytical problems the relationship holds well enough.

Properties of the Bouguer, Lambert, and Beer (Beer's law) relationship:

$$T = \frac{I}{I_0} = 10^{-\varepsilon c l}$$

where T = transmittance, I_0 = intensity of incident energy, I = intensity of transmitted light, ε = molar extinction coefficient (in $L \cdot mol^{-1}$ cm^{-1}), c = concentration (in $mol \cdot L^{-1}$), and l = pathlength (in cm). To simplify this equation into its more standard form, showing absorbance as a logarithmic term and, used to linearize the relationship between spectrophotometer response and concentration, gives the following expression as the relationship between absorbance and concentration.

$$\text{Abs.} = A = -\log\left(\frac{I}{I_0}\right) = -\log(T) = \varepsilon c l$$

The following statements hold true for what is most often termed Beer's law: (1) The relationship between transmittance and concentration is nonlinear, (2) yet the relationship between absorbance and concentration is linear. Beer's law is the common basis for *quantitative analysis*. Knowledge of Beer's law allows us to calculate the maximum theoretical dynamic range for an instrument using a few simple mathematical relationships.

The goal in the design of an optical spectrometer is to maximize the energy (or radiant power) from a light source through the spectrometer to the detector. The optical throughput for a spectrometer is dependent upon multiple factors, such as the light source area, the apertures present within the light path, lens transmittance and mirror reflectance losses, the exit aperture, and the detector efficiency. A simplified model to describe such a system is given below. From *paraxial optical theory* it is known that:

$$A_s \Omega_{\text{Ent}} = A_d \Omega_{\text{Exit}}$$

This relationship applies when A_s = the illuminated area of the light source, A_d = the illuminated detector area, Ω_{Ent} = the solid angle subtended by the source at the entrance aperture of the spectrometer, and Ω_{Exit} = the solid angle subtended by the detector by the exit aperture. This relationship is essential in determining the distances of the source and detector from the main spectrometer optical system, as well as the size of the entrance and exit apertures (or slits).

Several terms are useful in any discussion of spectrometry: *selectivity* = specific sensor response to the *analyte of interest; sensitivity* = the *quantifiable level of response* from a sensor with respect to the concentration of a specified analyte; and *detection limit* = the smallest concentration difference that can be detected above the background noise level of a sensor. This is often estimated using 2–3 times the background RMS noise as a signal and estimating the concentration for this signal level using a calibration curve. Additional details on the physics of spectrometers can be found in Blaker (1970), Bracey (1960), Braun (1987), Ditchburn (1965), Fogiel (1981), and Wist (1986).

TYPES OF SPECTROMETERS

The reader is referred to Bracey (1960), Braun (1987), and Wist (1986) for details on spectrometer design.

Discrete Photometers

Discrete photometers consist of an irradiance source, discrete interference filters, a sample compartment, and detector, along with appropriate electronics for signal amplification and stabilization of detector signals. The optical configuration for a generic photometer is shown in Figure 1.1.

Single-Beam Spectrometers

Single-beam spectrometers have a single optical channel that is configured to measure either the sample or reference channel, but not both simultaneously. The resultant spectrum is the ratio of the transmission spectra from sample and reference measurements. In practice the response of the detector measured with zero photon energy flux is measured as the *dark current*. The final transmission (in *T* units) spectrum from this device is given as:

$$\text{Reflectance or Transmittance Spectrum} = \frac{\text{Sample}_T - \text{Dark}}{\text{Reference}_T - \text{Dark}}$$

If the spectrometer has any instabilities (either optical, mechanical, or electronic), the time constant between sample and reference measurements becomes critical. Single-beam instruments must either be more inherently stable or alternate between sample and reference measurements at

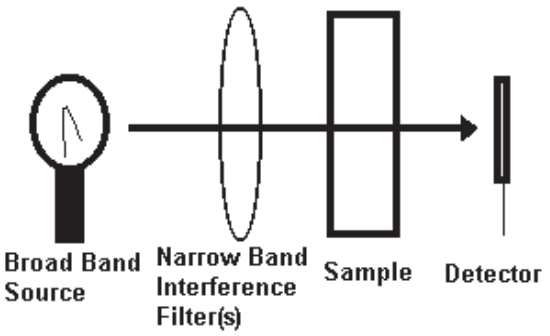


Fig. 1.1 Interference filter photometer optical configuration.

a frequency that makes negligible the rate of change of the spectrometer. Designs for single and double monochromator spectrophotometers, as well as a diode array design, are shown in Figures 1.2a–1.2c. These designs can be configured as single- or double-beam spectrometers.

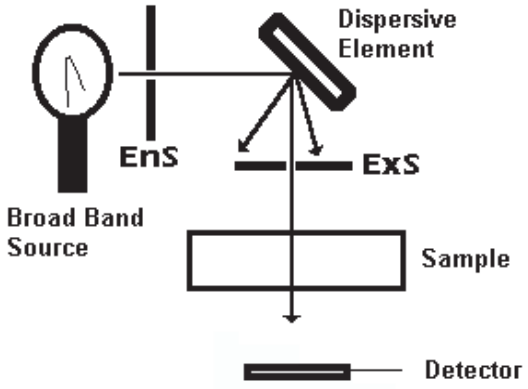


Fig. 1.2a Single monochromator system (dispersive) optical configuration. EnS and ExS designate entrance slit and exit slit, respectively.

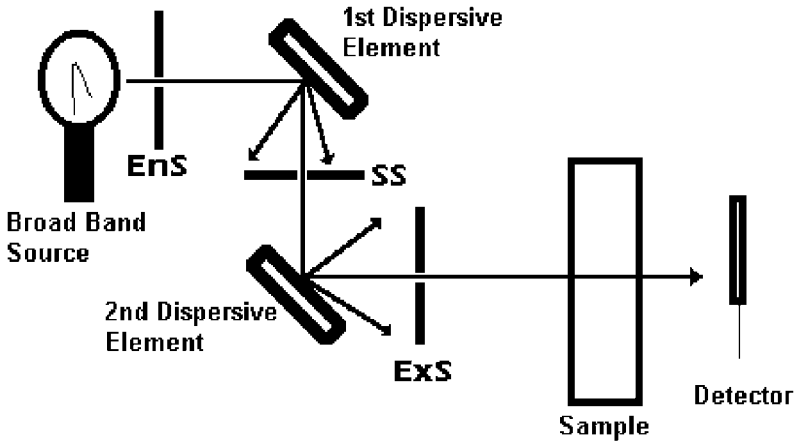


Fig. 1.2b Double monochromator system (dispersive) optical configuration. EnS, SS, and ExS designate entrance slit, second slit, and exit slit, respectively.

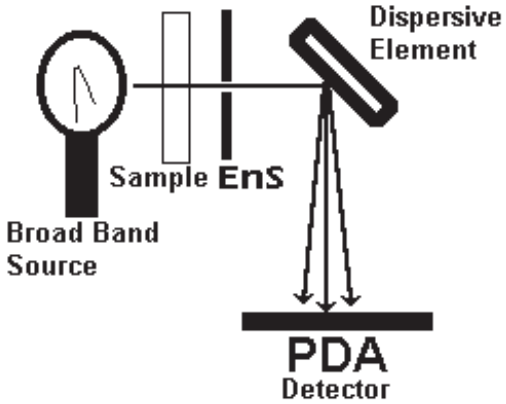


Fig. 1.2c Diode array spectrophotometer (dispersive) optical configuration. EnS designates the entrance slit.

Double-Beam Spectrometers

A double-beam spectrometer consists of both sample and reference channels and measures both channels simultaneously. The separation of the light beam from the source is accomplished using a beam splitter such that roughly 50% of the source's emitted energy is divided to both sample and reference channels. The use of the dual-beam concept compensates for instrument instabilities inherent in all spectrometers. The resultant spectrum is the ratio of the sample and reference channels in transmission. As in the case of single-beam instruments, in practice the final spectrum from this device is given as:

$$\text{Reflectance or Transmittance Spectrum} = \frac{\text{Sample}_T - \text{Dark}}{\text{Reference}_T - \text{Dark}}$$

Double-Beam/Dual-Wavelength Spectrometers

Double-beam/dual-wavelength spectrometers are capable of making measurements at two nominal frequencies simultaneously. They possess the capability of illuminating a specimen with two wavelengths (λ_1 and λ_2) while measuring the spectrum at both wavelengths. This is accomplished by using two dispersive elements (e.g., diffraction gratings) to disperse the incident energy from the source onto the sample specimen at the two different wavelengths. A shutter interrupts one of the beams while the other is incident to the detector. The signals from λ_1 and λ_2 are processed in such a manner that the displayed signal is a differential absorbance of $D\lambda_1 - \lambda_2$; this differential absorbance is proportional to concentration. Dual-wavelength scanning is used to cancel the effects of background when measuring turbid samples, for quantitative determination of a single component in multicomponent mixtures, or for quantitative determination of high-speed reactions.

Interferometer-Based Spectrometers

Interferometry is often used to measure wavelengths; this type of measurement is conducive to spectroscopic measurements. In spectroscopy, the accuracy of wavelength measurements can be critical to from 6 to 10 significant figures. Wavelength measurements from interferometer-type devices are much more accurate than those generally made using dispersive-type instruments. The most common type of interferometer is the Michelson type shown in Figure 1.3. A parallel beam of light from the spectrometer source (SS) is directed into a bean splitter (BS) at an angle of 45° from normal. The beam splitter consists of a 50% reflective surface. The transmitted portion of light from the beam splitter is directed to a highly reflective movable mirror (MM), whereas the light reflected from the beam splitter strikes a fixed mirror (FM). From the perspective of the detector (D) the following phenomena are "observed." If the pathlengths to FM and MM are the same, an observer at the D position will see a bright fringe. If the pathlengths are not the same, the phase difference will determine whether the fringe observed at the detector is light or dark. The fixed mirror is adjusted only for tilt, to keep it normal to the incident beam. As MM is moved, the path becomes different and the phase changes. A movement of MM (d) is related to the number of fringes in the interference pattern ($\#f$) observed at D by the relationship:

$$\frac{d}{\#f} = \frac{\lambda}{4}$$

when λ = the wavelength of parallel incident light entering the interferometer and d is expressed in units identical to wavelength units. The optical design configuration for an interferometer-based instrument is shown in Figure 1.3.

Open-Path and Emission Spectrometers

An open-path spectrometer is designed according to the features of dispersive and interferometer-based instruments, with the exception that the sample is located remotely from the instrument

and the light source is either sunlight or laser power. The basic configuration for sampling in the open path design is shown in Figure 1.4.

DETAILS OF SPECTROMETER COMPONENTS

Light Sources

All materials emit electromagnetic radiation, the nature of which depends upon the material and its temperature. Gases or vapors of atoms at less than atmospheric pressure emit radiation at discrete wavelengths when an electric current is passed through them. Excited gases containing molecules emit spectra consisting of multiple lines very close together, which comprise emission bands. Solid materials emit or radiate continuous spectra across all frequencies. The following relationships define the physics of light sources.

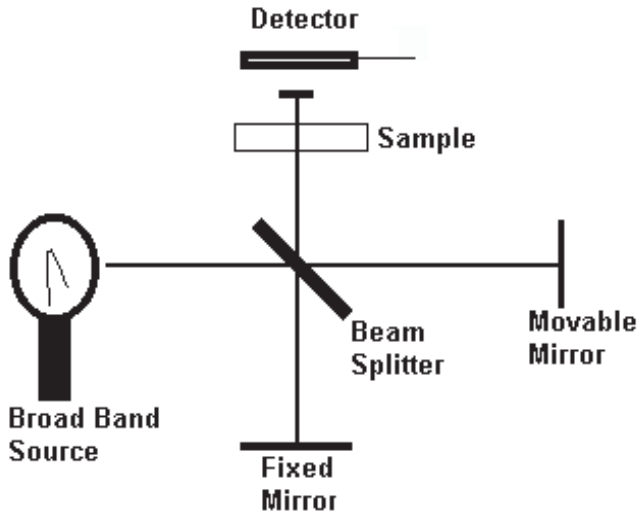


Fig. 1.3 Fourier transform spectrophotometer (interferometer) optical configuration.

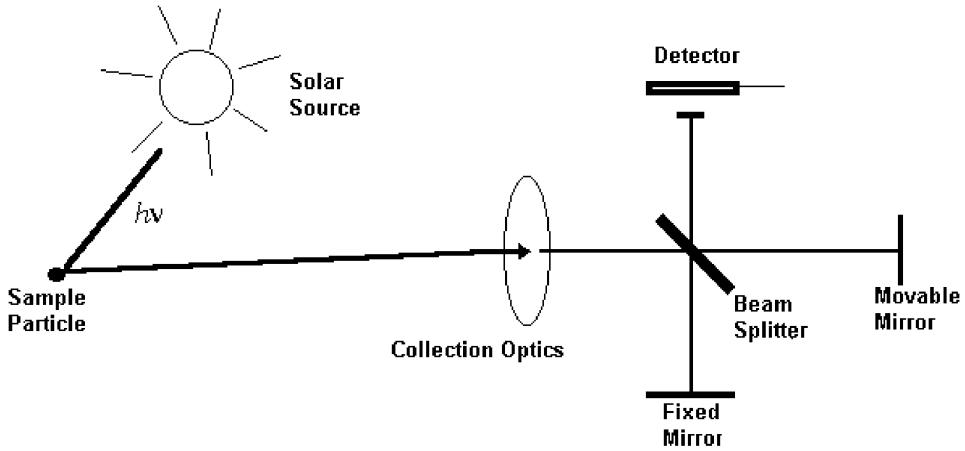


Fig. 1.4 Open-path sampling configuration using an interferometer.

Kirchoff's law states that for a thermal radiator at constant temperature and in thermal equilibrium, the emissivity (e) at any given frequency is equal to the absorptance (a) for radiation from the same direction; so $e = a$, where *absorptance* is defined as the ratio of energy absorbed by the surface to that of incident energy striking the surface.

An ideal surface absorbing all the energy that strikes its surface could be termed a *black body* (no radiation is reflected or emitted, thus it appears perfectly black at all frequencies). It would also stand from Kirchoff's law that this black body would also be the ideal emitter of radiation.

The *emissivity* (ε) for any black body source is defined as the ratio of the emitted radiance (ρ) by the source to the radiance of a black body (BB) at the same temperature and frequency (wavelength). The following equation illustrates this:

$$\varepsilon = \frac{\rho}{\rho_{\mathrm{BB}}}$$

The spectral radiance (P) is defined as the radiant power (or photon flux density) emitted per unit source area per unit solid angle (the conventional units for expressing this are watts/m^2/steradians).

Max Planck derived the principles of quantum mechanics and expressed this using the concept of light quanta as discrete energy "packets" that are transferred with an energy (E) proportional to the frequency of the electromagnetic radiation involved. Thus *Planck's law* has become one of the most widely known concepts in the physics of electromagnetic radiation. The expression is given as:

$$E = h\nu$$

where E = the energy content of each light quantum, and Planck's constant (h) = 6.63 . . . $\times 10^{-34}$ joule-sec.

From Planck's work, the *Planck's radiation formula* is used to calculate the radiance emitted by from a black body at a given wavelength (λ), emissivity (e), and temperature (T) as:

$$\rho_{BB} = 3.745 \times 10^4 \lambda^{-5} \left(\frac{1.4388 \times 10^4}{e^{\lambda T} - 1} \right)$$

where the units for ρ_{BB} are watts/cm^2/μm.

From Planck's radiation formula other relationships for a black body spectrum can be derived. The *Stefan Boltzmann law* gives the total radiance emitted (ρ_{BB}) from a black body as a function of the black body temperature:

$$\rho_{BB} = \sigma \times T^4$$

where σ = Stefan's constant = 5.67×10^{-8} watts/m^2 °K^4. *Wien's displacement law* demonstrates the shift (to shorter wavelengths) of the maximum peak position for a black body spectrum as a function of temperature:

$$\lambda_{\max} = \frac{2898\mu \times {}^{\circ}\mathrm{K}}{T}$$

where $\lambda_{\max}$ is the peak maximum position from the black body spectrum in microns (μ) and T is in °K. Note: T(°K) = T(°C) + 273.

Detectors

There are two basic types of photon detectors: photoemissive and solid state. The photoemissive type is generally represented by the photomultiplier tube detectors; the solid-state type is represented by photodiode detectors, pyroelectric detectors, and infrared detectors.

In defining the physics of detector devices, several terms deserve explanation. First, the term *specific detectivity* (or D^*) is essential. This *D-star* value is defined as the detectivity of a radiation detector as a function of the square root of the product of the active detector element

area (A) and the bandwidth (ω, in cycles per second), divided by the noise equivalent power (NEP) of the detector element. D* is reported in cm. $Hz^{1/2}$ $watts^{-1}$ units.

$$D^* = \frac{\sqrt{A \times \omega}}{\text{NEP}}$$

Other concepts important to the discussion of detectors include: *spectral sensitivity, radiant sensitivity, detectivity, dark current,* and *quantum efficiency.*

Filters

Two basic types of interference filters exist: *bandpass filters* and *edge filters.* Bandpass filters transmit light for only a defined spectral band. The transmitted spectral bands may be from less than 1 nm FWHM (**f**ull band**w**idth at **h**alf **m**aximum transmission band height) to 50 nm or more FWHM. Edge filters transmit light either above or below a certain wavelength region; these are referred to as "cut-on" and "cut-off" types, respectively. These filters transmit efficiently throughout a broad region until the transmission limit of the filter substrate material is reached.

Interference filters consist of a solid Fabry–Perot cavity. This is a device made of a sandwich of two partially reflective metallic layers separated by a transparent dielectric spacer layer. The partially reflective layers are of higher refractive index than the dielectric spacer layer and are $\lambda/4$ in thickness, where λ is the peak wavelength (wavelength of maximum transmission) for the filter. The lower-refractive-index spacer layer is made to $\lambda/2$ thickness. The thickness of the dielectric spacer layer determines the actual peak transmission wavelength for the filter. Only the $\lambda/2$ light transmits with high efficiency; the other wavelengths experience constructive interference between the multiple-order reflections from the two partially reflective layers.

The wavelength position of the transmittance peak (λ_t) through either a Fabry–Perot interferometer or a bandpass interference filter is given as:

$$\lambda_t = \frac{2 \times n_\varepsilon t_\sigma \cos\alpha}{o}$$

where λ_t = the wavelength of maximum transmittance for the filter, n_ε = the refractive index of the surrounding medium (air = 1.0003), t_σ = the thickness of the dielectric spacer in microns, sometimes referred to as the *effective refractive index*; α = the angle of incidence of the light impinging onto the dielectric spacer; o = the order number for the interference (a nonzero integer as 0, 1, 2 . . .).

The assumption for all interference filters is that incident energy striking the filter is collimated and at a normal incidence. The wavelength of peak transmittance for the filter can be moved by varying the angle of incidence (α) of light impinging upon the surface of the filter. The relationship defining the peak position of the maximum transmission is given by:

$$\lambda_{new} = \lambda_{max}\sqrt{1-\left(\frac{n_\varepsilon}{n_\sigma}\right)\sin^2\alpha}$$

where λ_{New} = the wavelength of the new peak transmission position at incident angle α (when the incident angle, α, is nonnormal $\geq 0°$), λ_{Max} = the wavelength of the current peak maximum for the interference filter with impinging light at an incident angle $\alpha = 0°$, n_ε = the refractive index of the surrounding medium (air = 1.0003), n_σ = the refractive index of the dielectric spacer, sometimes referred to as the *effective refractive index,* and α = the angle of incidence of the light impinging onto the filter surface.

Gratings

When incident light strikes a diffraction grating, the light is separated into its component wavelengths, with each wavelength scattered at a different angle. To calculate the particular angle

(θ_d) at which each wavelength of light (λ_a) is scattered from a diffraction grating, the following expression is used:

$$\theta_d = \sin^{-1}\left(\frac{o \times \lambda_a}{s}\right)$$

where θ_d = the angle of diffracted light from the normal angle, o = the order number (integers as 1, 2, 3, etc.), s = the spacing of the lines on the grating (in the same units as wavelength), λ_a = the wavelength of the incident light in air (for white light it represents the wavelength of interest for calculation of the diffraction angle).

The dispersion of a grating refers to how broadly the monochromator disperses (or spreads) the light spectrum at the sample specimen position. Dispersion is generally expressed in units of nm per mm. Dispersion depends upon the groove density (number of grooves per mm) of the grating.

The intensity distribution of light (I) from the surface of a diffraction grating is given by:

$$I = \left[\left(\frac{I_0}{S^2}\right)\left(\frac{\sin b}{b}\right)\left(\frac{\sin S \times a}{\sin a}\right)\right]$$

where I = the intensity from the surface, I_0 = the incident energy intensity, S = the number of slits passed by the light following diffraction, $\mathrm{b} = \frac{\pi \times s}{\lambda_a}$, s = the spacing of the lines on the grating (in the same units as wavelength), λ_a = the wavelength of the incident light in air (for white light it represents the wavelength of interest for calculation of the diffraction angle).

$$a = \frac{\pi \times \sigma}{\lambda_a}$$

where σ is the spacing of the slits (in the same units as wavelength) and λ_a = the wavelength of the incident light in air (for white light it represents the wavelength of interest for calculation of the diffraction angle).

The resolution (P) of a diffraction grating is given by the following expression:

$$P = oN = \frac{\lambda}{\Delta\lambda}$$

where o = the order of the interference pattern, N = the total number of lines on the diffraction grating surface, λ = the wavelength at which the resolution is determined, $\Delta\lambda$ = the distance in wavelength between the lines or optical phenomena to be resolved.

Beam splitters

Beam splitters are optical devices used to divide and recombine an optical beam (or beams) of light. They can be produced using half-silvered mirrors, which reflect approximately 50% of the energy incident to them; the remaining 50% is transmitted through the beam splitter.

Prisms

Prisms can be used either to disperse light into its spectral components or as right-angle prisms with reflective coatings on the hypotenuse side to bend light at a 90° angle. The dispersive properties of prisms have been known since the late 17th century. The deflection or dispersion of a prism is described using *Snell's law* at each optical surface of the prism, taking into account the refractive index of the prism at each wavelength. Snell's law describes the change in the path of light crossing an interface between two different materials (with different refractive indices) when the incident angle of the first surface is other than 90°. The wavefronts or wave propagation angle through the interface must move toward the normal angle as shown in Figure 1.5.

The numerical explanation of Snell's law is given by:

$$n_1 \sin \alpha_1 = n_2 \sin \alpha_2$$

where n_1 = the refractive index of the first medium at the interface of two materials of varying refractive indices, n_2 = the refractive index of the second medium at the interface of two materials of varying refractive indices, α_1 = the angle of incidence and reflection of the impinging light onto the surface of the second medium, and α_2 = the angle of refraction of the light passing through the interface of two materials of varying refractive indices.

For an equilateral (dispersive) prism, the *wave propagation angle* (δ) (shown in Figure 1.6) through the prism is given by:

$$\delta = \alpha_1 + \sin^{-1} [(\sin \theta) (n^2 - \sin^2\alpha_1)^{\frac{1}{2}} - (\sin\alpha_1 \cos\theta] - \theta$$

where α_1 = the angle incident to the surface of the prism, θ = the wedge angle of the prism (60° for an equilateral triangle), n = the refractive index of the prism at the frequency (wavelength) of incident energy, and δ = the wave propagation angle (angle of refraction) through the prism.

Interferometer Assemblies

Multiple interferometer types exist in modern spectrometers, the reader is referred to Candler (1951), Jamieson (1963), Steel (1983), and Strobel (1989) for a more exhaustive description of

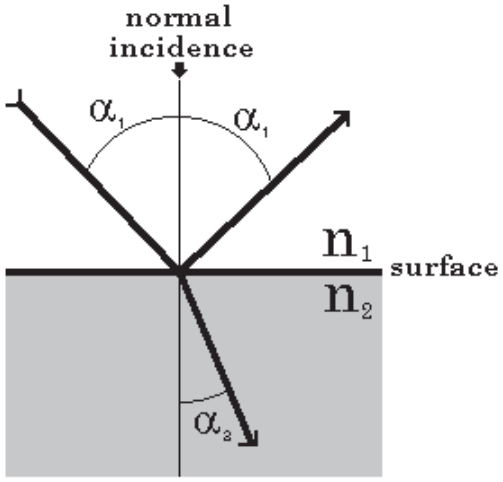


Fig. 1.5 Demonstration of Snell's law showing the light wave propagation angles of reflection and refraction at the interface between materials of differing refractive indices n_1 and n_2.

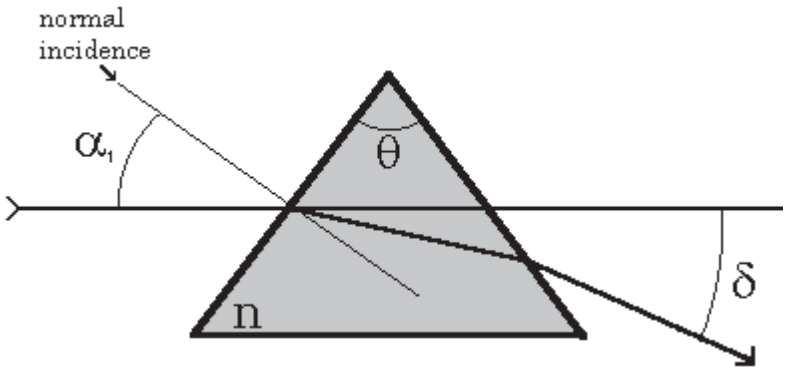


Fig. 1.6 Wave propagation angles through an equilateral prism for light dispersion.

the optical configurations for these devices. The classical interferometer design is represented by the Michelson interferometer, as shown in Figure 1.3. A movable mirror (MM) is displaced linearly minute distances to yield an interference pattern (or interferogram) as a series of sine waves when the interference pattern is observed from a specific field of view Figure 1.7a).

The interferogram is plotted as the intensity of light (*y*-axis) versus the mirror position (*x*-axis); thus the signal from the interferometer is a function of time (because the mirror is in motion at a constant velocity (Figure 1.7b). The raw interferogram, as it is sometimes termed, is converted to a spectrum using the Fourier transform, and a spectrum is determined by ratioing a spectrum determined with a sample in the beam (as the sample spectrum) to a spectrum determined with no sample in the beam (as the background spectrum).

A laser beam of known frequency is used to signal a separate sensor to provide near-perfect sampling of the mirror position. The resolution of the interferometer depends upon the distance of motion in the movable mirror. The precise distance traversed by the movable mirror can be determined using the number of fringes observed to pass a given field of view in time (*t*), given the wavelength of light from the laser. The distance traversed by the mirror is given as

$$d = \frac{f\lambda}{2}$$

where d = the distance traveled, f = the number of fringes in an interference pattern passing a specified field of view, and λ = the wavelength of laser light used.

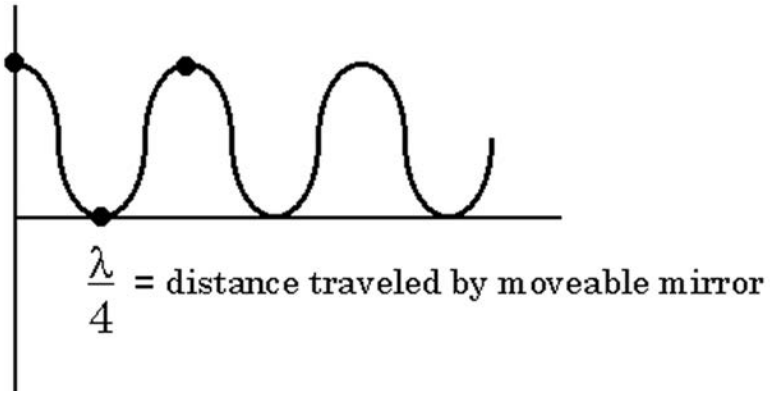


Fig. 1.7a Interferometric output as a sine wave function.

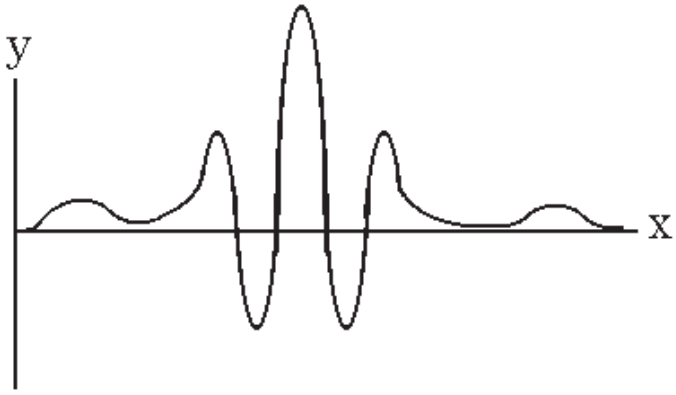


Fig. 1.7b The interferogram is plotted as a function of the light intensity (*y*-axis) versus the mirror position (*x*-axis); thus the signal is a function of time (because the mirror is moved at a constant rate). The raw interferogram is subjected to the multiple-step Fourier transformation, and a spectrum results.

High resolution involves moving the mirror at integral cycles over greater distances per unit time as compared to low-resolution measurements. A complete explanation of the physics of interferometry is beyond the scope of this chapter. The reader is referred to Griffiths (1986).

Polarizers

A diversity of polarizing elements exists for the purpose of rotating or selecting light of a specific electronic vector orientation. When the electronic direction vector of light incident to a surface is parallel to the electronic field vector of the surface, increased interaction of the incident light (absorption) occurs. This principle is important in characterizing the surface chemistry for optical components, thin films, metal surfaces, and semiconductor interfaces. The use of a quartz plate can act as a polarizer that will rotate the plane of linearly polarized light. This rotation (P) can be described using the following relationship:

$$\mathrm{P} = \left[\pi t\left(\left|n_{\Lambda} - n_{P}\right|\right)\right] \times \frac{1}{\lambda_0}$$

where t = the thickness of a quartz plate cut perpendicularly to the optical axis, n_{Λ} = the refractive index for right circularly polarized light, n_P = the index of refraction for left circularly polarized light, and λ_0 = the vacuum wavelength of light entering the quartz plate. *Note:* for quartz, $n_{\Lambda} = 1.5582$ and $n_P = 1.5581$.

Electronic Components Used in Spectrometry

For references describing the electronic components of optical spectrometers, see Braun (1987), Jamieson (1963), and Strobel (1989). Two special categories of electronic devices deserve representation when discussing spectrometers. The first category is detectors and detector electronics that produce a current of voltage signal proportional to the photon flux striking the detector. Detector stability is provided by correct electronic circuitry that allows the detector signal to be selectively amplified with the minimum introduction of noise; thus electronic circuitry enhances the signal-to-noise ratio of the detector signal.

Digital microcomputers comprise the second essential electronic element for modern spectrometers. With the addition of appropriate software, sophisticated instrument control and data processing can enhance the usefulness and user friendliness of spectrometers. These issues are described in detail in Jamieson (1963) and Strobel (1989).

PROPERTIES OF SPECTROMETERS

Aperture Diameter

To calculate the aperture (a) required by an optical system to resolve two objects with known linear separation, the Rayleigh criterion for resolution is used:

$$\mathrm{a} = \frac{1.22 \times \lambda}{\alpha_R}$$

where α_R = the angle of separation from the measuring device exit aperture to the objects to be resolved (which is calculated as $\tan \alpha = \frac{\text{opp.}}{\text{adj.}}$, where α_R is expressed in radians), λ = the wavelength of light observed from the objects, and a = the aperture of the optic system.

Entrance and Exit Pupils

The *entrance pupil* refers to the size and location of the entrance aperture between the light source and the remainder of an optical system. The *exit pupil* refers to the size and location of the exit aperture within an optical train just prior to the detector.

Bandpass and Resolution

The terms *bandpass* and *resolution* are used to express the capability of a spectrometer to separate spectral bands or lines that are separated by some finite distance. For an instrument that disperses energy over a prespecified spectral region of the electromagnetic spectrum, the bandpass of a spectrometer is used to describe which portion of the spectrum can actually be isolated by the spectrometer in a "pure" wavelength form. The spectrometer bandpass is dependent upon the dispersion of the grating (see the earlier section on gratings) and the entrance and exit slit widths. An illustration is often given to elucidate the problem associated with measuring monochromatic light using conventional spectrometers. If the ideal spectrometer were used to measure a bright-line emission spectrum at a single wavelength (λ_1), the spectrum would appear as a single line (Figure 1.8). What really occurs when such a spectrum is measured using a conventional spectrometer is a broad band spectrum, as shown in Figure 1.9. The spectrum assumes a Gaussian-like (or bell-shaped) curve. This characteristic broadening of a line spectrum through the spectrometer is an illustration of the spectrometer bandpass. The actual bandpass for any instruments assigned a value by determining the full width at half maximum (FWHM) height of the bell-shaped spectrum. Thus for the band in Figure 1.9 the FWHM could be empirically determined by finding the wavelength where maximum intensity occurs and measuring the peak height at this position. This height measurement is divided in half and the bandwidth measured at this height on the band, as illustrated in Figure 1.10.

The actual shape of a band is the result of several instrumental characteristics, including the overall quality of the optics and detector systems as well as the width and positions of the entrance and exit slits. Every dispersive spectrometer consists of a dispersive element (e.g., diffraction grating) in combination with an entrance and an exit slit. The image of the entrance slit and exit slit determines the spectrometer bandpass, which is sometimes referred to as the *slit function*. Actually the slit function is the result of the convolution (combination) of the images of these two slits. The bandshape of a dispersive spectrometer is shown in Figures 1.9 and 1.10. Other factors associated with optical and electronic quality cause a rounded overall shape. The bandpass of a spectrometer is equal to the FWHM. Often texts dealing with instrumentation will state that the bandpass of a spectrometer is approximated by the product of the linear dispersion of the monochromator and the entrance or exit slit width (whichever is larger).

The resolution of a spectrometer can be defined as the minimum distance between two peaks that can be detected by the spectrometer under designated operational performance settings. Resolution is calculated by multiplying the slit width (generally expressed in mm) by the dispersion of the monochromator (in nm per mm). Due to practical issues and nonideal optics, the actual resolution of a spectrometer must be slightly greater (poorer) than the theoretical value.

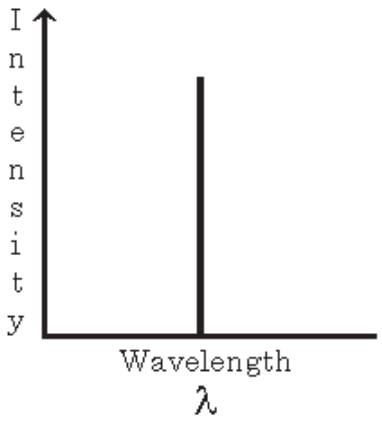


Fig. 1.8 Bright-line emission spectrum at a single wavelength as it would appear in an ideal spectrophotometer.

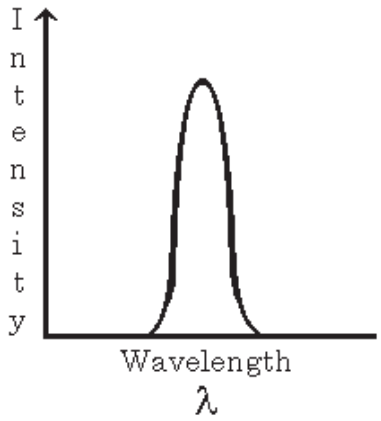


Fig. 1.9 Spectrum of a bright-line emission source (e.g., deuterium lamp). The characteristic broadening is an illustration of the bandpass of a spectrophotometer.

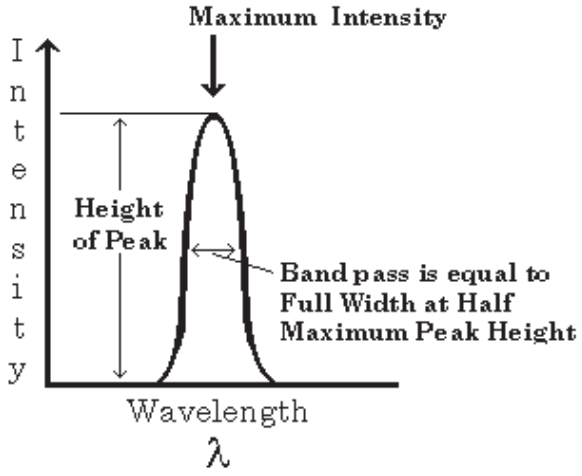


Fig. 1.10 Illustration of the determination of bandpass using the bell-shaped peak obtained by using a bright-line source projected through a monochromator optical system.

To summarize, bandpass and resolution are identical in practice. Only the resolution specification of a spectrometer is the expression of bandpass under the specified measuring conditions of an instrument dependent upon the slit width settings.

The empirical resolution of a spectrometer is determined by measuring the FWHM in mm for two narrow bands that are completely resolved (to the baseline) using the spectrometer. The spatial difference between the maximum absorbance (lambda max.) is determined between the bands (in mm), simultaneously noting the difference between the lambda max. points in nm. The various measurements required for this calculation are shown in Figure 1.11 and illustrated by the following relationship:

$$\text{Bandpass} = \text{Resolution} = \frac{\text{Band difference in nm}}{\text{Band difference in mm}} \times \text{FWHM in mm}$$

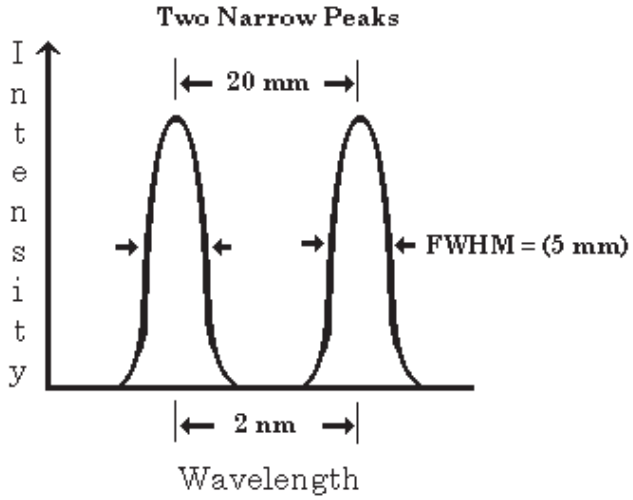


Fig. 1.11 Method to determine maximum resolution of a spectrometer under specific measurement conditions. In the example shown, Bandpass = Resolution = FWHM × Dispersion = (2 nm) / (20 mm) × 5 mm = 0.50 nm resolution.

Numerical Aperture

The numerical aperture (NA) is a measure of how much light can be collected by any optical system. The NA is expressed as the product of the refractive index of the incident material (n_i) and the sine of the ray angle maximum (θ_{Max}) from normal incidence; the function is given by

$$NA = n_i \times \sin\theta_{Max}$$

Attenuation (Light Transmittance Losses in Optical Systems)

Losses in transmitted light through spectrometers are due to absorption, reflection, scattering, and optical misalignment; the losses can vary with temperature and wavelength. The quantity of optical loss is expressed as an attenuation rate in decibels (dB) of optical power per unit distance (cm). Typical losses result from launch optics, temperature variations, optical couplings within the optical path, aging of mirrored surfaces, and soiled optical surfaces. The losses in energy transmitted through a spectrometer can be calculated by using Beer's law. Beer's law states that the irradiance of energy through an absorbing medium falls exponentially with the distance of transmission according to the following relationship:

$$I_d = I_0 \times 10^{(\alpha d/10)}$$

where I_d = the irradiance at distance (d) from the source, I_0 = the source irradiance at $d = 0$, α = the attenuation (absorption) coefficient in units of dB/cm, and d = distance in cm.

Attenuation losses are wavelength dependent; thus the value for α is a function of the incident wavelength (λ).

Etendue

The etendue (or relative throughput advantage) for an optical system is the product of the potential illuminated surface area (A) and the solid angle of the optic. Traditionally, this is represented by the two following equations, where ε' represents the etendue, and Ω_S represents the solid angle. Thus the etendue is represented as

$$\varepsilon' = A \times \Omega_S$$

and the solid angle is given by

$$\Omega_S = 2\pi\left(1 - \sqrt{1 - \left(\text{NA}\right)^2}\right)$$

Therefore these two equations allow us to calculate the relative improvement for an optical spectrometer optical system. As can be seen, the NA and aperture diameter are preeminent factors for throughput in optical systems, as shown in Table 1.1.

Throughput

The relative throughput (T) represents the overall effectiveness of an optical system to transmit light relative to the amount of light introduced into the system (I_0) from the light source. It is defined as the ratio of light energy passing into an optical system to the light energy passing out of the optical system. For dispersive spectrometers, this relationship is defined by

$$T = \frac{\pi D w_s \left(R_1 \times R_2 \times \cdots \times R_k\right)\left(\Sigma_g\right)}{4f^2}$$

where D = the dispersion constant in mm/nm, w_s = the slit width in mm (or exit slit), f = the f/number of the optical system (f/number = $\frac{1}{2(\text{NA})}$), R_k = the reflectivity of mirrors or other optical surfaces, Σ_g = the spectral efficiency of the grating (approximately 0.80 at the blaze wavelength).

Signal-to-Noise Ratio

The theoretical total signal (S) from an optical system can be given by:

$$S = R_S B_\lambda \varepsilon' \tau q$$

where R_s = the light source spectral radiance, B_λ = the spectral bandwidth, ε' = the etendue of the spectrometer optical system, τ = transmission losses (and emissivity), and q = quantum efficiency.

The measured signal-to-noise ratio (s/n) from an optical system can be calculated as the full transmitted signal divided by the RMS noise (in transmittance units). Thus for a 100% line with RMS noise as 0.001%T, s/n = 100/0.01 = 10,000 : 1. This applies when RMS noise is calculated as

$$\text{RMS} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(T_i - \overline{T}\right)^2}$$

where T_i = the individual transmission value at each data channel i and T = the mean transmission value for all data channels.

Table 1.1 Etendue and Relative Throughput as a Function of Numerical Aperture (NA)

NA (Numerical Aperture)	Relative Etendue (ε′) for 1-mm-diameter aperture	Relative Throughput
0.20	0.10	1
0.40	0.40	4
0.60	1.00	10

Dynamic Range

The range of a specified analyte concentration over which a sensor response is directly proportional to a change in concentration is the dynamic range of a spectrometer. Dynamic range is limited by stray light and noise. Knowledge of Beer's law (previously discussed) allows us to calculate the maximum theoretical dynamic range for an instrument using a few simple mathematical relationships and a calculation of the relationship to stray light and maximum observable absorbance value, given by the following. (*Note:* The error in a measurement due to stray light can be computed using this expression.)

$$A_i = \log\left(\frac{1+\frac{S_l}{I_0}}{\frac{I}{I_0}+\frac{S_l}{I_0}}\right) = \log\left(\frac{100+I_s}{T+I_s}\right)$$

where I_0 = incident light intensity, I = transmitted light intensity, S_l = stray light intensity, I_s = stray light as a percentage of I_0, and T = percent transmittance of the measurement under test.

Error in a measurement is also attributable to the combined noise of the measurement system, given as:

$$\text{Noise (as \%)} = \frac{\text{RMS}\times 100}{A}$$

The relative dynamic range of a spectrometer is written as:

$$A_{DR} = \log\left(\frac{100+I_s}{I_s}\right) - k\left(\text{RMS}_A\right)$$

where k = multiplier for desired confidence level and RMS_A = root mean square noise measurement (in A). *Note:* To simplify calculations, the $-k$(RMS) term can be dropped, yielding an estimated value for dynamic range.

Stray Radiant Energy

The relationship between absorbance and stray light is given by:

$$A_i = \log\left(\frac{1+\frac{S_l}{I_0}}{\frac{I}{I_0}+\frac{S_l}{I_0}}\right) = \log\left(\frac{100+I_s}{T+I_s}\right)$$

This calculation relationship in absorbance units applies where I_0 = incident light intensity, I = transmitted light intensity, S_l = stray light intensity (as a fraction of I_0), I_s = stray light (as fraction of I_0), and T = percentage of transmittance. The relationship between transmittance (T) and Absorbance (A) is given in Table 1.2.

Table 1.2 Relationships between %*T*, *T*, and *A*

% Transmittance	Transmittance	Absorbance
100.0	1.0	0.0
10.0	0.1	1.0
1.0	0.01	2.0
0.1	0.001	3.0
0.01	0.0001	4.0
0.001	0.00001	5.0
0.0001	0.000001	6.0

The calculation of percentage error in a measurement due to stray radiant energy is given by

$$E\,(\%) = 100\left[1 - \left(\frac{\log\left(\frac{100 + I_S}{T + I_S}\right)}{A_t}\right)\right]$$

where I_s = stray light as a percentage of I_0, T = percentage transmittance of measurement, and A_t = true absorbance level of sample specimen measured.

Wavelength Accuracy

The accuracy in wavelength measurements is determined by taking a standard reference material (or emission line spectrum) of known wavelength position (λ_k) and making measurements of these known positions using the spectrometer. The difference between the known position(s) and the positions as measured using the spectrometer (λ_s) is reported as the wavelength accuracy of the spectrometer, expressed as ($\lambda_k - \lambda_s$).

Wavelength Repeatability

Wavelength repeatability (λ_r) is the precision with which a spectrometer can make repeated measurements at the same nominal wavelength over temporal and environmental changes. This specification is calculated as

$$\lambda_r = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\lambda_i - \bar{\lambda}\right)^2}$$

where λ_i = wavelength determined at each of multiple measurements taken n times and $\bar{\lambda}$ = mean wavelength determined using each of the multiple measurements taken n times.

Photometric Accuracy

The accuracy in photometric measurements is determined by taking a standard reference material of known transmission (T_k) values and making measurements at specific wavelengths of these known photometric values using the spectrometer. The difference between the known transmission and the transmission as measured using the spectrometer (T_s) is reported as the photometric accuracy of the spectrometer, expressed as ($T_k - T_s$).

Photometric Repeatability

Photometric repeatability (T_r) is the precision with which a spectrometer can make repeated measurements at the same nominal transmission value over temporal and environmental changes. This specification is calculated as

$$T_r = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(T_i - \bar{T}\right)^2}$$

where T_i = transmission determined at each of multiple measurements taken n times and $\bar{T}$= mean transmission determined using each of the multiple measurements taken n times.

REFERENCES

Blaker, J. W. (1970). *Optics II—Physical and Quantum Optics.* New York: Barnes and Noble, 91 pp.
Bracey, R. J. (1960). *The Techniques of Optical Instrument Design.* London: English Universities Press, 316 pp.
Braun, R. D. (1987). *Introduction to Instrumental Analysis.* New York: McGraw-Hill, 1004 pp.

Candler, C. (1951), *Modern Interferometers.* Glasgow: Hilger and Watts, 502 pp.
Ditchburn, R. W. (1965). *Light,* 2nd ed. London: Blackie, 833 pp.
Fogiel, M. (ed.) (1981). *The Optics Problem Solver.* New York: Research & Education Association, 817 pp.
Griffiths, P.R., de Haseth, J.A. (1986). *Fourier Transform Infrared Spectrometry.* New York: Wiley, pp. 1–55.
Jamieson, J. A., McFee, R. H., Plass, G. N., Grube, R. H., Richards, R. G. (1963). *Infrared Physics and Engineering.* New York: McGraw-Hill, 673 pp.
Steel, W. H. (1983). *Interferometry.* 2nd ed., Cambridge: Cambridge University Press, 308 pp.
Strobel, H. A., Heineman, W. R. (1989). *Chemical Instrumentation: A Systematic Approach,* 3rd ed. New York: Wiley, 1210 pp.
Wist, A. O., Meiksin, Z. H. (1986). *Electronic Design of Microprocessor-Based Instruments and Control Systems.* New York: Prentice-Hall, 287 pp.

FURTHER READING

Ball, C. J. (1971). *An Introduction to the Theory of Diffraction.* Oxford: Permagon Press, 134 pp.
Clark, G. L. (ed.) (1960). *The Encyclopedia of Spectroscopy.* New York: Reinhold, 787 pp.
Cohen, J. B. (1966). *Diffraction Methods in Material Science.* New York: Macmillan, 357 pp.
Guild, J. (1960). *Diffraction Gratings as Measurement Scales.* London: Oxford University Press, 211 pp.
Jenkins, F. A., White, H. E. (1957). *Fundamentals of Optics.* New York: McGraw-Hill, 637 pp.
Johnson, Jr., C. S., Pedersen, L. G. (1986). *Problems and Solutions in Quantum Chemistry and Physics.* New York: Dover, 429 pp.
Knowles, A., Burgess, C. (eds.) (1984). *Practical Absorption Spectrometry.* New York: Chapman and Hall, 234 pp.
Mach, E. (1926). *The Principals of Physical Optics.* New York: Dover, 324 pp.
Smith, R. J. (1980). *Electronics: Circuits and Devices.* 2nd ed. New York: Wiley, 494 pp.

2.

SOURCES, DETECTORS, WINDOW MATERIALS, AND SAMPLE PREPARATION FOR UV-VIS, NIR, IR, AND RAMAN SPECTROSCOPY

Table 2.1 UV-Vis, NIR, IR Emission Sources.

Source type	Useful emission range (microns)	Useful emission range (cm^{-1})
Quartz tungsten-halogen monofilament lamp	0.22–2.7	45,455–3,704
DC deuterium lamp for UV	0.185–3.75	54,054–2,667
Pulsed xenon arc lamp	0.18–2.5	55,556–4,000
DC arc lamp	0.20–2.5	50,000–4,000
Globar	1.0–100	10,000–100
Nernst glower	0.30–35	33,333–286
Carbon arc	0.50–100	20,000–100
Mercury lamp	0.30–100	33,333–100
Visible and NIR lasers	Helium-neon (He:Ne) @ 632.8 nm and visible lasers at 768 nm	15,802.8 13,020.8
	Neodymium:yttrium aluminum garnet (Nd:YAG) @ 1064 nm or 532 nm, generally 0–3 W output power	9,398.5 18,797.0

Useful wavelength and frequency working ranges are given.

Table 2.2 UV-Vis, NIR, and IR Detectors.

Detector type	Useful detection range (microns)	Useful detection range (cm^{-1})
Silicon	0.30–1.1	33,333–9,091
PbS (Lead sulfide)	1.1–3.0	9,091–3,333
InAs (Indium arsenide)	1.7–5.7	5,882–1,754
InGaAs (Indium gallium arsenide)	0.90–1.7	11,111–5,882
Ge:X (Germanium)	2–40	5,000–250
Ge:Au (Germanium gold)	2–9	5,000–1,111
Ge:Cd (Germanium cadmium)	2–24	5,000–417
PbSe (Lead selenide)	1.7–5.5	5,882–1,818
Ge:Zn (Germanium zinc)	2–40	6,667–250
InSb (Indium antimonide)	1.8–6.8	5,556–1,471
PbTe (Lead telluride)	1.5–4.5	6,667–2,222
DTGS/KBr (Deuterated triglycine sulfate)	0.83–25	12,050–400
DTGS/PE (Deuterated triglycine sulfate)	10–120	1,000–83
MCT (Mercury cadmium telluride) or HgCdTe (photovoltaic)	1–17	10,000–588
TGS (Triglycine sulfate)	10–120	1,000–83
PLT (Pyroelectric lithium tantalate) ($LiTaO_3$)	1.5–30	6,667–333

Useful wavelength and frequency working ranges are given.

Table 2.3 UV-Vis, NIR, and IR Window Materials.

Window material	Useful transmittance range (microns)	Useful transmittance range (cm^{-1})
CsI (Cesium iodide)	0.3–50	33,333–200
PbS (Lead sulfide)	1.1–3.0	9,091–3,333
KBr (Potassium bromide)	0.25–26	40,000–385
KCl (Potassium chloride)	0.25–20	40,000–500
NaCl (Sodium chloride)	0.25–16	40,000–625
KRS-5 (Thallium Bromide-Iodide)	0.6–40	16,667–250
Ge (Germanium)	1.1–30	9,091–333
As_2S_3 (Arsenic sulfide)	0.6–15	16,667–667
MgF_2 (Magnesium fluoride) (IRTRAN-1)	0.6–9.5	16,667–1,053
ZnSe (Zinc selenide) (IRTRAN-4)	0.6–26	16,667–385
BaF_2 (Barium fluoride)	0.2–13	50,000–769
ZnS (Zinc sulfide) (Cleartran or IRTRAN-2)	0.6–15	16,667–667
CaF_2 (Calcium fluoride) (IRTRAN-3)	0.2–9	50,000–1,111
Al_2O_3, Aluminum oxide (Sapphire)	0.2–7	50,000–1,429
SiO_2 (Fused silica or quartz)	0.2–4	50,000–2,500
AgBr (Silver bromide)	0.5–35	20,000–286
Polyethylene (high density)	16–333	625–30

Useful wavelength and frequency working ranges are given.

Table 2.4 Fiber-Optic Materials

Fiber-optic material	Useful transmittance range (microns)	Useful transmittance range (cm^{-1})
SiO_2 (Fused silica or quartz)	0.2–1.25	50,000–8,000
SiO_2 (Anhydrous quartz)	0.4–2.64	25,000–3,788
ZrF (Zirconium fluoride)	0.9–4.76	11, 111–2,100
Chalcogenide	2.22–11.1	4,505–901

Useful wavelength and frequency working ranges are given.

SELECTION OF USEFUL MEASUREMENT PATHLENGTHS (BASED ON PURE HYDROCARBONS)

Fourth-overtone NIR, 650–800 nm, 10 cm
SW-NIR, 800–1100 nm, 5–10 cm
LW-NIR, 1050–3000 nm, 0.1–2 cm
MIR, 2500–20K nm, 0.1–4 mm
Raman, 2500–20K nm, N/A

MATRIX/MEASUREMENT TECHNIQUES OF CHOICE

Gases: long-path MIR (0.5–20 m)
Solids: diffuse reflectance, diffuse transmittance, HATR
Liquids: all techniques
Organics: all techniques
Wastewater: sparging MIR
Caustics: MIR-ATR
Optically dense materials/opaque materials: HATR

SOLVENTS FOR SPECTROSCOPIC MEASUREMENTS

UV-Vis Solvent Cut-off Wavelengths (in Nanometers)

Chloroform (235–240 nm)
Hexanes (195–202 nm)
Methanol and ethanol (205 nm)
Water (190 nm)
1,4-Dioxane (205 nm)
Acetonitrile (190 nm)

Solvents for Near-Infrared Spectroscopy

For liquid measurement in the NIR, solvents most nearly transparent from 1.0 to 3.0 microns include: carbon tetrachloride, tetrachloroethylene, carbon disulfide, and chlorofluorocarbons.

None of these materials can be considered safe from an environmental or health perspective, and thus they are not recommended. The only signal for any of the solvents above occurs at 2.21 microns for carbon disulfide (which should not be used at pathlengths above 1 cm. All of the preceding solvents are transparent, even to 10-cm pathlengths, over the NIR spectral region, with the only exception so noted. Solvents such as chloroform, methylene chloride, dioxane, di (*n*-butyl) ether, triethylene glycol (dimethyl ether), heptane, benzene, acetonitrile, dimethylformamide, and dimethyl sulfoxide have all been used as solvents for a portion of the NIR region, generally from 1.0 to 1.6 microns and from 1.8 to 2.2 microns. Silicone lubricants and Nujol (liquid paraffin) have also been suggested as solvents, meeting the requirements of nontoxicity, low cost, and availability. These materials, however, are far from ideal spectroscopically.

For solid samples, oven-dried (105°C for 2 hours) sodium chloride, potassium bromide, or potassium chloride can be used to dilute samples across the entire 1.0–3.0 micron range. *Note:* Diffuse reflectance is often used for near-infrared measurements of solid samples. The instruments can be easily configured for such measurements, and the requirements for optimum spectra include small sample size (preferably less than 50-micron-diameter mean particle size) and an infinite pathlength (generally 5–10 mm).

Solvents for Infrared Spectroscopy

Common solvents used for infrared analysis include chloroform, acetone, methanol, and hexane. Cast films are often made by dissolving or extracting a soluble solid or liquid sample in one of the above solvents, filtering the extract, evaporating most of the solvent in a suitable hood, and casting a thin film (by evaporation) onto an infrared-transparent window material. Chloroform has IR bands at 3020 (weak), 1215, and 755 cm^{-1} (strong). Acetone has several sharp IR bands near 3000 (weak), 1715 (strong), 1420 (weak), 1365 (strong), 1220 (strong), 1090, and 535 wavenumbers. Methanol has bands at 3650–2750 (three broad and strong bands), 1450 (medium broad), 1120, 1030 (strong), and 640 cm^{-1} (broad). Hexanes have bands at 2750–3000 (strong and sharp), 1445 (strong), 1250 (weak), 900 (weak), and 850 cm^{-1} (weak).

For solid samples, oven-dried (105°C for 2 hours) potassium chloride or potassium bromide can be used to dilute samples across the entire range from 40,000 to 385 cm^{-1}.

ANALYTICAL METHOD AND SAMPLE PREPARATION FOR INFRARED SPECTROSCOPY

Infrared spectra must be measured in such a manner as to reduce to negligible, or preferably to eliminate altogether, the carbon dioxide doublet at 2349 cm^{-1} and the multiple bands due to water vapor, indicated by multiple sharp bands centered near 3600 cm^{-1} and 1640 cm^{-1}. This can be accomplished either by means of extended sample compartment purge times using dry air or dry nitrogen as a purge gas or by carefully referencing out the background under identical reference and measurement ambient air conditions. *Note:* A background reference using air can serve to accommodate for small amounts of water vapor and carbon dioxide, but the resultant sample spectrum should not exhibit these bands as noticeable.

The spectrum of the unknown and the reference spectrum should in no case obtain a minimum percentage transmittance (%*T*) of less than 2% so as to provide structural detail of the most prominent peaks. If spectra exceed this limit (by exhibiting a %*T* of less than 2%), then a thinner film should be recast in the case of thin-film measurements, or the sample must be further diluted in the case of KBr pellet sample preparation. If these procedures are followed closely, an accurate comparison of the test sample spectrum and reference spectrum can be made.

Powders

Each sample is prepared by finely grinding a small quantity of each powder (~0.01 g) using a mortar and pestle and then adding several drops of mineral oil (Nujol) and continuing to grind

the sample until it becomes a white paste, something like cold cream. The paste is then evenly distributed between a pair of infrared windows (either KBr or NaCl) to form a thin translucent film. If the film is totally opaque, then the particle size of the powder is still too large and the material must be subjected to further grinding. Once the thin translucent film is formed between the windows, the transmission spectrum is measured. The maximum absorbance of the infrared spectrum for each sample must not exceed 1.2 Au. If this occurs, the windows must be further compressed (taking care not to break the windows) to decrease the pathlength between the windows until the absorbance is within range.

Pastes and Creams

Each sample is prepared by placing a small droplet of material onto an infrared window (either KBr or NaCl). The windows are gently pressed together to form a thin transparent film. Once the thin transparent film is formed between the windows, the transmission spectrum is measured. The maximum absorbance of the infrared spectrum for each sample must not exceed 1.2 Au. If this occurs, the windows must be further compressed (taking care not to break the windows) to decrease the pathlength between the windows until the absorbance is within range.

Solvent-Soluble Materials

Each sample is prepared by weighing approximately 0.05 g of sample into a 50-mL beaker and dissolving it with 20 mL chloroform on a hot plate (~60°C). Once dissolved, indicated by a clear solution with no solid present (~5–10 minutes), a few drops of the solution are added to an infrared window (KRS-5, KBr or NaCl) and a slight film of the material is cast onto the window. Once the thin translucent film is formed on the window, the transmission spectrum is measured. The maximum absorbance of the infrared spectrum for each sample must not exceed 1.2 Au. If this occurs, a drop of chloroform is added to the window to disperse the film further until the absorbance is within range.

Liquids

A few tiny dropletss of the solution are added to an infrared window (KRS-5, KBr or NaCl) and a slight film is cast onto the window. Once the thin translucent film of the material is formed on the window, the transmission spectrum is measured. The maximum absorbance of the infrared spectrum for each sample must not exceed 1.2 Au. If this occurs, a drop of chloroform is added to the window to disperse the wax further until the absorbance is within range.

SOLVENTS FOR RAMAN SPECTROSCOPY

Most organic solvents have some spectral signature using Raman spectroscopy. Water and alcohols are low-scattering materials for Raman emission spectroscopy and thus are useful as solvents. Glass and silica are also useful as containers for measuring solids or neat liquids using Raman spectroscopy, for the Si–O–H is a poor scattering material and thus has little to no Raman signal intensity. Small glass NMR sample vials are often used for Raman measurements and are especially serviceable because they are considered disposable.

II.

SAMPLE IDENTIFICATION AND SPECIALIZED MEASUREMENT TECHNIQUES

3.

INFRARED MICROSPECTROSCOPY

The IR microscope can be used for many samples typically measured using the IR macro bench. It can be faster and easier than using the benchtop system for routine samples due to the lesser requirements for sample preparation. The only restrictions for microscope use is the size and hardness of the sample. Most samples are compatible for measurement using the microscope. A variety of objectives are available for IR measurements using transmittance, diffuse and specular reflectance, reflectance-absorption, ATR (attenuated total reflectance), and grazing angle. Visible objectives are used for finding and aligning the sample into the proper position for measurement. A description of microscope terms and procedures is outlined next. Microscopic measurements are more optimized by purging the measurement area using dry nitrogen to eliminate absorption bands due to carbon dioxide and water vapor. A plastic ring is often employed between the microscope objective and sample stage for purging. Purge gases can also blow the sample out of the field of view.

ALIGNMENT

Alignment is the procedure used to maximize energy throughput and thus to increase the signal-to-noise ratio of the microscope. The noise is measured and recorded over time to ensure optimal microscope performance.

Alignment Procedure for Microscope Optics for Use in Reflectance Measurements

A gold-coated mirror is generally used for microscope alignment in the reflectance mode. Use the upper source (reflectance configuration on single-illumination systems) to focus a bright image on the gold surface. Perform standard noise-performance measurement as outlined next.

Alignment Procedure for Microscope Optics for Use in Transmission Measurements

The alignment procedure for IR microscopes follows three main steps and uses the optical survey mode of the microscope.

1. Focus the objective onto the sample (generally a 100-micron pinhole) by moving the stage in the *x-, y-,* and *z*-directions to adjust the sample to the focal spot of the objective. This step is performed with upper illumination (reflectance configuration on single-illumination systems)

and with the upper aperture removed. With proper alignment the pinhole should be exactly on center and appear as a dark spot.

2. Focus the condenser to the focal point of the sample. This step is performed using the lower source (transmittance configuration on single-illumination systems) and with the lower aperture present. No sample or pinhole should be present for this step. Move the condenser focus knob in the *z*-direction until the brightest possible spot is observed. The condenser thumbscrews are then used to complete the *x*- and *y*-adjustments to exactly center the bright spot.
3. Position both the upper and lower apertures in place and align the condenser image on the center focal point of the objective. This step is performed using the 100-micron pinhole in exact center position with both the upper and lower light sources (transmittance configuration on single-illumination systems). Once completed, this configuration should provide the maximum energy throughput for the microscope optics and thus provide the highest signal-to-noise ratio.

APERTURE

The aperture is the opening within the microscope optics that is responsible for producing an optical image and for specifying the area of the sample to be measured using transmittance or reflectance. The aperture is generally made of aluminum or steel coated with carbon black to absorb extraneous IR light. Coated-glass apertures are available to allow visual observation of a sample without allowing IR energy to pass the area around the opening. The size of the aperture should be compatible with the image size generated by the specific objective and condenser optics. Table 3.1 presents the objective or condenser magnification and the corresponding compatible aperture.

Table 3.1 Objective/Condenser Magnification and Compatible Aperture.

Objective/condenser magnification	Compatible aperture
10 times	1000 microns (1.0 mm)
15 times	1500 microns (1.5 mm)
20 times	2000 microns (2.0 mm)
25 times (some ATR)	2500 microns (2.5 mm)
30 times (some grazing angle)	3000 microns (3.0 mm)
32 times	3200 microns (3.2 mm)

COMPENSATION RING

The compensation ring is a focusing mechanism for both the objective and condenser optics that is used to adjust for the refraction of light caused by a specific window thickness over or under the sample. If a window is used over the sample, the compensation ring of the objective is used to adjust for the refraction of light. If a window is placed under the sample, the compensation ring of the condenser is used to adjust for the refraction of light. To calculate the compensation setting required for each window thickness and material, use the following relationship:

$$\text{Compensation setting} = \text{Thickness of window (in mm)} \times \frac{\text{Refractive index of window}}{1.5}$$

COMPRESSION CELL

The compression cell allows two sample windows with flat surfaces to be squeezed together for the flattening of samples to make them transparent or to flatten them for easier transmittance measurement. KBr and diamond are common window materials.

DICHROISM

Dichroism is the use of infrared polarized light (as p- and s-polarized light) to measure the molecular orientation of crystalline polymers and other highly oriented molecules. For microscope work, the requirements are (1) the capability to generate p- and s-polarized infrared energy, and (2) the capability of minimizing the stray radiant energy (stray light) by using redundant apertures (see earlier discussion of this term). The difference between a spectrum of a material taken with p-polarized light ($A_{||}$) and the spectrum of a material illuminated with s-polarized light ($A_{\perp}$) can reveal molecular orientation sensitivities of the measured molecules. When the electronic field vector of the sample molecules is perpendicular to the field direction vector of the infrared energy there is very little interaction and slight or no absorption of the light. When the electronic field vector of the sample molecules is parallel to the field direction vector of the infrared energy there is a large interaction and significant absorption of the light. One can measure the effect of stretching on molecular orientation by making dichroic measurements before and after stretching the sample. The dichroic ratio in (arbitrary units) can be measured from +1.0 to –1.0 by using the following ratio:

$$\text{Dichroic ratio} = \frac{A_{\text{II}} - A_{\perp}}{A_{\text{II}} + A_{\perp}}$$

GAIN SETTING OF DETECTOR

The gain is the voltage amplification of a detector (above a nominal voltage setting) that has the effect of increasing the signal strength of the detector in a linear fashion over the linear range of a detector. For example, if the signal-to-noise ratio of a measurement A is 50% of a previous measurement B, moving the gain setting to 2 for this more difficult measurement A will yield the same voltage at the detector as measurement B. This gain enhancement will provide increased detector sensitivity for measurements over a limited linear detection range.

LIGHT SOURCES

Microscopes are generally provided with an upper light source and a lower light source. The upper source is used to view the objective image for alignment or for the reflectance measurement mode. The lower source is used to view the condenser image for alignment or for the transmittance mode measurement. Some microscopes have a single visible light source. For aligning this microscope configuration, the reflectance mode is used for objective focusing and the transmittance mode is used for condenser focusing.

MOTORIZED STAGE

The motorized stage allows the microscope to “memorize” the point position (or positions) on a sample and to find a position reproducibly.

NOISE PERFORMANCE MEASUREMENT

The optical performance of the microscope is evaluated by completing the alignment procedure to optimize energy throughput and the signal-to-noise ratio. Once optimized, the noise of the microscope system is recorded for later performance comparisons. The noise and spectrum are recorded over time to verify continuous optical performance of the system. The measurement parameters should be measured in exactly the same manner for each measurement. The number of scans, resolution, apodization function, data format, and frequency range should always be adjusted to the same parameter setup each time a noise measurement is made. The noise should be recorded as the peak-to-peak (maximum percentage transmittance minus minimum percentage transmittance) over a prespecified frequency region of the spectrum. Generally, peak-to-peak noise should be less than 0.1 %*T* for most IR microscopes. *Note:* Peak-to-peak noise should approximately double when moving from benchtop IR transmittance measurements to microscope IR transmittance measurements. The peak-to-peak noise factor should increase approximately four times when moving from the benchtop IR transmittance measurements to microscope IR reflectance measurements.

OBJECTIVE

The optical device taking the light from the microscope and focusing it onto the sample is called the objective. The higher the magnification, the lower the throughput. A 15× objective is a general-use objective. Higher magnification allows more specific sample position measurements, but reduces the energy throughput and signal-to-noise ratio. Types of objectives include standard, specular reflectance, ATR, and grazing-angle.

ATR Objective

ATR is used for surface analysis using physical contact with a sample surface. The objective utilizes a crystal of material for the actual physical contact with the sample. Typical crystal materials include diamond (Di), germanium (Ge), silicon (Si), and zinc selenide (ZnSe). ATR measurements can yield excellent-quality spectra provided that the contact pressures of the ATR crystal and the sample are held constant; reproducible data requires reproducible contact pressure. Commercial contact gauges are available from suppliers of microscope accessories. One percent reproducibility is typically achieved when keeping the contact pressure constant. ATR crystals are generally ZnSe with a refractive index of 2.42 and an angle of incidence of 45°. Sample penetration is around 40 microns using this ATR crystal. An air background is taken for ATR measurements. The depth of penetration is calculated for ATR crystals using the following relationship:

$$\text{Depth of penetration for ATR} = \frac{\text{Wavelength of incident light}}{2\pi \times n_1(\sin^2 \alpha - n_2^2)^{1/2}}$$

Where n_1 = refractive index of the ATR crystal; refractive indices for typical ATR crystals are: ZnSe (2.42), Di (2.2), Si (3.6), Ge (4.0)
n_2 = refractive index of the sample
α = angle of incidence (and reflection) of the ATR crystal, typically 45°

Grazing-Angle Objective

A grazing-angle objective involves a measurement configuration where light from the objective strikes the sample at a high angle of incidence for measuring the coating or surface characteristics of a sample. This measurement technique has a sample penetration of typically less than 1 micron. This technique can be used to measure very thin surface characteristics of reflective samples. The technique is qualitative and is not particularly reproducible and thus

not useful for quantitative analysis. The grazing-angle objective typically is configured for a 65–80° angle of incidence. Apertures are not generally used when using grazing-angle measurements; there is a fivefold pathlength increase when using grazing-angle as compared to standard transmittance measurement geometry.

Visible Objective

A visible microscope objective is for use in a visual survey of the sample for the purpose of inspection, sample positioning, and alignment. Crosshair features are often available for measuring sample distances, sample size, and sample alignment.

REDUNDANT APERTURES

A pair of apertures can reduce the diffraction of light caused when the light passing through an aperture is of a wavelength close to the size of the aperture. A second aperture matched to the first aperture can reduce or eliminate the spurious energy due to diffraction. Use of a second aperture (termed the *redundant aperture*) allows a single sample layer less than 50 microns to be measured on a routine basis.

REFRACTION OF LIGHT

Refraction is the change in direction of light due to a change in the velocity of the light while passing through two or more materials of differing refractive indices. In microscope systems this phenomenon causes the offset of the light based on the refractive index and thickness of the sample and sample accessory window material(s). The refracted light must be adjusted for by using a compensation ring, which is a feature of both the objective and condenser optics.

SPURIOUS ENERGY

Spurious energy in a microscope system is unfocused light caused by the diffraction of IR energy passing through an aperture that is nearly the same size as the wavelength of the IR energy. If the wavelength of light is longer than the size of an aperture, the incident light will be reflected back. The closer the wavelength is to the size of the aperture, the greater is the percentage of light diffracted. Typically for microscopes, apertures of less than 50–60 microns are too small and create diffraction problems. The use of multiple apertures can reduce the diffraction of light passing through the microscope optics.

STRAY LIGHT

Stray light is dealt with in other sections of this text; however, the main source of stray light (also termed *stray radiant energy*) within the IR microscope is the spurious energy caused by diffraction of infrared energy through small apertures.

WINDOW MATERIALS FOR MICROSCOPY

Typical window materials for microscopy include: barium fluoride (BaF_2) for use with polar solvents (including water); potassium bromide (KBr) for solids and nonpolar solvent use; zinc selenide (ZnSe) with its high refractive index for use in diamond cell background measurement; and diamond for compression cell work, where higher pressures are required.

4.

DICHROIC MEASUREMENTS OF POLYMER FILMS USING INFRARED SPECTROMETRY

INTRODUCTION

Infrared dichroism is the use of infrared polarized light as p- (90°) and s- (0°) polarized light to measure the molecular orientation of polymer films and other highly oriented molecules. The capability to generate p- and s-polarized infrared energy is required to complete these measurements. The difference between a spectrum of a polymer film taken with s-polarized light ($A_{||}$) and the spectrum of a film illuminated with p-polarized light ($A_{\perp}$) will reveal the molecular orientation of the polymer backbone and attached side groups. When the electric field vector of the infrared-active molecule (dipole) is perpendicular to the field direction vector of the infrared energy there is no interaction of the incident infrared energy with the dipole and thus no infrared absorption. When the electronic field vector of the infrared-active molecule (dipole) is parallel to the field direction vector of the infrared energy there is a large interaction and significant infrared absorption. The molecular orientation of the various molecules in a polymer film can be measured by making dichroic (s- and p-polarized light) measurements before and after stretching of the film sample.

THEORY

The intensity of an infrared absorption band is proportional to the square of the transition moment (or infrared-active dipole moment). The absolute intensity of an infrared band also depends upon the direction of the transition moment (dipole electric field vector) and the field direction vector (electric field vector) of the incident infrared radiation. The proportion of the transition moment (TM_P) in the direction of the infrared electric field direction vector (**E**) is given as

$$TM_P = tm \times \cos \beta$$

noting that $(tm \times \cos \beta)^2$ is proportional to the infrared absorbance, that tm is the transition moment of the molecule (dipole) of interest, and that β is the included angle. An illustration of the dipole electric field vector and the electric field vector of the s-polarized infrared radiation (with respect to the angle of polymer stretching) is illustrated in Figures 4.1 and 4.2.

Polymer films may be stretched, resulting in uniaxial elongation (orientation along the stretching axis). During the process of stretching, the polymer backbone will align in a parallel manner along the direction of stretching. However, under the stretching conditions, the attached groups

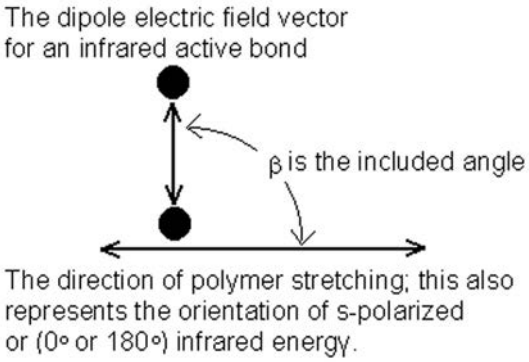


Fig. 4.1 Illustration of the bond angle between the dipole electric field vector and the direction of polymer stretching.

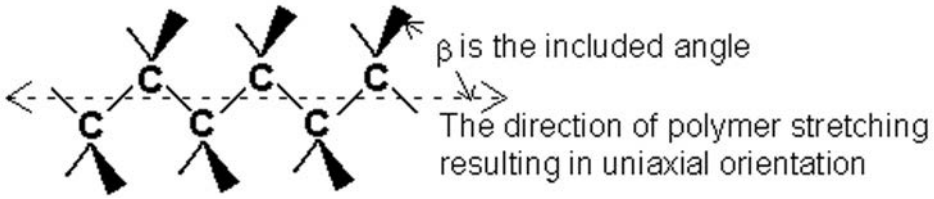


Fig. 4.2 Illustration of Fig. 4.1 for a molecular example.

may assume no preferred orientation with respect to the direction of stretching. For polymer films the direction of stretch is generally chosen as the horizontal (↔) direction. When this stretching direction is chosen, the s-polarized or (0° or 180°) infrared energy is designated as the infrared polarizer orientation for measuring $A_{||}$ (absorbance spectrum representing parallel polarized light). Likewise, the p-polarized or (90° or 270°) IR energy is the orientation for measuring $A_{\perp}$ (absorbance spectrum representing perpendicularly polarized light) [1].

METHODS OF CALCULATING DICHROIC PARAMETERS

The dichroic calculations (in arbitrary units) can be reported using the following relationships for polymer films:

$$\text{Dichroic ratio } (R) = \frac{A_{||}}{A_{\perp}} \tag{1}$$

$$\text{Dichroic difference } (\Delta A) = A_{||} - A_{\perp} \tag{2}$$

$$\text{Dichroic difference ratio } (\Delta A_r) = \frac{A_{||} - A_{\perp}}{A_{||} + A_{\perp}} \quad (\text{from} +1.0 \text{ to } -1.0) \tag{3}$$

$$\text{Polymer orientation ratio } (P_r) = K_1 \frac{R-1}{R+2} \tag{4}$$

Where K_1 = $2/(3\cos^2\beta^{-1})$ and β = the bond moment angle (in degrees) between the absorption band of interest and the polymer backbone. *Note:* if β is unknown, replace constant K_1 with the value 1.0 for calculation of P_r. *Note:* R is the Dichroic Ratio.

Calculation of Bond Moment Angles (β) [1]

$$\text{Dichroic ratio } (R) = \frac{A_{\parallel}}{A_{\perp}} = 2\left(\frac{\cos^2 b}{\sin^2 b}\right) = 2\ \cot^2 b \tag{5}$$

Thus by using Eq. (5), the values for R at any angle β can be calculated, where β is the included angle or the infrared dipole angle relative to the direction of stretch (i.e., s-polarized orientation). The angle β and the corresponding R values are shown in Table 2.2.

Table 2.2 Dichroic Ratio Versus Infrared Dipole Angle Relative to Direction of Stretch.

β (infrared dipole angle relative to direction of stretch)	R (dichroic ratio)
90°	0.000
80°	0.062
70°	0.265
60°	0.667
54°44'	1.000
50°	1.407
40°	2.843
30°	3.000
20°	15.094
10°	64.667
$\beta \rightarrow 0°$	$R \rightarrow \infty$

GRAPHICAL REPRESENTATION

The polymer stretching ratio (α) is plotted on the abscissa versus the (P_r) polymer orientation ratio as the ordinate. The polymer stretching ratio (α) is given by $\alpha = L_s/L_o$ = Stretched length/Initial length. The initial length should be set to approximately 1.0 cm by making two marks 1.0 cm apart directly on the polymer area to be stretched. The value for the polymer stretching ratio (α) for no stretching should be 1.0.

METHOD

Infrared Polarizers

Two basic types of infrared polarizers are commercially produced. These include the Brewster angle cross-plate polarizers and the wire grid polarizers. The Brewster angle cross-plate polarizers use a high-refractive-index material (such as germanium) and have a useful transmission range of 5000–500 cm^{-1}. The wire grid polarizers consist of 0.2-micron-wide aluminum strips separated by a space of approximately 0.4 microns. The matrix for placing the aluminum strips is often KRS-5 with a useful working range of 5000–285 cm^{-1} [2].

All polarizers are placed within the sample compartment directly in the infrared beam. They are mounted for complete 360° rotation. At 0° or 180° rotational settings the transmitted electric field vector (**E**) of the infrared energy is horizontal (s-polarized). This represents $A_{||}$, or the absorbance spectrum for parallel polarized light. Likewise, at 90° or 270° rotational settings the transmitted electric field vector (**E**) of the infrared energy is vertical (p-polarized). This represents $A_{\perp}$, or the absorbance spectrum for perpendicularly polarized light.

Polymer Stretching

If an automated polymer stretcher is available, this is used per manufacturer's instructions. If no special apparatus is available, the following manual technique is used. A manual device is constructed for stretching the polymer film over a small area for measurement. The polymer films are cut in strips approximately 4 cm (width) by 6 cm (length). Each side of the strip is mounted, using standard adhesive tape, to two metal pieces (approximately 4 cm by 7 cm). The polymer is measured at initial length (without stretching) using both 0° ($A_{||}$) and 90° ($A_{\perp}$) polarizer settings. Then, by gently stretching the polymer film in approximately 2–3-mm increments, the sample is repeatedly measured for each length change using both 0° ($A_{||}$) and 90° ($A_{\perp}$) polarizer settings. The stretch length change is determined by marking the film at initial length with two marks approximately 1 cm apart. These marks are best made using a fine-point permanent marker and are manually measured as each 2–3 mm stretch is completed. The spectral measurements are best made using a standard infrared film holder during measurement.

Infrared Measurements and Reporting

Measurements are made as 0° ($A_{||}$) and 90° ($A_{\perp}$) starting at $\alpha = 1.0$ (initial) and at 5 or more increasing stretch lengths (L_s). Select the functional group bands of interest on the infrared spectrum (*Note:* The absorbance of the band should be less than or equal to 0.2*A* and the bandwidth at half-height should be less than or equal to 30 cm^{-1}. This ensures the highest linearity and photometric accuracy of the infrared instrument). The absolute absorbance values are measured for these selected infrared bands for both the 0° ($A_{||}$) and 90° ($A_{\perp}$) spectra. When these results are tabulated, calculate and plot P_r (ordinate) versus α (abscissa). Report final dichroic results in tabular form as R, ΔA, ΔA_r, and P_r for all six measurements for two bands per sample following the equations given in the previous equations.

REFERENCES

1. N. B. Colthup, L. H. Daly, and S. E. Wiberley. *Introduction to Infrared and Raman Spectroscopy.* San Diego, CA: Academic Press, 1990, p. 99.
2. J. Harric. Harrick Scientific Corp., Ossining, NY, 1995.

5.

CHEMICAL IDENTIFICATION AND CLASSIFICATION TESTS

INTRODUCTION

Identification tests are used to supplement spectroscopic information to make positive identification of materials as belonging to specific classes of compounds. Confirmatory tests are useful to ensure accuracy when identifying materials.

The alkanes, alkenes, alkynes, and benzenoid aromatic compounds, aldehydes, ketones, amines, carbohydrates, alcohols, and halogen organics can be identified using standard chemical tests. For each of these tests, special emphasis is placed on the reactivity or nonreactivity of each of these different hydrocarbon classes. As a result of these classification tests and the determination of certain physical constants (i.e., melting point, boiling point, density, and refractive index) the chemist is able to identify unknown compounds with reasonable certainty even without additional sophisticated analytical instrumentation.

REAGENTS

The following reagents are required for the testing of each compound class mentioned.

Tests for aromaticity

1. Concentrated sulfuric acid
2. Fuming sulfuric acid
3. Chloroform and aluminum chloride

Tests for unsaturation

4. Bromine solution (in water or chloroform)
5. 2% Potassium permanganate in water

Test for terminal C≡C triple bond

6. Copper ammonium chloride

Tests for aldehydes and ketones

7. 2, 4-Dinitrophenylhydrazine
8. Tollen's reagent
9. Sodium bisulfite reagent

Tests for amines

10. Dilute (0.1 N) Hydrochloric acid
11. Hinsberg reaction (benzenesulfonyl chloride)

12. Nitrous acid
13. Bromine (5%) in water

Tests for carbohydrates
14. Fehling's reagent

Tests for alcohols
15. Lucas' test reagent

GENERALIZED TEST PROCEDURE

A few milliliters (1–2) of the reagent is placed in a large-mouth test tube (i.e., 7–10 cm) and the test material is added dropwise (1–5 drops). Careful observation of the results is made using safety glasses and appropriate safety protection. *Note:* Refer to each test for specific procedures.

SAFETY ISSUES

Individuals performing these tests must be adequately trained in the use, storage, and disposal of reagent chemicals. DANGER! Some reagents are poisonous and react violently with water. Refer to an appropriately trained chemical safety specialist for safety guidelines. The chemist performing these tests must be cautioned to use appropriate safety protection and to comply with all safe practices in the use of chemicals. This test document does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use. Appropriate professional training is essential for the proper use of these procedures. Refer to local, state, and federal laws affecting the proper use and disposal of chemicals.

TESTS FOR AROMATICITY

1. Concentrated Sulfuric acid

Procedure

To 40–60 drops (2–3 mL) of concentrated sulfuric acid add 3–4 drops of test solution.

> *Insoluble or no reaction* (i.e., no heat liberated) indicates an alkane, benzene, monoalkyl-aromatic, or possibly a dialkyl-aromatic.
>
> *Soluble or reaction* (i.e., heat liberated) indicates polyalkylaromatic, alkene, cyclopropane, or alkynes.

Examples
a. Toluene yields no reaction.
b. 2-Pentene forms a clear yellow liquid and evolves heat.
c. *p*-Xylene yields no reaction.
d. Styrene forms a clear yellow liquid and evolves heat.
e. Mesitylene forms a clear yellow liquid and evolves heat.
f. Napthalene yields no reaction.

2. Fuming Sulfuric acid

Procedure

To 40 drops (2 mL) of fuming sulfuric acid add 10 drops (0.5 mL) of test solution.

> *Soluble* indicates aromatic compound.
>
> *Insoluble or reaction* (i.e., heat liberated) indicates aliphatic compound.

Examples

a. Aalkanes (C21–C40) yield no reaction.
b. Cyclohexane decomposes into a yellow precipitate in solution.
c. Benzene forms a clear brown solution.
d. Napthalenes form a cloudy white solution.

3. Chloroform and Aluminum chloride

Procedure

To 2 drops (0.1 mL) of test solution add 40 drops (2 mL) chloroform. Following vigorous agitation, add 0.5 g of dry, anhydrous aluminum chloride. Add the aluminum chloride to the test tube so that some of the solid goes into the chloroform and some remains along the top of the liquid line in the test tube.

> *Reaction* indicates an aromatic system that does not have any strong deactivating groups (for example, $–NO_2$, –CXO, –CN)

Examples

a. Benzene yields an orange or red solution.
b. Naphthalene yields a blue solution.
c. Biphenyl yields a purple solution.
d. Anthracene yields a green solution.
e. Phenanthrene yields a purple solution.
f. Cyclohexane yields no color reaction.
g. Cyclohexene yields no color reaction but gives a brown precipitate.
h. Toluene yields an orange solution.
i. Styrene yields an orange solution.

TESTS FOR UNSATURATION

4. Bromine (5%) Solution (in Water or Chloroform)

Procedure

To 4 drops (0.2 mL) of the test solution add 40 drops (2 mL) of chloroform. Add 5% bromine in chloroform, drop by drop, carefully shaking the test tube between each drop. If two or more drops are decolorized in less than 1 minute, the test is positive.

> *Reaction* indicates unsaturated carbons (i.e., alkenes or alkynes); aromatics *do not* react. The brown bromine solution changes to colored or colorless quite dramatically.
>
> *No reaction* indicates saturated carbons (i.e., alkanes).

Examples

a. Cyclohexene yields a colorless solution.
b. Styrene yields a yellow solution.
c. Toluene yields no reaction.

5. Potassium Permangenate (2%) in Water

Procedure

To 2 drops (0.1 mL) of test solution add 40 drops (2mL) ethanol. Add 2% potassium permangenate solution in water, drop by drop, carefully shaking the test tube between drops. A positive test is indicated if the purple permangenate color disappears rapidly after the addition of 2–3 drops of the 2% permangenate reagent.

Reaction indicates unsaturated carbons (i.e., alkenes or alkynes); aromatics *do not* react. A positive reaction is also indicated if the purple solution changes to a brown precipitate quite dramatically.

No reaction indicates saturated carbons (i.e., alkanes).

Examples

a. Benzene yields no reaction.
b. Cyclohexene yields a brown precipitate.
c. Toluene yields no reaction.
d. Terpentine yields a brown precipitate.
e. Naphthalene yields no reaction.

TEST FOR TERMINAL C≡C TRIPLE BOND

6. Copper Ammonium Chloride

Reagent Recipe

Dissolve 0.3 g sodium hydrogen sulfite in 60 drops (3 mL) of 5% aqueous sodium hydroxide. In a separate vessel, dissolve 1.2 g $CuSO_4 \bullet 5H_2O$ and 0.3 g NaCl in 4 mL of warm deionized water. With careful stirring, slowly add the bisulfate solution to the copper sulfate solution. Cool to room temperature, decant the supernatant, and wash the copper chloride precipitate with 2-mL portions of 5% aqueous sodium hydroxide solution. Dissolve the precipitate in a mixture of 3 mL water and 4 mL concentrated ammonium hydroxide. Keep this solution tightly stoppered so as to prevent air oxidation.

Procedure

For the test liquid add 2 drops (0.1 mL) of unknown into 1 mL of ethanol and 1 mL of ammoniacal cuprous chloride reagent. Formation of a green precipitate indicates a positive test.

Reaction forming a green precipitate indicates a terminal **C≡C**.

No reaction indicates the absence of such a bond.

Examples

a. 1-Hexyne yields a green precipitate.
b. Cyclohexene yields no reaction.
c. Phenylacetylene yields a green precipitate.
d. Styrene yields no reaction.

When the results of these tests are combined with boiling point and/or refractive index, many organic liquids can be positively identified.

TESTS FOR ALDEHYDES AND KETONES

7. 2, 4-Dinitrophenylhydrazine

Reagent Recipe

Add 1 mL of concentrated sulfuric acid to 0.2 g of 2, 4-dinitrophenylhydrazine. Water (2 mL) is added dropwise with mixing. To the warm solution, add 5 mL of ethanol (95%).

Procedure

Add 3 drops of the unknown liquid to 3 mL of 95% ethanol. Add this combined solution to the reagent mixture. Agitate, and observe for the following.

> *Reaction* forming a bright red or orange precipitate indicates that either functional group is present.
>
> *No reaction* indicates that no aldehyde or ketone groups are present.

Examples

a. Diethylketone yields a red-orange precipitate.
b. Benzaldehyde yields a red-orange precipitate.
c. Toluene yields no reaction.
d. Acetone yields a yellow precipitate

8. Tollen's Reagent

Reagent Recipe

Add 4 mL of a 5% silver nitrate solution to 2 drops of a 10% sodium hydroxide solution. To this mixture add a 2% solution of ammonia dropwise, with constant agitation, until the silver oxide precipitate dissolves. An excess of ammonia is to be avoided to retain the sensitivity of the test. The reagent should be prepared just prior to testing.

Procedure

In a very clean test tube add the Tollen's reagent and 3–4 drops of the test liquid, and warm the mixture gently. A mirrored surface indicates an aldehyde. If the inside of the tube is not clean, a precipitate will result, also indicating the presence of the aldehyde.

> *Reaction* forming a white silver precipitate indicates the presence of an aldehyde but not ketone functional group(s).
>
> *No reaction* indicates that no aldehyde groups are present.

Examples

a. Benzaldehyde yields a white precipitate.
b. Diethylketone yields no reaction.
c. Formaldehyde yields a mirrored surface or precipitate.

9. Sodium Bisulfite Reagent

Reagent Recipe

Add 5 mL of ethanol (95%) to 10 mL of a 40% aqueous sodium bisulfite solution. Decant the liquid from a small quantity of precipitate that forms, and use the liquid for the procedure.

Procedure

Add 5 drops of the test liquid to 1 mL of the sodium bisulfite reagent. Stopper the test tube (container) and shake vigorously.

Reaction liberating heat indicates that either functional group (aldehyde or ketone) is present. *Note:* Some methyl ketones and cyclic ketones yield a positive test. Aryl ketones and sterically hindered ketones do not react.

No reaction indicates that no aldehydes, methyl ketones, or cyclic ketones are present

Examples

a. Diethylketone yields no reaction.
b. Benzaldehyde yields heat.
c. Toluene yields no reaction.
d. Methylpropylketone yields heat.

TESTS FOR AMINES

10. Dilute (0.1 N) Hydrochloric Acid

Reaction forming amine hydrochloride salt occurs—sample is soluble.

No reaction indicates that no amine groups are present—sample remains insoluble.

Examples

a. Methylamine yields methylamine hydrochloride salt.
b. Aniline yields aniline hydrochloride salt.
c. Ethylamine yields ethylamine hydrochloride salt.

11. Hinsberg Reaction (Benzenesulfonyl chloride)

Reagent Recipe

To 0.6 mL of aniline add 5 mL of 10% sodium hydroxide solution and 0.8 mL of benzenesulfonyl chloride. This mixture is added to a test tube, stoppered, and shaken vigorously. The mixture should be maintained below 20°C to prevent the formation of a purple dye. Make certain the solution is basic and that any residue or solid material is filtered from the liquid and the liquid is retained.

Reaction of primary or secondary amines form N-substituted benzenesulfonamide precipitate.

No reaction indicates that no primary or secondary amine groups are present. *Note:* Tertiary amines do not react to form precipitate.

Examples

a. Methylamine (primary amine) yields N-substituted benzenesulfonamides precipitate.
b. Aniline (primary amine) yields N-substituted benzenesulfonamides precipitate.
c. Ethylamine (primary amine) yields N-substituted benzenesulfonamides precipitate.
d. Secondary amines yield N-substituted benzenesulfonamides precipitate.
e. Tertiary amines yield no reaction.

12. Nitrous Acid

Perform the test reaction at 0°C.

Reaction of aromatic primary amines form brilliant orange aniline dyes; primary aliphatic amines liberate nitrogen gas bubbles. Secondary aliphatic and aromatic amines yield yellow N-nitroso derivatives.

No reaction indicates that no primary amine groups are present. *Note:* Secondary and tertiary amines do not react to form highly colored dyes.

Examples

a. Primary aromatic amines form brilliant orange aniline dyes.
b. Primary aliphatic amines liberate nitrogen gas bubbles.
c. Secondary aliphatic and aromatic amines yield yellow N-nitroso derivatives.
d. Tertiary amines do not react.

13. Bromine (5%) in Water

Reaction of aromatic amines removes the color from bromine water, yielding a yellow precipitate. Aromatic amines are oxidized to benzonitriles.

No reaction indicates that no amine groups are present. *Note:* Phenols can also react with bromine water, and this test should not be used to distinguish phenols from amines

Examples

a. Aromatic amines remove the color from bromine water, yielding a yellow precipitate.
b. Benzylamine removes the color from bromine water, yielding a yellow precipitate.
c. Phenol removes the color from bromine water, yielding a yellow precipitate.

TESTS FOR CARBOHYDRATES

14. Fehling's Reagent

Recipe

Fehling's reagent is made by mixing equal volumes of each of the following solutions:

a. Copper sulfate solution: 6.9 g of copper sulfate (hydrated) in 100 mL of water
b. 34.6 g of sodium potassium tartrate plus 14 g of sodium hydroxide pellets in 100 mL water

Procedure

Add 0.1 g of test material to 3 mL water and 3 mL Fehling's reagent. Heat the mixture to boiling. Observe the results as the liquid cools.

Reaction of all monosaccharides and most disaccharides with Fehling's reagent causes a red precipitate of cuprous oxide to form.

No reaction indicates that no monosaccharide or disaccharide groups are present.

Examples

a. D-Fructose causes a red precipitate of cuprous oxide to form.
b. D-Glucose causes a red precipitate of cuprous oxide to form.
c. Starch yields no reaction.

TESTS FOR ALCOHOLS

15. Lucas' Test Reagent

Reagent Recipe

To 30 g of concentrated hydrochloric acid, dissolve 37.5 g of anhydrous (fused) zinc chloride. Stir gently in a water bath at near 0°C to avoid loss of HCl. This volume of material (approximately 39 mL) is sufficient for approximately 10–12 tests.

Procedure

To 0.5 mL of the test alcohol add 3 mL of the Lucas' reagent at 25–28°C. Add the mixture to a test tube, stopper, and shake vigorously for several seconds, and then allow it to stand. Observe immediately, in 5 minutes, and in 1 hour.

> *Reaction* of tertiary alcohols is at once, yielding a milky white precipitate of alkyl chloride. Secondary alcohols react within 5 minutes to give the same result, with a second layer separating in the test tube after several minutes. Primary alcohols do not react at near room temperature and the solution remains clear. Separation of a secondary layer of a milky white precipitate of alkyl chloride occurs only in the presence of secondary or tertiary alcohols.
>
> *No reaction* indicates that no secondary or tertiary alcohol groups are present.

Examples

a. Ethanol yields no reaction.
b. Methanol yields no reaction.
c. Isobutyl alcohol does react.
d. 2-methyl-1-propanol does react.

REFERENCES

1. James S. Swinehart. *Organic Chemistry and Experimental Approach.* Englewood Cliffs, NJ: Prentice-Hall, 1969.
2. Ervin Jungreis. *Spot Test Analysis: Clinical, Environmental, Forensic, and Geochemical Applications.* New York: Wiley, 1985.
3. Ralph L. Shriner, Reynold C. Fuson, David Y. Curtin. *The Systematic Identification of Organic Compounds: A Laboratory Manual.* New York: Wiley, 1948.
4. Emile M. Chamot, Clyde W. Mason. *Handbook of Chemical Microscopy.* New York: Wiley, 1931. *Note:* This reference is most useful for inorganic cation and anion identification.
5. Roger Adams, John R. Johnson, Charles F. Wilcox, Jr. *Laboratory Experiments in Organic Chemistry.* New York: Macmillan, 1967.

III.

UV-VIS SPECTROSCOPY

6.

COMPARING ULTRAVIOLET-VISIBLE AND NEAR-INFRARED SPECTROMETRY

INTRODUCTION

Ultraviolet (190–380 nm), visible (380–750 nm), short-wave near-infrared (750–1100 nm), long-wave near-infrared (1100–2500 nm), infrared (4000–400 cm^{-1}), and Raman spectroscopy comprise the bulk of the electronic and vibrational mode measurement techniques. The measurement modes for UV-Vis-NIR spectroscopy are given in Table 6.1.

Basic spectroscopic measurements involve the instrumental concepts of bandpass and resolution, signal-to-noise ratio, dynamic range, stray light, wavelength accuracy and precision, and photometric accuracy and precision. These concepts are described in Chapter 1.

INSTRUMENTATION

Optical configurations

The basic optical configuration for UV-Vis-NIR instrumentation is shown in the schematic diagrams in Figures 6.1 through 6.5. Double-monochromator instruments (dispersive, Figure 6.1) provide a traditional means for reducing the stray light of an instrument at the point of measurement. Double-monochromator instruments are generally used for applications where high degrees of photometric accuracy and repeatability are required. Optical components with strict specifications are often tested using double monochromator systems. These systems demonstrate extremely low-stray-light specifications on the order of 0.0001% *T* or better.

Table 6.1 Basic UV-Vis-NIR Measurement Modes.

Instrument measurement mode	Description of measurement
Scan	Absorbance vs. wavelength
Time drive	Absorbance vs. time at a specific wavelength
Individual Wavelength(s)	Individual absorbance(s) at selected wavelength(s)
Chemometrics and Quantitative methods	Concentration of analyte vs. absorbance
Kinetics	Kinetic rates of reaction

Single monochromators (dispersive, Figure 6.2) are lower cost than double-monochromator systems and are generally used as workhorses within the laboratory. These systems typically meet the basic requirements for routine quantitative and qualitative work for relatively undemanding applications. The dynamic range of these systems is stray light limited.

Diode array detection (dispersive, Figure 6.3) offers the advantage of the absence of moving parts extending the longevity and reliability of such systems over more traditional spectropho-

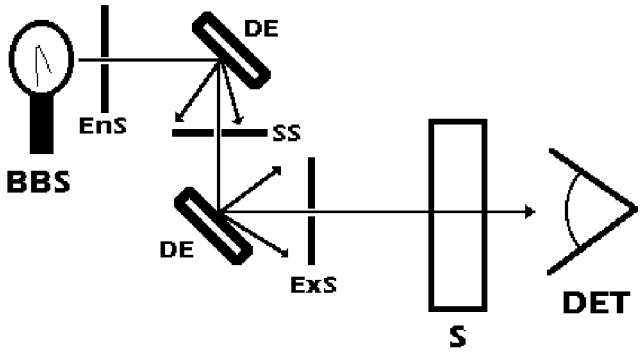


Fig. 6.1 Double-monochromator System (dispersive) optical configuration.

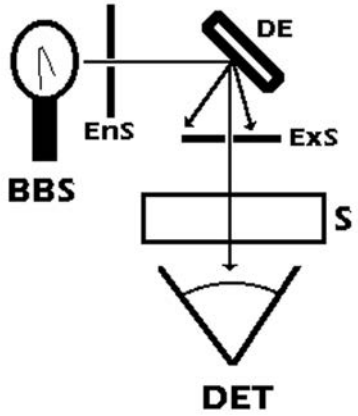


Fig. 6.2 Single-monochromator system (dispersive) optical configuration.

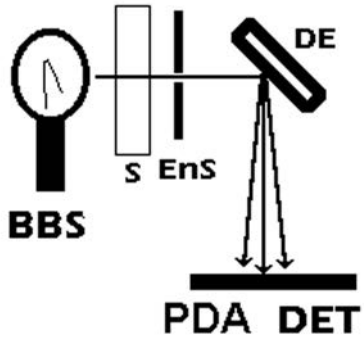


Fig. 6.3 Diode array spectrophotometer (dispersive) optical configuration.

tometers. The main advantage of these instruments is the rapidity with which data can be collected (e.g., in milliseconds) versus the traditional scanning instrument, which makes spectral measurements in seconds to minutes.

Interferometers (Figure 6.4) provide the main optical element for Fourier transform infrared spectrophotometry. Interferometer-based Fourier transform spectrophotometers are extremely accurate in the frequency scale, but have sometimes lacked precision in the photometric domain when compared to their older counterparts, the dispersive-based instruments. However, interferometry is an extremely accurate means for measuring spectra with respect to frequency-dependent measurements.

Interference filter photometers (dispersive, Figure 6.5) provide a low-cost, rugged alternative to grating- or interferometer-based instrumentation. These instruments typically contain from 5 to 40 interference filters that select the proper wavelengths for quantitative analysis based on previous work with scanning instruments, or based on theoretical positions for absorption.

Light-emitting diodes (LEDs) provide emission of prespecified wavelengths of light. Interference filters are sometimes used to further reduce the bandwidth of the light used for analysis.

Basic instrument configurations are shown in Figures 6.1 to 6.5. The key for abbreviations used in the figures are: BBS (broad band source), EnS (entrance slit), DE (dispersive-element,

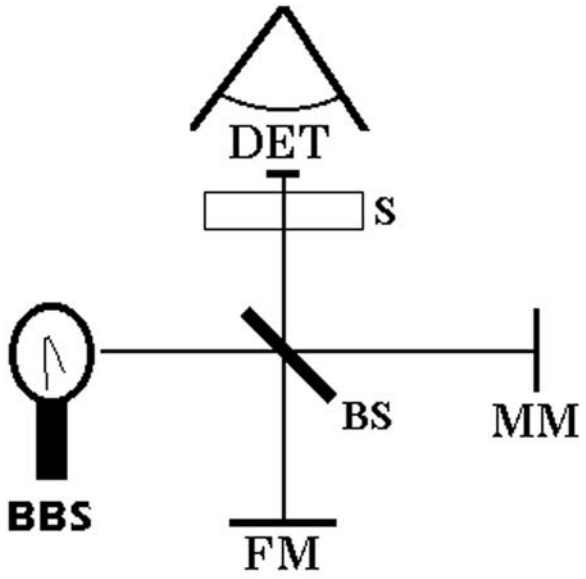


Fig. 6.4 Fourier transform spectrophotometer (interferometer) optical configuration.

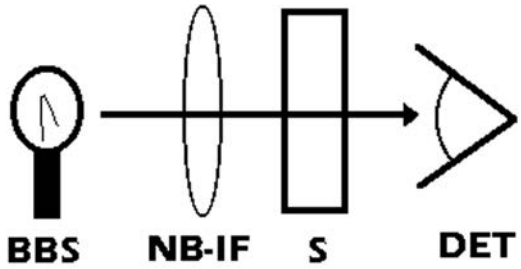


Fig. 6.5 Interference filter photometer optical configuration.

grating, or prism type), SS (second slit), ExS (exit slit), S (sample), DET (detector), PDA DET (photodiode array detector), bs (beam splitter), fm (fixed mirror), mm (movable mirror), NB-IF (narrow bandpass interference filter).

Typical Lamp Sources (Useful Working Ranges)

Most instrumentation used to measure the region from 190 to 2500 nm utilizes a combination of the quartz tungsten-halogen lamp for the visible and near-infrared regions (approximately 350 to 2500 nm), and the DC deuterium lamp for the ultraviolet region (from 190 to 350 nm). The useful working ranges for the most common sources are as follows:

Quartz tungsten-halogen filament Lamp (220–2700 nm)

DC deuterium lamp for UV (185–375 nm)

Pulsed xenon arc lamp (180–2500 nm)

DC arc lamp (200–2500 nm)

Unusual Sources

Unusual sources can be found when making measurements within the ultraviolet or near-infrared region. These can include lasers, such as the nitrogen laser (337.1 nm), and a variety of dye lasers (350–750 nm).

Calibration Lamps (with Emission Line Locations in Nanometers)

Calibration lamps are used to check the wavelength accuracy for ultraviolet and visible spectrophotometers. The main lamps used include the following two:

Mercury (argon) lamp (253.7, 302.2, 312.6, 334.0, 365.0, 404.7, 435.8, 546.1, 577.0, 579.0)

Mercury (neon) lamp (339.3, 585.2, 793.7, 812.9, 826.7, 837.8, 864.7, 877.2, 914.9, 932.7, 953.4)

Detectors (Useful Working Ranges)

A variety of detectors are available for UV-Vis-NIR measurements. High-performance instruments utilize photomultiplier tube technology from the ultraviolet into the visible region. Lead sulfide is the detector of choice for near-infrared measurements. The more common detectors follow, with their useful operating ranges indicated. More information on detector performance is given in Chapter 1 on Optical Spectrometers.

Silicon photodiode (350–1100 nm)

Photomultiplier tubes (PMTs) (160–1100 nm)*

PbS (lead sulfide) (1000–3000 nm)

CCDs (charge-coupled devices) (180–1100 nm)

Photodiode arrays (silicon-based PDAs) (180–1100 nm)

InGaAs (Indium gallium arsenide) (800–1700 nm)

Window and Cuvet Materials

A variety of window materials are available for sample cells and optical elements within spectrophotometers. These materials are selected for their optical clarity for use in specific wave-

*Total detection range using PMT technology.

length regions as well as their strength and cost characteristics. Table 6.2 lists data for the most common materials used for sample cells and optical components in UV-Vis-NIR instrumentation.

Fiber Optic Materials (Useful Working Range)

New core and cladding materials are becoming available for use in fiber-optic construction. The most common materials in current use include those listed here. The useful working range for typical samples of these materials is given in parentheses.

SiO_2 (pure fused silica or quartz) (0.2–1.25 microns)

Anhydrous quartz (0.4–2.64 microns)

ZrF (zirconium fluoride) (0.9–4.76 microns)

Chalcogenide (2.22–11.1 microns) for use in NIR-IR measurements

Methods for Testing UV/Vis Instrumentation

Linearity checks are performed by using three neutral-density glass filters available from NIST as SRM 930D. These glasses have nominal percentage transmittance at 10, 20, and 30%. Solutions of nickel and cobalt in nitric and perchloric acids are available as SRM 931. Metal on quartz transmittance standards are available as SRM 2031 with nominal percentage transmittance at 10, 30, and 90%.

Photometric accuracy is measured for UV using SRM 935 consisting of a solution of potassium dichromate in perchloric acid. An additional material consisting of potassium acid phthalate in perchloric acid is available as SRM 84.

Wavelength accuracy is measured using ASTM Practice E 275–83, Practice for Describing and Measuring the Performance of Ultraviolet, Visible, and Near-Infrared Spectrophotometers.

Table 6.2 Characteristics of Window/Cuvet Materials.

Optical material	Transmittance range (in nm)	Refractive index at 600 nm	Relative rupture strength (sapphire = 100)
Methacrylate	250–1100	—	—
UV-Grade fused silica	200–2500	1.4580	10.9
Synthetic fused silica	230–2500	1.4580	10.9
Crystalline quartz (SiO_2)	240–2500	1.5437	2.3
Quartz, extremely low O-H	190–2500	1.5437	2.3
Flint glass (SF 10)	380–2350	1.7268	3.8
Flint glass (SF 8)	355–2350	1.6878	3.8
BK 7 glass	315–2350	1.5165	3.7
Optical crown glass	320–2300	1.5226	3.7
Borosilicate crown glass	360–2350	1.4736	3.7
Pyrex®	360–2350	1.4736	3.8
Tempax®	360–2350	1.4736	3.8
Sapphire (Al_2O_3)	150–5000	1.7677	100
Sodium chloride	250 nm–16 μm	1.5400	0.5
Suprasil 300®	190–3600	1.54	3.8
Diamond	220–4000	2.40	83.7

A second method used is E 958–83, Practice for Measuring Practical Spectral Bandwidth of Ultraviolet-Visible Spectrophotometers. Potassium dichromate in perchloric acid at pH 2.9 exhibits known maxima at 257 and 350 nm, with minima at 235 and 313 nm. Samarium perchlorate can be used for the wavelength region 225–520 nm with excellent results. Holmium oxide glass filters exhibit bands at 279.3, 287.6, 333.8, 360.8, 385.8, 418.5, 446.0, 453.4, 536.4, and 637.5 nm. In addition, the holmium glass exhibits bands within the 750–1200-nm region. Didymium oxide glass filters are available for use from 250 to 2000 nm.

Stray light measurements are made using a sharp cut-off filter. Examples of such filter materials include saturated solutions of such compounds as potassium ferromanganate and lithium carbonate. Other solutions exhibiting abrupt cut-off wavelengths include: KBr, KCl, NaI, $NaNO_3$ solutions, and acetone. Refer to ASTM E 169-87, Practice for General Techniques of Ultraviolet-Visible Quantitative Analysis.

SAMPLING CONSIDERATIONS

Sample Presentation Geometry

A variety of sample presentation methods are available to the analyst. These include: transmission (straight and diffuse), reflectance (specular and diffuse), transflectance (reflectance and transmission), and interactance (a combination of reflectance and transmission).

Cuvet Cleaning

Light cleaning: Detergent wash, followed by multiple pure-water rinses.

Heavy cleaning: Repeat the light cleaning followed by cleaning with a chromic-sulfuric acid solution wash and multiple pure-water rinses.

Table 6.3 Typical Sampling Accessories.

Sample cells	Outer dimensions (in mm)	Pathlength (in mm)	Capacity (in mL)
Standard transmittance	45 (H) × 12.5 (W) × 3.5 (L)	1.0	0.3
	45 (H) × 12.5 (W) × 7.5 (L)	5	1.5
	45 (H) × 12.5 (W) × 12.5 (L)	10	3.0
Semimicro	45 (H) × 12.5 (W) × 12.5 (L)	10	1.0 or 1.5
Micro cell	25 (H) × 12.5 (W) × 12.5 (L)	10	0.5
Cylindrical cells	10 (L) × 22 (Diameter)	10	3.1
	20 (L) × 22 (D)	20	6.3
	50 (L) × 22 (D)	50	16
	100 (L) × 22 (D)	100	31
Microflow cell	50 (H) × 12.5 (W) × 12.5 (L)	10	0.4 or 0.6
Round	75 (H) × 12 (D)	~10	5.9
	105 (H) × 19 (D)	~17	23.8
	150 (H) × 19 (D)	~17	34.0

Selection of Pathlength

UV, 190–350 nm, 1 mm–10 cm

SW-NIR, 800–1100 nm, 5–10 cm

LW-NIR, 1050–3000 nm, 0.1–2 cm

Matrix/Measurement Techniques

Clear solids (optical materials): transmittance

Translucent or opaque solids: diffuse reflectance or diffuse transmittance (for turbid samples)

Reflecting optical surfaces: specular reflectance

Clear liquids: transmittance

Translucent or opaque liquids: reflectance or diffuse transmittance

High optical density (highly absorbing): tiny pathlengths in transmittance

Sample Optical Properties

Clear samples are measured using transmission spectroscopy.

Colored samples are generally measured using transmission spectroscopy, unless the optical density exceeds the linear range of the measuring instrument. At this point either dilution or narrowing of the pathlength is the technique of choice.

Fine scattering particulates within a sample are measured by diffuse transmission or diffuse reflectance methods. The scattering produces a pseudo-pathlength effect that must be compensated for by using scatter correction data-processing methods when making quantitative measurements on scattering materials.

Large scattering particulates present a challenge for measurements, for the particles intercept the optical path at random intervals. Signal averaging can be employed to compensate for random signal fluctuations as well as careful monitoring and tracking of signal changes. Reflectance spectroscopy can be used to measure the size, velocity, and concentration of scattering particulates within a flowing stream.

High-absorptivity (optically dense) materials with absorbances above 4–6 OD are difficult to measure accurately without the use of double-monochromator instruments with stray light specifications below 0.0001% *T*. Measurements can be made with extremely slow scanning speeds and by opening the slits during measurement. These measurements should be avoided by the novice unless high-performance instrumentation and technical support are available.

APPLICATIONS

The grouping of atoms producing a characteristic absorption is called a chromophore (*chromo* = color + *phore* = producer). A specific grouping of atoms produces a characteristic absorption band at specific wavelengths. The intensity and location of these absorption bands will change with structural changes in the group of atoms and with solvent changes. Typical UV solvents are given in Table 6.4. The location of bands associated with UV chromophores is shown in Table 6.5.

Table 6.4 Typical UV Solvents and Approximate UV Cut-Off Wavelengths.

Solvent	UV cut-off (in nm)
Acetonitrile	190
Water	190
Cyclohexane	195
Isooctane	195
n-Hexane	201
Ethanol (95 vol. %)	205
Methanol	205
Trimethyl phosphate	210
Acetone	220
Chloroform	240
Xylene	280

Table 6.5 Absorptions of UV Chromophores (160–210 nm).

Chromophore	Absorption Band (in nm)
Nitriles (R-C≡N)	160*
Acetylenes (—C≡C—)	170*
Alkenes (>C=C<)	175–185*
Alcohols (R—OH)	180 (175–200)*
Ethers (R—O—R)	180*
Ketones (R—C=O—R′)	180*, 280
Amines, primary (R—NH_2)	190* (200–220)
Aldehydes (R—C=O—H)	190*, 290
Carboxylic acids (R—C=O—OH)	205
Esters (R—C=O—OR′)	205
Amides, primary (R—C=O—NH_2)	210
Thiols (R—SH)	210
Sulfides (R_2S)	210–215
Unsaturated aldehydes (C=C—C=O)	210–250
Nitrites (R—NO_2)	270–275
Carbonyl (R_2>C=O)	270–310
Azo-group (R—N=N—R)	340–350

*Absorptions below the cut-off for common solvents would not be observed in solvent solution measurements.

Use of UV-Vis for Life Sciences

Enzymatic Methods

Table 6.6 gives a summary of the various enzymatic methods of analysis using spectroscopic technique.

Table 6.6 Spectroscopic Assay Measurements for Enzymes.

Enzyme name	Reaction type and assay wavelength (in nm)
Lactate dehydrogenase	Direct absorbance at 350
Alkaline phosphatase	Direct absorbance at 550
NADH and NADPH	Direct absorbance at 340
Alcohol dehydrogenase	Increased Abs.—NADH at 340
Aldolase	Increased Abs. at 240; decreased Abs. at 340
D-Amino acid oxidase	Decreased Abs.—NADH at 340
L-Amino acid oxidase	Change in Abs. at 436
Alpha-Amylase	Color reaction at 540
L-Arginase	Color reaction at 490
Arylsulfatase	Hydrolysis reaction at 405
Catalase	Liberation of H_2O_2—Abs. at 240
Cholinesterases	Color reaction at 340
Alpha-Chymotrypsin	Hydrolysis products at 280
Creatine kinase	Increased Abs.—NADH at 340
Deoxyribonuclease I	Depolymerization product at 260
Diamine oxidase	Decreased Abs.—NADH at 340
Beta-Galactosidase	Hydrolysis Product at 405, or 436
Glucose oxidase	Color reaction at 436
Glucose-6-phosphate dehydrogenase	Increased Abs.—NADH at 340
Glucose phosphate isomerase	Increased Abs.—NADPH at 340
Beta-Glucosidase	Increased Abs.—NADPH at 340
Beta-Glucuronidase	Color reaction at 540
Glutamate-oxaloacetate transaminase	Decreased Abs.—NADH at 340
Glutamate pyruvate transaminase	Decreased Abs.—NADH at 340
Gamma-Glutamyl transferase	*p*-Nitroaniline at 400
Hexokinase	Increase in Abs.—NADH at 340
Alpha-Hydroxybutyrate dehydrogenase	Decreased Abs.—NADH at 340
Isocitrate dehydrogenase	Increase in Abs.—NADH at 340
Lactate dehydrogenase	Decrease in Abs.—NADH at 340
Leucine amino peptidase	Presence of *p*-nitroalinine at 405
Lipase	Color reaction at 540
Monoamine oxidase	Color reaction at 456
Pepsin	Color reactions at 578, 691, and 750
Peroxidase	Color reaction at 460
Acid phosphatase	Increased Abs. at 300
Alkaline Phosphatase	Color reaction at 530
Pyruvate kinase	Decreased Abs. at 340
Sorbitol dehydrogenase	Decrease in Abs.—NADH at 340
Trypsin	Increased Abs. at 280
Urease	Color reaction at 580, 630
Xanthine oxidase	Color reaction at 530–580

Use of SW-NIR for Organic Composition Analysis

Table 6.7 demonstrates the basic functional group measurements that have useful signal in the short-wavelength near-infrared (SW-NIR) region (800–1100 nm) of the electromagnetic spectrum. As can be seen from the table, the SW-NIR region is used to measure molecular vibrations as combination bands for C—H groups, for second overtones of O—H and N—H groups, and for third overtone C—H group measurements. All NIR spectroscopy is used to measure these basic organic functional groups resulting from molecular vibrations (Table 6.8). The advantages of SW-NIR include high signal-to-noise ratio from readily available technologies, typically 25,000 : 1; as well as high throughput using fiber-optic cabling. An additional advantage of SW-NIR over other IR regions includes the use of flow-cell pathlengths sufficiently large for industrial use (most often 5–10 cm). This range of pathlengths is useful in obtaining representative sample size measurements and in preventing fouling of internal cell optics. Representative SW/NIR spectra are shown in Fig. 6.6 on p. 60.

Approximate LW-NIR Band Location for Common Organic Compounds

Traditional LW-NIR spectroscopy included the use of filter instruments to measure the major constituents of nutritional interest in grain and forage materials. Selected wavelengths com-

Table 6.7 C—H, N—H, and O—H Stretch Absorption Bands for Specific Short-Wavelength NIR (800–1100 nm) functional groups (2nd or 3rd Overtones).

Structure	Bond vibration	Approx. band locations (in nm)
ArCH . (Aromatics)	C–H str. 3rd overtone	857–890
. CH—CH (Methylene)	C–H str. 3rd overtone	930–935
. CH_3 (Methyl)	C–H str. 3rd overtone	912–915
"	C–H combination	1010–1025
R—OH (Alcohols)	O–H str. 2nd overtone	940–970
ArOH (Phenols)	O–H str. 2nd overtone	947–980
HOH (Water)	O–H str. 2nd overtone	960–990
Starch	O–H str. 2nd overtone	967
Urea	Sym. N–H str. 2nd overtone	973
. $CONH_2$ (Primary amides)	N–H str. 2nd overtone	975–989
.CONHR (Secondary amides)	N–H str. 2nd overtone	981
Cellulose	O–H str. 2nd overtone	993
Urea	Sym. N–H str. 2nd overtone	993
$ArNH_2$ (Aromatic amines)	N–H str. 2nd overtone	995
. NH (Amines, general)	N–H str. 2nd overtone	1000
Protein	N–H str. 2nd overtone	1007
Urea	N–H str. 2nd overtone	1013
RNH_2 (Primary amines)	N–H str. combination	1020
Starch	O–H str. combination	1027
CONH (Primary amides)	N–H str. combination	1047
$=CH_2$ (Methylene)	C–H str. combination	1080