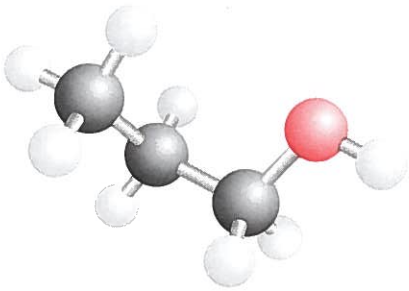
CHAPTER

14



Ultraviolet Spectroscopy, Refractive Indices, and Qualitative Instrumental Organic Analysis

Online Study Center

This icon will direct you to techniques, equipment setups, and online resources at http://college.hmco.com/PIC/williamsonMMOE5e.

PRELAB EXERCISE: In the identification of an unknown organic compound, certain procedures are more valuable than others. For example, far more information is obtained from an IR or NMR spectrum than from a UV spectrum or a refractive index measurement. Outline, in the order of priority, the steps you will employ in identifying an organic unknown.

Ultraviolet Spectroscopy

UV: Electronic transitions within molecules

Ultraviolet (UV) spectroscopy produces data about electronic transitions within molecules. Whereas absorption of low-energy infrared (IR) radiation causes bonds in a molecule to stretch and bend, the absorption of short-wavelength, high-energy ultraviolet (UV) radiation causes electrons to move from one energy level to another with energies that are capable of breaking chemical bonds. Many molecules produce only a major absorption signal (*see* Figs. 14.2 to 14.7 on pages 294–298) due to a single, extended structural feature involving π bonded systems: carbon-carbon single bonds, carbon-oxygen or carbon-nitrogen double bonds, triple bonds, and aromatic rings. Because many different combinations of these π systems can absorb at nearly the same wavelength, the structure is usually elucidated by other techniques, and its UV spectrum is interpreted based on this structure.

We are most concerned with transitions of π electrons in conjugated and aromatic ring systems. These transitions occur in the wavelength region of 200–800 nm (nanometers, 10^{-9}meters). Most common UV spectrometers cover the region of 200–400 nm as well as the visible spectral region of 400–800 nm. Below 200 nm air (oxygen) absorbs UV radiation; spectra in that region must therefore be obtained in a vacuum or in an atmosphere of pure nitrogen.

Consider ethylene, even though it absorbs UV radiation in the normally inaccessible region at 163 nm. The double bond in ethylene has two *s* electrons in a π molecular orbital and two, less tightly held, *p* electrons in a π molecular orbital. Two unoccupied, high-energy-level, antibonding orbitals are associated with these orbitals. When ethylene absorbs UV radiation, one electron moves up from the bonding π molecular orbital to the antibonding π* molecular orbital (Fig. 14.1). As Figure 14.1 indicates, this change requires less energy than the excitation of an electron from the σ to the σ* molecular orbital.

By comparison with IR spectra and nuclear magnetic resonance (NMR) spectra, UV spectra are mostly featureless (Fig. 14.2). This condition results as molecules in a number of different vibrational states undergo the same electronic transition to produce a band spectrum instead of a line spectrum.

Band spectra

Unlike IR spectroscopy, UV spectroscopy lends itself to precise quantitative analysis of substances. The intensity of an absorption band is usually given by the molar extinction coefficient, ε, which, according to the Beer-Lambert law, is equal to the absorbance (*A*) divided by the product of the molar concentration (*c*) and the path length (*l*) in centimeters.

Beer-Lambert law

$$\varepsilon = \frac{A}{cl}$$

λ_{max} = wavelength of maximum absorption

The wavelength of maximum absorption (the tip of the peak) is given by λ_{max}. Because UV spectra are so featureless, it is common practice to describe a

FIG. 14.1
The electronic energy levels of ethylene.

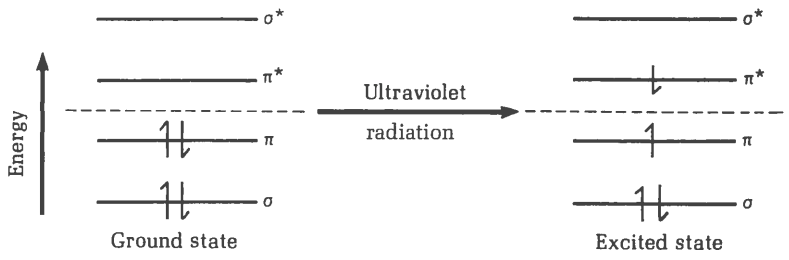
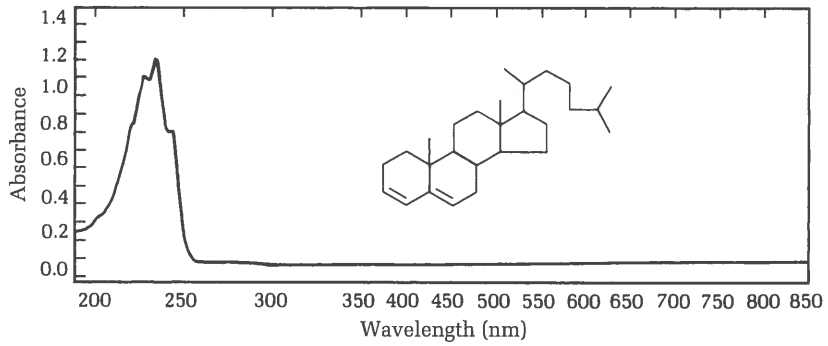


FIG. 14.2
The UV spectrum of cholesta-3,5-diene in ethanol.



spectrum like that of cholesta-3,5-diene (Fig. 14.2) as λ_{max} = 234 nm (ε = 20,000) and not bother to reproduce the actual spectrum.

ε, extinction coefficient

The extinction coefficients of conjugated dienes and enones are in the range of 10,000–20,000, so only very dilute solutions are needed for spectra. In the example in Figure 14.2, the absorbance at the tip of the peak is 1.2, and the path length is the usual 1 cm; so the molar concentration, c, needed for this spectrum is 6×10^{-5}mol/L,

The path length (l) is the distance (in centimeters) that light travels through a sample.

$$c = \frac{A}{l\varepsilon} = \frac{1.2}{20{,}000} = 6 \times 10^{-5} \text{ mol/L}$$

which is 0.221 mg/10 mL of solvent. The usual laboratory balance cannot accurately weigh such small quantities; therefore sample preparation usually requires the quantitative serial dilution of more concentrated solutions.

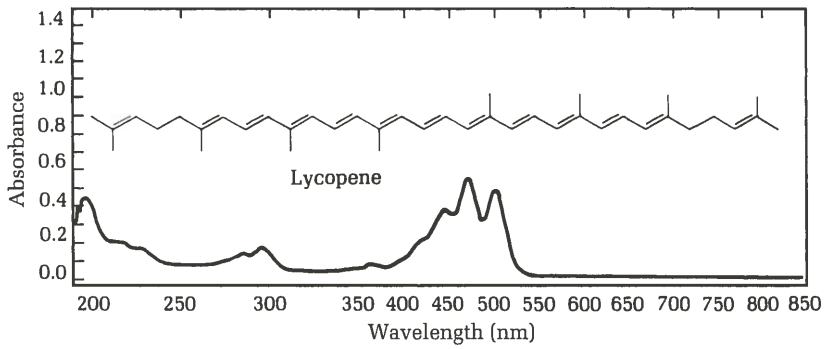
Spectro grade solvents

The usual solvents for UV spectroscopy are 95% ethanol; methanol; water; and saturated hydrocarbons such as hexane, trimethylpentane, and isooctane. The three hydrocarbons are often especially purified to remove impurities that absorb in the UV region. Any transparent solvent can be used for spectra in the visible region.

UV cells are expensive; handle with care.

Sample cells for spectra in the visible region are made of glass or clear plastic, but UV cells must be composed of the more expensive fused quartz because glass absorbs UV radiation. The cells and solvents must be clean and pure because very little of a substance produces a UV spectrum. A single fingerprint will give a spectrum!

Ethylene has λ_{max} = 163 nm (ε = 15,000), and butadiene has λ_{max} = 217 nm (ε = 20,900). As the conjugated system is extended, the wavelength of maximum absorption moves to longer wavelengths (toward the visible region). For example, lycopene, with 11 conjugated double bonds, has λ_{max} = 470 nm (ε = 185,000; Fig. 14.3). Because lycopene absorbs blue visible light at 470 nm, the substance appears bright red. It is responsible for the color of tomatoes; its isolation and analysis are described in Chapters 8 and 9.

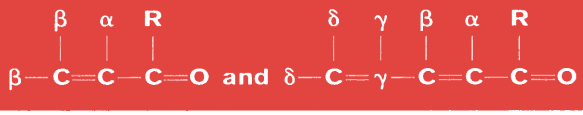


■ FIG. 14.3
The UV/Vis spectrum of lycopene in isooctane.

TABLE 14.1 • Rules for Predicting the λ_{max} for Conjugated Dienes and Polyenes

	Increment (nm)
Parent acyclic diene (butadiene)	217
Parent heteroannular diene	214
Double bond extending the conjugation	30
Alkyl substituent or ring residue	5
Exocyclic location of double bond to any ring	5
Groups: OAc, OR	0
Solvent correction (see Table 14.3)	( )
λ_{max}^{EtOH} =	Total

TABLE 14.2 • Rules for Predicting λ_{max} for Conjugated Enones and Dienones:



	Increment (nm)
Parent α,β-unsaturated system	215
Double bond extending the conjugation	30
R (alkyl or ring residue), OR, $OCOCH_3$	
α	10
β	12
γ, δ and higher	18
α-Hydroxyl, enolic	35
α-Cl	15
α-Br	23
exo-Location of double bond to any ring	5
Homoannular diene component	39
Solvent correction (see Table 14.3)	( )
λ_{max}^{EtOH} =	Total

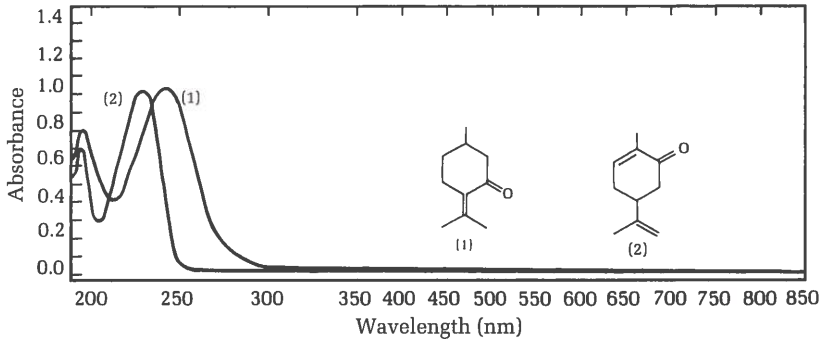


FIG. 14.4
The UV spectra of (1) pulegone and (2) carvone in hexane.

TABLE 14.3 • Solvent Correction

Solvent	Factor for Correction to Ethanol
Hexane	+11
Ether	+7
Dioxane	+5
Chloroform	+1
Methanol	0
Ethanol	0
Water	−8

TABLE 14.4 • Calculation of λ_{max} for Pulegone (See Fig. 14.4)

Parent α,β-unsaturated system	215 nm
α-Ring residue, R	10
β-Alkyl group (two methyls)	24
Exocyclic double bond	5
Solvent correction (hexane)	−11
Calculated λ_{max} =	245 nm; found = 244 nm

TABLE 14.5 • Calculation of λ_{max} for Carvone (See Fig. 14.4)

Parent α,β-unsaturated system	215 nm
α-Alkyl group (two methyls)	10
β-Ring residue	12
Solvent correction (hexane)	−11
Calculated λ_{max} =	226 nm; found = 229 nm

Woodward and Fieser rules for dienes and dienones

The wavelengths of maximum absorption of conjugated dienes and polyenes and conjugated enones and dienones are given by the Woodward and Fieser rules (Tables 14.1 and 14.2). The application of the rules is demonstrated by the spectra of pulegone (1) and carvone (2) in Figure 14.4. The solvent correction is given in Table 14.3. The calculations are given in Tables 14.4 and 14.5.

No simple rules exist for the calculation of aromatic ring spectra, but several generalizations can be made. From Figure 14.5 it is obvious that as polynuclear aromatic rings are extended linearly, λ_{max} shifts to longer wavelengths.

Effect of acid and base on λ_{max}

As alkyl groups are added to benzene, λ_{max} shifts from 255 nm for benzene to 261 nm for toluene to 272 nm for hexamethylbenzene. Substituents bearing nonbonding electrons also cause shifts of λ_{max} to longer wavelengths—for example, from 255 nm for benzene to 257 nm for chlorobenzene, 270 nm for phenol, and 280 nm for aniline (ε = 6200–8600). That these effects are the result of the

FIG. 14.5
The UV spectra of (1) naphthalene, (2) anthracene, and (3) tetracene.

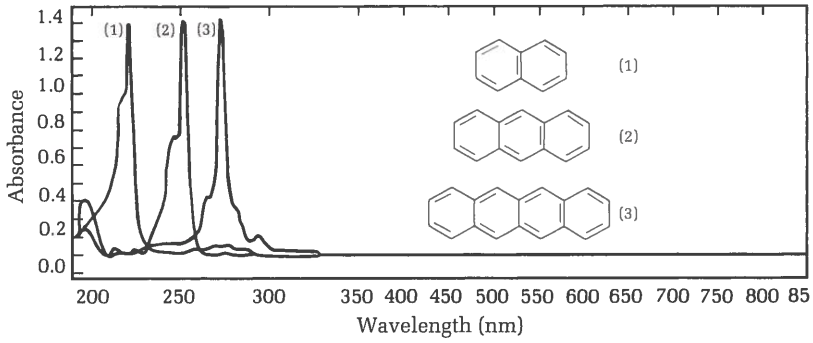


FIG. 14.6
The UV spectra of (1) neutral vanillin and (2) the anion of vanillin.

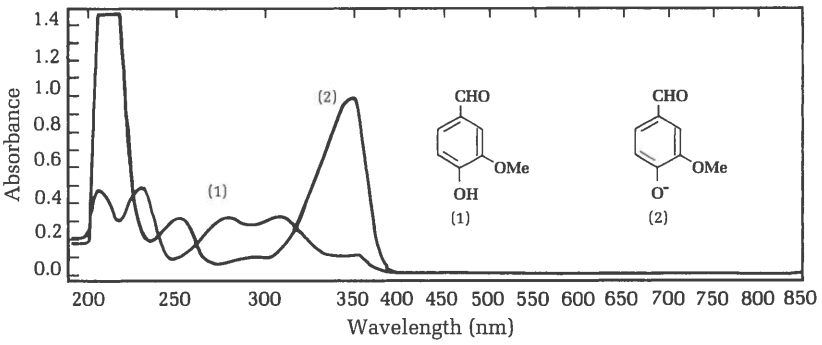
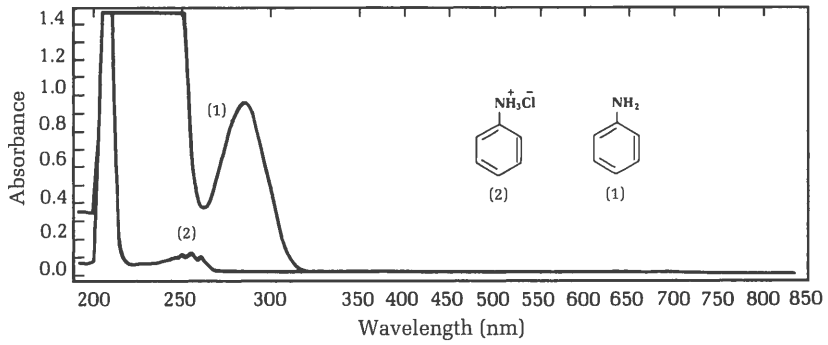


FIG. 14.7
The UV spectra of (1) aniline and (2) aniline hydrochloride.



interaction of the π-electron system with nonbonded electrons is seen dramatically in the spectra of vanillin and the anion derived by deprotonation of its phenolic OH (Fig. 14.6). The two additional nonbonding electrons in the anion cause λ_{max} to shift from 279 nm to 351 nm and ε to increase. Protonation of the nonbonding electrons on the nitrogen of aniline to give the anilinium cation causes λ_{max} to decrease from 280 nm to 254 nm (Fig. 14.7). These changes of λ_{max} as a function of pH have obvious analytical applications.

Intense bands result from π-π conjugation of double bonds and carbonyl groups with the aromatic ring. Styrene, for example, has λ_{max} = 244 nm (ε = 12,000), and benzaldehyde has λ_{max} = 244 nm (ε = 15,000).

EXPERIMENT

UV Spectrum of an Unknown Acid, Base, or Neutral Compound

Determine whether an unknown compound obtained from your instructor is acidic, basic, or neutral from the UV spectra in a neutral solvent such as pure ethanol or methanol as well as under acidic conditions (add 1 drop of 5% HCl to the solution in the cuvette and mix) and basic conditions (add 1 drop of 1 *M* NaOH to the acidic solution in the cuvette, mix, and check that the pH is basic).

Cleaning Up. Because UV samples are extremely dilute solutions in ethanol or methanol, they can normally be flushed down the drain.

Refractive Indices

The *refractive index*, symbolized by n, is a physical constant that, like the boiling point, can be used to characterize liquids. It is the ratio of the velocity of light traveling in air to the velocity of light moving in the liquid (Fig. 14.8). It is also equal to the ratio of the sine of the angle of incidence ϕ to the sine of the angle of refraction ϕ':

$$n = \frac{\text{velocity of light in air}}{\text{velocity of light in liquid}} = \frac{\sin \phi}{\sin \phi'}$$

The angle of refraction is also a function of temperature and the wavelength of light (consider the dispersion of white light by a prism). Because the velocity of light in air (strictly speaking, a vacuum) is always greater than that through a liquid, the refractive index is a number greater than 1, for example, hexane, $n_D^{20} = 1.3751$; diiodobenzene, $n_D^{20} = 1.7179$. The superscript 20 indicates that the refractive index was measured at 20°C, and the subscript D refers to the yellow D-line from a sodium vapor lamp, which produces light with a wavelength of 589 nm.

The measurement is made on a refractometer using a few drops of liquid. Compensation is made within the instrument for the fact that white light, not sodium vapor light, is used, and a temperature correction must be applied to the observed reading by adding 0.00045 for each degree above 20°C:

$$n_D^{20} = n_D^t + 0.00045(t - 20°C)$$

The refractive index can be determined to 1 part in 10,000, but because the value is quite sensitive to impurities, full agreement in the literature regarding the last figure does not always exist. For this reason, the refractive indices in this book have been rounded to the nearest 1 part in 1000, as have the refractive indices reported in the Aldrich catalog of chemicals. To master the technique of using the refractometer, measure the refractive indices of several known, pure liquids before measuring an unknown.

Specialized hand-held refractometers that read over a narrow range are used to determine the concentration of sugar, salt, or alcohol in water.

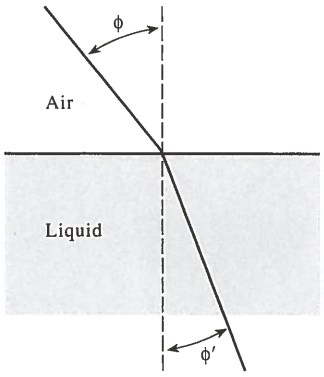


FIG. 14.8
The refraction of light.

Using a Refractometer

Refractometers come in many designs. In the most common, the Abbé design (Fig. 14.9), two or three drops of the sample are placed on the measuring prism using a polyethylene Beral pipette (to avoid scratching the prism face). The illuminating prism is closed, and the lamp is turned on and positioned for maximum brightness as seen through the eyepiece. If the refractometer is set to a nearly correct value, then a partially gray image will be seen, as shown in Figure 14.10a. Turn the index knob so that the line separating the dark and light areas is at the crosshairs, as shown in Figure 14.10b. Sometimes the line separating the dark and light areas is fuzzy and colored (Fig. 14.10c). Turn the chromatic adjustment until the

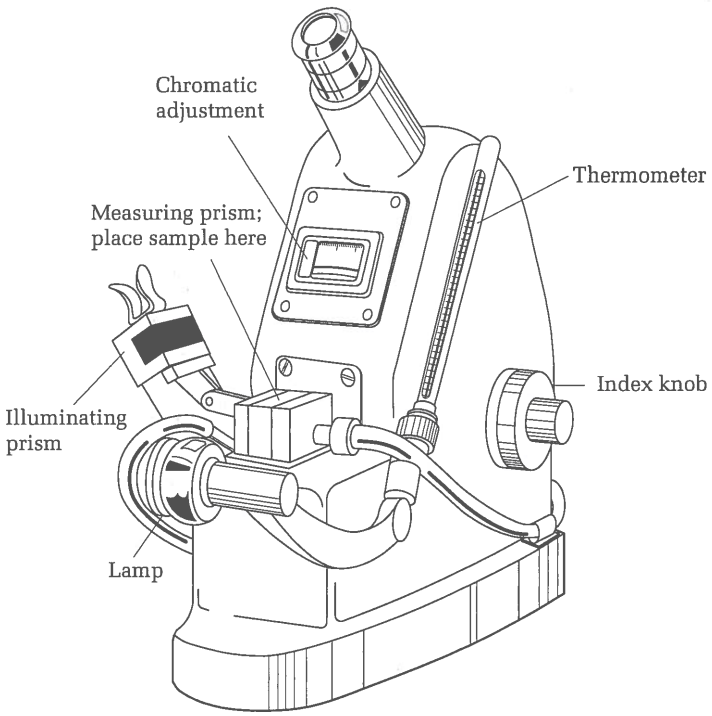


FIG. 14.9
An Abbé refractometer. The sample block can be thermostatted.

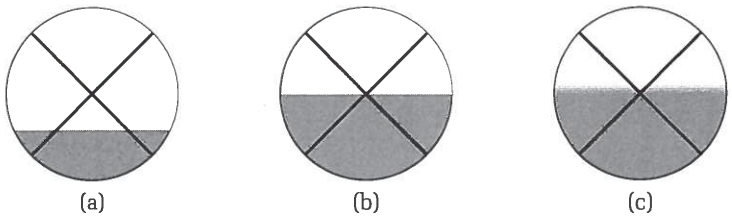


FIG. 14.10
(a) The view into a refractometer when the index knob is out of adjustment. (b) The view into a refractometer when properly adjusted. (c) The view when the chromatic adjustment is incorrect.

For most organic liquids, the index of refraction decreases approximately 0.00095 ± 0.0001 for every °C increase in temperature.

demarcation line is sharp and colorless. Then read the refractive index. On a newer instrument, press a button or hold down the on/off switch to light up the scale in the field of vision or give the digital readout. On older models, read the refractive index through a separate eyepiece. Read the temperature on the thermometer attached to the refractometer and make the appropriate temperature correction to the observed index of refraction. When the measurement is completed, open the prism and wipe off the sample with lens paper, using ethanol, acetone, or hexane only as necessary.

Qualitative Instrumental Organic Analysis



General Resources
Qualitative Organic Analysis

As indicated in many of the previous chapters, physical characterization and structural elucidation are major activities in organic chemistry. In many areas, such as drug metabolism studies and forensic or environmental chemistry, only milligram or microgram quantities of organic compounds are available. Fortunately, today's instrumental methods have the requisite sensitivity to meet this challenge. It may be possible to establish the structure of a compound on the basis of spectra alone (IR, NMR, MS [mass spectrometry], and/or UV), but often these spectra must be supplemented with other information about the unknown: physical state, solubility, and confirmatory tests for functional groups. Before spectra are run, other information about the unknown must be obtained. Is it pure or a mixture (test by thin-layer [TLC], gas, or liquid chromatography)? Once a substance is known to be homogeneous, it can often be identified by spectroscopy alone. What are its physical properties: melting point, boiling point, and color and solubility in various solvents, such as those commonly used in NMR? A mass spectrum can determine a compound's molecular weight and, if measured with sufficient accuracy, identify the elements present and the molecular formula.

For the millions of organic compounds that have been synthesized or isolated from nature, spectral data are included when they are reported in the chemical literature. Thousands of the more common chemicals that can be purchased or easily synthesized have had their IR, NMR, MS, and UV spectra printed in multivolume collections that can be searched by compound class, formula, or spectral features. Today, these collections have been converted into digital form that can be searched by computer and compared with the spectra obtained for an unknown, yielding a list of all closely matching compounds. If two substances have identical IR spectra, they can be regarded as identical. Such is not always true of other spectra. Many substances can have identical or nearly identical UV spectra, and it is possible for the MS or NMR spectra for two different substances to be almost identical. When new substances are encountered in research laboratories, their spectra can be compared with those in commercial databases. Even though the particular new compound is not represented in those databases, a list of very similar substances can be generated, which will guide the determination of the structure of the new substance.

EXPERIMENT

Identifying an Unknown Compound

Most students consider the identification of an unknown organic compound to be one of the most enjoyable and challenging organic lab activities. In this laboratory course you may receive an "unknown" substance, which, of necessity, is usually a commercially available compound. At least initially, you may not be given access to a commercial spectral database for searching and comparison, but you will use the skills you have learned to interpret the spectral data you acquire. After you have done the best you can with your IR, NMR, and UV spectra and have arrived at a short list of possible compounds, you will be provided with the mass spectrum of your unknown. Interpretation of this spectrum usually eliminates most of the candidate structures and should allow you to complete the identification of the unknown.

Physical State

Check for Sample Purity

Distill or recrystallize as necessary. A constant boiling point and sharp melting point are indicators of purity but beware of azeotropes and eutectic substances. Check homogeneity by TLC, GC, or HPLC.

Note the Color

Common colored compounds include nitro and nitroso compounds (yellow), diketones (yellow), quinones (yellow to red), azo compounds (yellow to red), and polyconjugated olefins and ketones (yellow to red). Phenols and amines are often brown to dark purple because of traces of air oxidation products.

Note the Odor

Some liquid and solid amines are recognizable by their fishy odors; esters are often pleasantly fragrant. Alcohols, ketones, aromatic hydrocarbons, and aliphatic olefins have characteristic odors. On the unpleasant side in terms of odor are thiols, isonitriles, and low-molecular-weight carboxylic acids.

Ignition Test

Heat a small sample on a spatula; first hold the sample near the side of a microburner to see if it melts normally and then burns. Then heat directly in the flame. If a large ashy residue is left after ignition, the unknown is probably a metal salt. Aromatic compounds often burn with a smoky flame.

Beilstein Test for Halogens

Although the presence of a halogen can usually be determined by mass spectrometry, this test is so simple that it can easily be run to confirm the MS data. Heat the tip of a copper wire in a burner flame until no further coloration of the flame is