CHAPTER 19

SPECTROPHOTOMETRY

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INTRODUCTION

The electromagnetic spectrum of energy extends from the gamma rays emitted by radioactive elements with wavelengths of less than 0.1 nanometer to radio waves with a wavelength greater than 250 millimeters. However, this chapter will deal only with a very small section of this spectrum, namely the ultraviolet (185 to 380 nanometers), the visible (380 to 800 nanometers) and the infrared (0.8 to 50 micrometers). A schematic diagram is shown in Figure 19-1.

The terms used in spectrophotometry are gradually changing, and unless one is familiar with the old and new forms, confusion may result. The new and old terms are shown in Table 19-1.

Table 19-1.
Terms in Common Usage in Spectrophotometry

New	*Old*	*Value*
	Angstrom, A	10^{-10} meter
Nanometer	Millimicron	10^{-9} meter
Micrometer	Micron	10^{-6} meter
Millimeter	Millimeter	10^{-3} meter
Nanogram		10^{-9} gram
Microgram	Gamma	10^{-6} gram
Milligram		10^{-3} gram

Visible light can be divided into the six principal colors as shown in Table 19-2. These are the principal colors seen when white light is diffracted into its primary colors by a prism or diffraction grating. Various shades and tones of these colors are possible. When visible light is absorbed by a compound, there are resulting energy changes involving the valence electrons.

The ultraviolet portion of the spectrum is that portion of the sun's energy which causes sunburn and similar skin damage. The absorption of ultraviolet energy causes energy changes involving the ionization of atoms and molecules.

Infrared radiation is the portion of the spectrum associated with the generation of heat. Absorption of infrared energy results in molecular vibrations, such as bending or stretching of the interatomic bonds, and molecular rotation. The type of vibration is dependent on the wavelength of the incident radiation.

Table 19-2.
Principal and Complementary Colors

Transmitted Color	*Wavelength (nm)*	*Complementary Colors*
Violet	380-435	Yellowish Green
Blue	435-480	Yellow
Green	500-560	Purple
Yellow	580-595	Blue
Orange	595-650	Greenish Blue
Red	650-780	Bluish Green

When certain types of molecules absorb ultraviolet energy, energy in the ultraviolet or visible regions is emitted as the excess energy is released. This is called fluorescence and is a valuable tool for the chemist in identifying and quantitating such compounds.

The absorption of energy by solutions follows two basic laws. The Bouguer (1729), or Lambert (1760), law states that when a beam of plane-parallel monochromatic light enters an absorbing medium at right angles to the plane surfaces of the medium, the rate of decrease in intensity with the length of the light path through the absorbing medium is proportional to the intensity of the beam. Mathematically this can be expressed as

$$I = I_o\, e^{-Kb}$$

where

I = Unabsorbed Intensity
I_o = Incident Intensity
K = Constant
b = Cell Thickness (light path length)

Bernard's (1852), or Beer's (1852), law

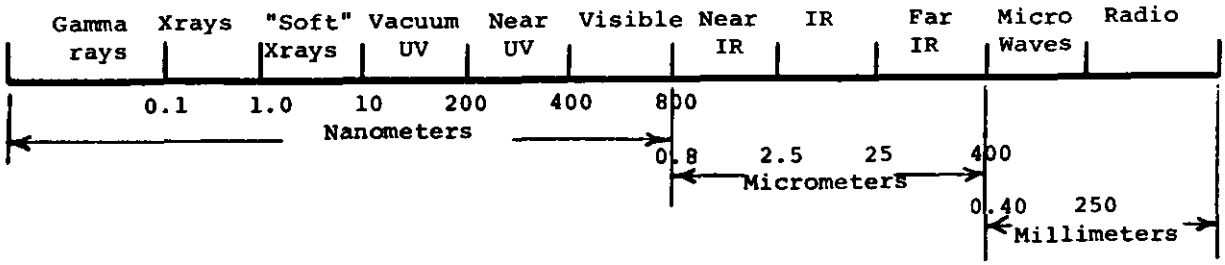


Figure 19-1. Schematic Diagram of Electromagnetic Spectrum. Note that the wavelength scale is not linear.

states that the intensity of the energy decreases exponentially with the increase in concentration. Mathematically this can be written as

$$2.303 \log \frac{I_o}{I} = K' C$$

Where C is the concentration of the absorbing material.

Combination of these two laws forms the basic law of spectrophotometry. It takes the form of:

$$\log \frac{I_o}{I} = a\,b\,c$$

where —a— is the absorptivity, a constant dependent upon the wavelength of the radiation and the nature of the absorbing material, whose concentration —c— is expressed in grams per liter. The product of the absorptivity and the molecular weight of the absorbing substance is called the "molar absorptivity" (ε).

Absorbance A is the product of the absorptivity, the optical pathlength and the concentration; i.e.,

$$A = a\,b\,c$$

The absorbance of a 1-cm layer of a solution containing 1 percent by weight of the absorbing solute is represented by the term $A\ \frac{1\%}{cm}$. The term "transmittance %" is the percent of the incident light passing through the absorbing solution and is related to the concentration exponentially. Thus, when the transmittance is plotted against concentration on semi-logarithmic graph paper a straight line should result if the system follows Beer's law.

Absorbance is directly related to concentration and can be plotted against concentration on linear coordinate graph paper. When such a plot gives a straight line the system follows Beer's law.

Most dilute systems will follow Beer's law over a limited range of concentration. Beer's law requires monochromatic radiation. However, most spectrophotometers and all filter photometers employ a finite group of light frequencies. The wider the band of the radiation, the greater will be the deviations from Beer's law. Temperature changes, ionization of the solute, stray light and changes in the pH of the solute, may cause deviations from Beer's law.

While it is desirable to have the analytical system follow Beer's law, it is not essential if a good reproducible calibration curve can be prepared.

VISIBLE LIGHT SPECTROPHOTOMETRY

Introduction

Analytical methods utilizing the visible section of the electromagnetic spectrum are of great importance. Most methods for the determination of metals in trace concentrations involve the production of a colored complex with some organic reagent. To be of value for analytical purposes the color-producing reaction should have the following characteristics:

1. Reagent and the color complex should be stable.
2. The reaction should be stoichiometric.
3. The color development should be rapid and color should resist fading.
4. The reaction should be specific for the element to be determined.
5. The reaction should show no more than minor variation with pH, temperature and other factors.
6. The color complex should be soluble in a solvent which is transparent in the area of spectral absorbance of the complex.
7. The color complex should have a sharp absorbance band.

Methods of color development generally fall into the following categories:

1. redox methods
2. complex formation
3. diazo and coupling reactions
4. condensations and addition
5. salt formation
6. chromophoric changes in valence
7. substitution.

Color procedures used may be classed as either single or mixed color. In the single color procedure, the color producing reagent is either colorless or the excess reagent is removed from the solution by suitable extractions. An example of this is the color complex formed with hexavalent chromium by s-diphenylcarbazide. Here the reagents are colorless but react with hexavalent chromium to produce a red complex. Oxidation of manganese to permanganate is another example of a single color method. The absorbance spectrum of the permanganate ion is shown in Figure 19-2. This ion shows a strong absorbance at 525 nanometers.

The determination of lead with dithizone (diphenylthiocarbazone) is an example of the mixed color technique. Dithizone dissolved in chloroform has a bright green color with a maximum absorbance at 625 nanometers as shown in Figure 19-3. In this figure Curve 1 is the absorbance spectrum of the dithizone solution which has been used to extract the reagent blank. It shows a zero absorbance or 100 percent transmittance at 610 nanometers and an absorbance at 515 nanometers which is due to traces of lead in the reagents. Curve 2 is the spectrum of the dithizone after extracting a solution containing 10 micrograms of lead. The increase in absorbance at 515 nanometers is evident. Figure 19-4 illustrates the change in absorbance as the lead dithizonate increases from 0.0 to 3.0 micrograms per ml of chloroform. The absorbance is set at 0.0 with the reagent blank. This will correct for any lead in the reagents. Then the absorbance is measured for the three standard solutions. It is evident that the standards follow Beer's law.

As a general rule, the complementary color is used to measure any colored complex. For example, if the solution to be measured is red, green light should be used.

When any colored reaction is used, it is advisable to determine the absorbance spectrum of the reaction product with the spectrophotometer to be used for the analysis. From this spectrum the proper wavelength to be used for the pro-

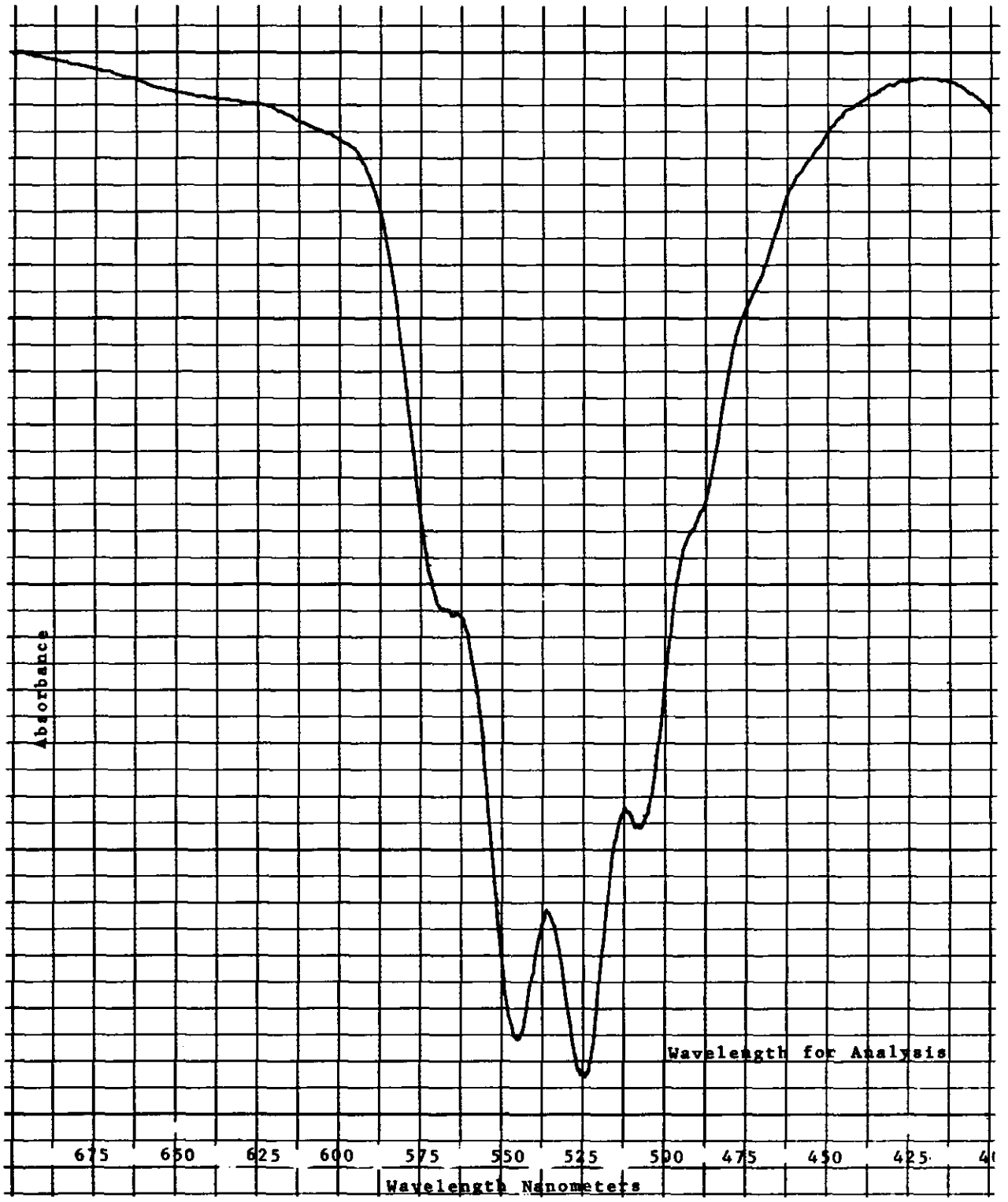


Figure 19-2. Visible Absorbance Spectrum of Permanganate Ion in Water.

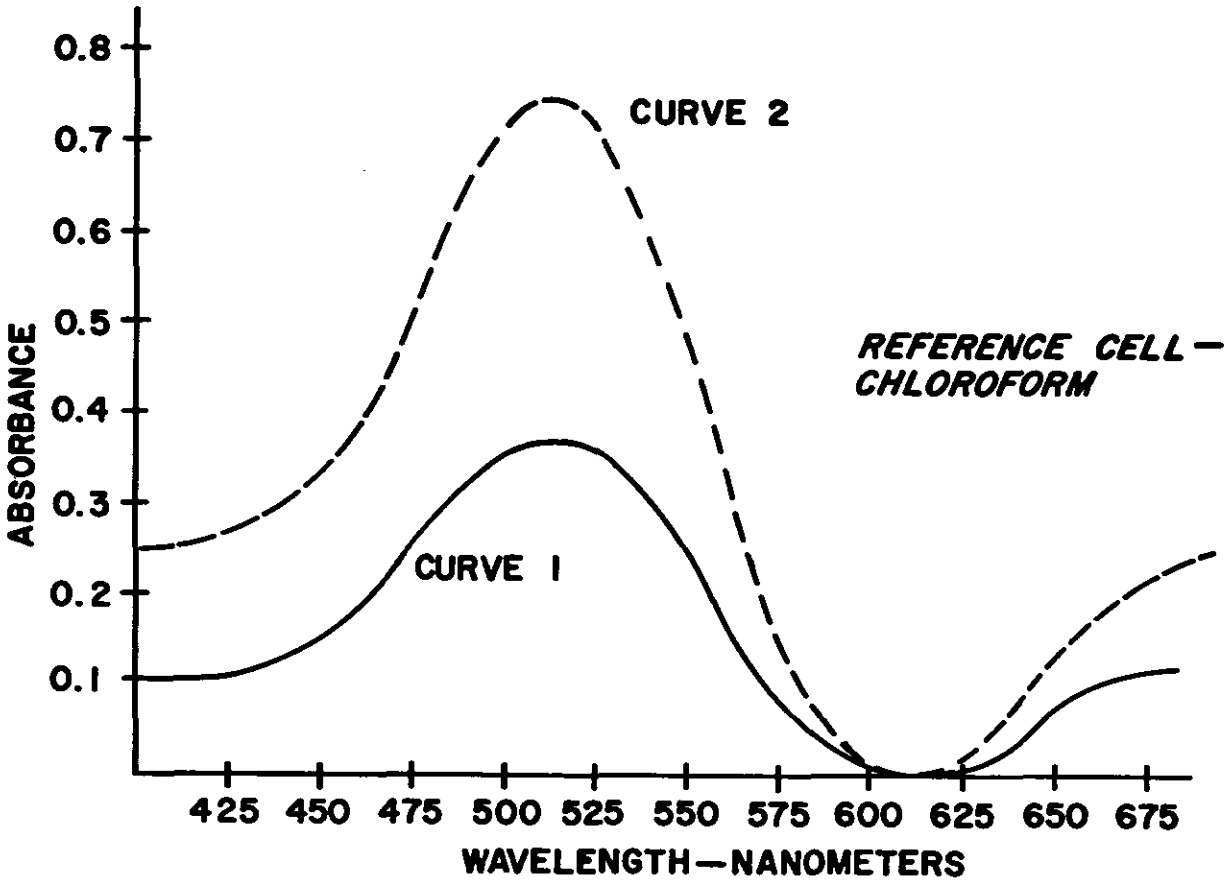


Figure 19-3. Absorption Spectra of Dithizone and Lead Dithizonate in Chloroform.

cedure will be evident and any instrumental variation or shifts in the wavelength scale of the particular instrument will be corrected.

In some instances, it is possible to utilize the bleaching effect of some ion on a colored organometallic complex to measure the concentration of the ion of interest. An example of this is the bleaching effect of fluoride ion on thorium or zirconium alizarin lakes. In this case, the loss of color of the lake is directly proportional to the amount of fluoride present.

While many color-producing reactions are available, some ions of interest do not form colored complexes that are suitable for analytical procedures. Frequently it is possible to produce a suspension of a finely divided uniformly sized precipitate. When a beam of light is passed through such a suspension, energy is lost due to light scattering. Under proper conditions this loss is proportional to the amount of precipitate. This analytical technique is called nephelometry. Such procedures require very rigid control of all conditions such as temperature, pH and concentrations of reagents to produce uniform size precipitates or reproducible results cannot be obtained. Procedures for the determination of chloride, as silver chloride, and sulfate, as barium sulfate, are examples of the application of this technique.

Instrumentation

The first techniques of spectrophotometry involved the direct comparisons of colors produced in unknown solutions with those of standards prepared under similar conditions. Observations were made with the naked eye using a common light source. Such techniques can still be used to obtain a rough estimate of the concentration. From this beginning, Nessler tubes developed. The color reaction is carried out in both a series of standards and unknowns. The solutions are placed in a series of long flat-bottom tubes and diluted so that the column of solution is either 10 or 20 centimeters in depth. After mixing, the color of the unknowns is matched against the standards. The unknown solutions can be bracketed between two standards and a rough approximation of the concentration can be made.

The Duboscq colorimeter developed from this technique. Light illumination from a common light source is passed up through the bottom of a pair of matched cups, through the solution and through a matched set of glass plungers. A prism system brings the light beams to a common axis. Light from each cup illuminates one-half of the viewed field. The intensities of the two halves of the viewed field are matched visually by raising or lowering the plunger in one cup. The depth is

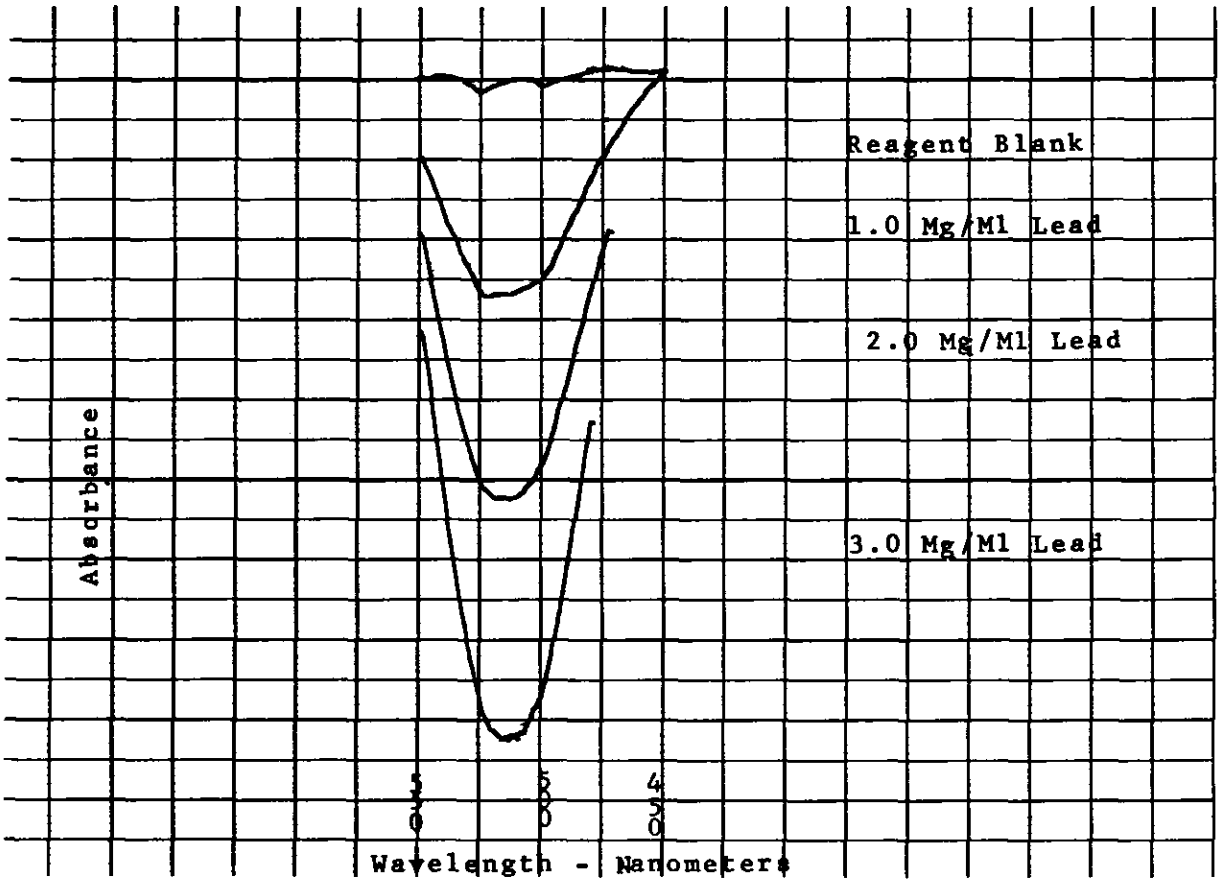


Figure 19-4. Absorption Spectra of Lead Dithizonate.

measured on a scale. From previously prepared calibration curves it is possible to estimate the concentration. The ability of the eye to match intensities varies with wavelength and intensity. The eye is best at about 500 nanometers and under the best conditions can be accurate to within 1 or 2 percent.

Another version of this technique is the wedge comparator. The light beam is split into two segments. One passes through the standard solution and one through the unknown. A neutral wedge of glass is moved into the exit beam of the standard solution to attenuate the light intensity until it matches the unknown.

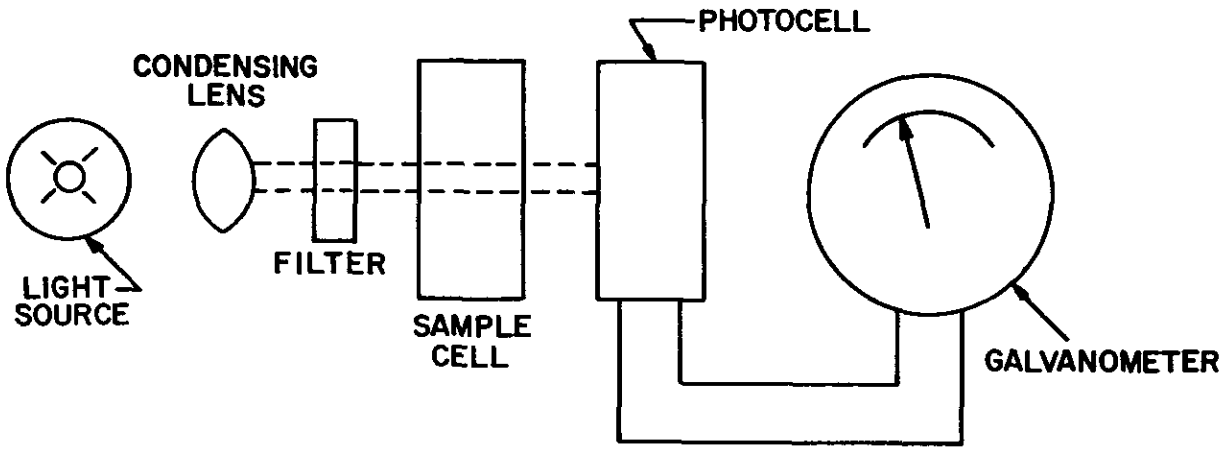
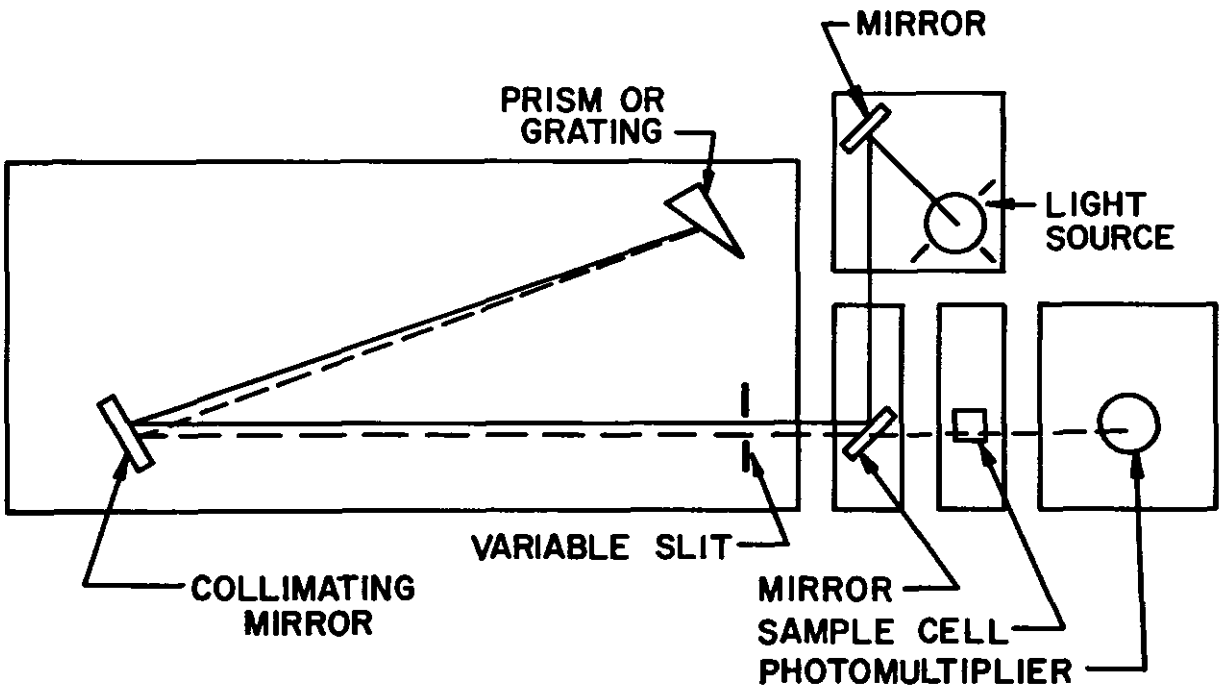


Figure 19-5. Schematic Diagram of Filter Photometer.

The next instrumental development was the single-beam photometer. The basic design of this type of instrument is shown in Figure 19-5. The light from the source passes through a filter, through the solution, and strikes a photocell. With the solvent in the light path and the proper filter in position the light intensity is adjusted to give a reading of 100 on the scale. Next the standards are inserted and the scale readings are recorded. Then the unknown solutions are inserted and read. The intensity of the light source can be adjusted either by a rheostat in series with the light source or a diaphragm in the light path.

To eliminate errors due to variations in the light source with time, the double-beam photometer was developed. In this instrument the filtered light is separated into two beams. One beam is reflected to a reference photocell. The remaining beam passes through the solution to be measured and strikes a second photocell. The net output of the two photocells, connected in opposition, is balanced by a variable resistor to give a zero reading on a galvanometer for the blank solution. The standards and unknowns are inserted into the beam and the deflections of the galvanometer are read; from the calibration curve the concentrations of the unknown can be determined. Filters available for these instruments were generally wide band-pass filters and lacked the narrow spectral band width required by Beer's Law.



Beckman Instruments, Inc.: Bulletin 134-D. Fullerton, California.

Figure 19-6. Schematic Diagram of Prism or Grating Spectrophotometer.

The next development was the prism or grating single-beam spectrophotometer. The basic instrumental design is shown in Figure 19-6. In this instrument light from the source is refracted by a prism or diffracted by a grating into its spectrum. A series of adjustable exit slits limits the wavelengths striking the sample. This spectrophotometer has a narrow band-pass which improves the conformity to Beer's Law. The position of the grating or the prism is set to the proper wavelength and the blank is inserted in the beam. After balancing the instrument with the shutter closed, the shutter is opened and the meter set to 100 percent transmittance or 0 absorbance by adjustment of the slit width and the sensitivity control. The standards are inserted in the beam and the absorbance determined for each concentration. The absorbance of the unknowns can be measured and the concentration determined from the calibration curve.

The light-measuring devices used in this type of instrument are either photocells or photo-multiplier tubes. Generally two photocells are available to cover the entire spectral range. These instruments are much more expensive than photometers but give greater accuracy and reproducibility as well as monochromatic character of the light used in the analysis.

A more recent development has been the ratio-recording spectrophotometer. In this instrument the light beam from the source is refracted by a prism and strikes a rotating segmented disc. One half of the disc is open allowing the beam to pass through a reference cell and eventually strike a detector. The other half of the disc is a mirror that reflects the light through the sample cell to the detector. The detector system produces a reading which is the ratio of the two beams. Such instruments eliminate all variations due to voltage or electronic fluctuations. Any of the newer in-

struments can be coupled to a recorder to give a permanent record of the results.

Applications

Visible spectrophotometry has many uses in the analyses needed in environmental control work. Several examples are discussed.

1. Biological Analysis.

Frequently, the measurement of some ion in a biological specimen is the best indicator of an exposure. The analysis of blood or urine for lead is an excellent means of evaluation of the worker's exposure. The blood or urine is ashed, the ash dissolved and extracted with a chloroform solution of dithizone at pH of 9.5. The lead reacts with the dithizone to form lead dithizonate. The lead dithizonate in the dithizone-chloroform solution is determined at 510 nanometers. By proper control of pH and use of complexing agents it is possible to eliminate interferences from other metallic ions.

The determination of manganese in urine is a useful measure of the exposure of workers to manganese. The urine is ashed and the manganese is oxidized to permanganate ion. The color of permanganate can be measured at 525 nanometers.

The determination of mercury in urine has taken on increased importance with the recent emphasis on mercury pollution. The urine is ashed under a reflux condenser, extracted with dithizone in carbon tetrachloride. The dithizone mercury solution is further extracted with 9N ammonium hydroxide twice to remove the unreacted dithizone leaving the single color of the mercury dithizonate. This is measured at a wavelength of 475 nanometers.

Many other ions of interest in environmental control work may be determined by spectrophotometric procedures.

2. Air Sample and Sample Analysis.

Lead can be determined in air samples by dithizone after ashing and solution of the sample. Under proper conditions the reaction is specific.

Several methods are available for the determination of iron. Under proper conditions, iron as ferric chloride in 28 percent hydrochloric acid can be measured directly at 460 nanometers. This method is not specific, for other colored metals in solution may affect the results. Specific reagents such as dipyridyl and ortho-phenanthroline are available for the determination of iron in trace quantities.

As mentioned earlier, manganese can be oxidized to permanganate ion whose concentration can be measured at 525 nanometers. While the reaction is specific for manganese, the presence of easily oxidized materials can reduce the permanganate ion and seriously affect the results.

Arsenic can be vaporized as arsine and absorbed in a solution of silver diethyldithiocarbamate in chloroform solution. The color produced is measured at 560 nanometers and the arsenic content determined from a calibration curve.

Chromium, in the hexavalent state, can be complexed with s-diphenylcarbazide and read at 540 nanometers. The reaction under the proper conditions is specific.

Aldehydes after absorption in sodium bisulfite solution can be determined using Schiff's reagent. The reaction is not specific for any one aldehyde in the presence of other aldehydes. The measurement is made at 560 nanometers.

Sulfates as barium sulfate, or chloride as silver chloride may be measured by nephelometric techniques under carefully controlled conditions. All reagents, except the precipitating agent, are added and the solution diluted to volume. The absorbance is measured at 500 nanometers. The precipitating agent is then added and the precipitate allowed to form for a specific time period. The absorbance is again measured at the same wavelength. The difference in absorbance is a measure of the sulfate or chloride ion.

ULTRAVIOLET SPECTROPHOTOMETRY

Many compounds containing specific types of chemical bonds will absorb ultraviolet light strongly at very specific wavelengths. The ultraviolet spectrum from 210 to 380 nanometers is of most interest since the ultraviolet spectrophotometers in use are not capable of operation below 210 nanometers. Some compounds of environmental interest, such as ketones, aldehydes, esters and organic acids absorb below this point.

However, many other compounds of great interest do absorb ultraviolet light above 210 nanometers. In general, all aromatic compounds such as benzene, toluene, and xylene have strong absorbing bands. The absorbance spectra of benzene and toluene vapors are shown in Figure 19-7. The spectra of these two compounds show many relatively intense absorption bands with good resolution. While these two compounds are homologs, their spectra are quite different. Figure 19-8 gives the spectra of different concentrations of benzene dissolved in cyclohexane. While the resolution is not as good as that observed in the vapor state, it is still sufficient to identify the compound. Even at a concentration as low as 0.20 milligram per milliliter appreciable absorption occurs at 254.6 nanometers. Phenolic type compounds also exhibit strong absorbance bands in the ultraviolet region.

Some inorganic materials show strong absorbances in the ultraviolet range. Iodine absorbs strongly at 352 and 440 nanometers. Nitrates and nitrites absorb at 270 and 225 nanometers, respectively.

One of the prime requirements for analytical work in this section of the spectrum is a solvent that is relatively transparent to ultraviolet light. A solvent must have a cutoff point well below the absorbing band to be measured. The cutoff point in the ultraviolet region is the wavelength at which the absorbance of a 10-mm path length approaches unity with water as the reference. Table 19-3 gives the cutoff wavelengths for many of the more common solvents.

Generally the solvents of greatest use are methanol, ethanol, isopropanol, isooctane, cyclohexane, sulfuric acid and water. Since absolute ethanol is distilled with benzene, it usually contains traces of benzene which make it unsatisfactory as a solvent.

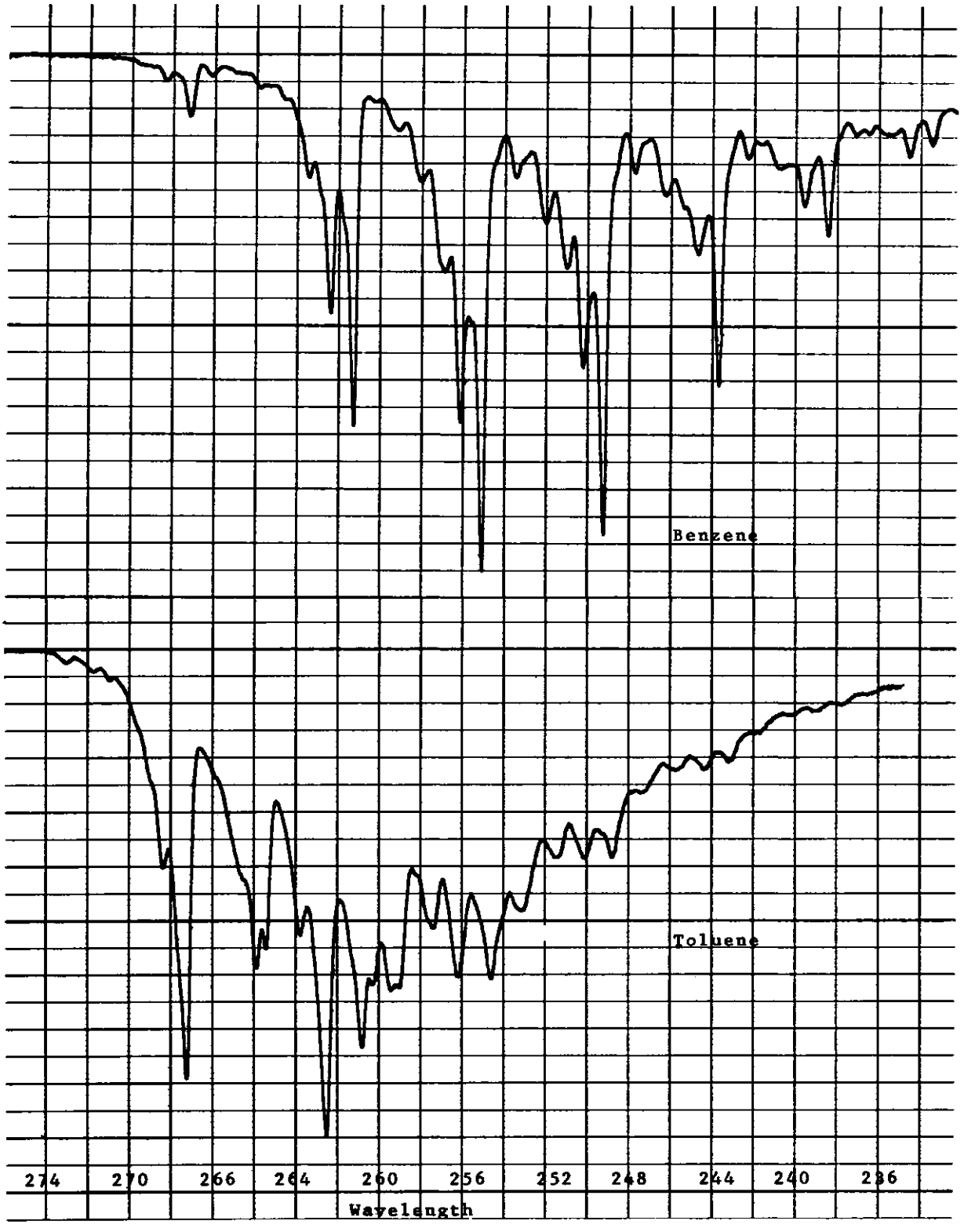


Figure 19-7. Ultraviolet Spectra of Benzene and Toulene Vapor.

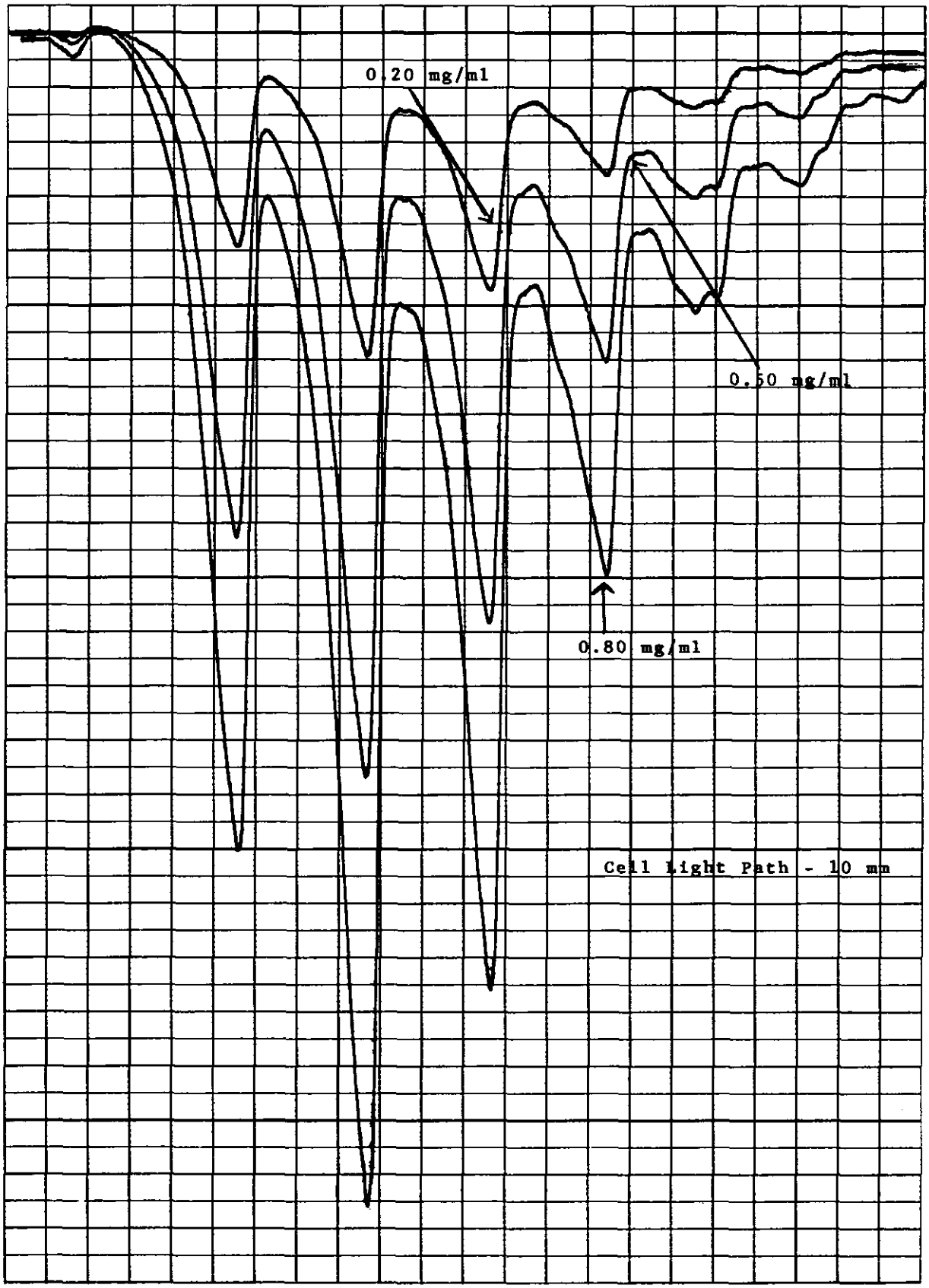


Figure 19-8. Ultraviolet Spectra of Benzene in Cyclohexane.

Table 19-3. Ultraviolet Cutoff Wavelength, Nanometers

Solvent	Grade	
	Reagent	Spectrographic
Acetone	327	330
Benzene	279	280
N-butanol	268	210
Carbon tetrachloride	263	265
Chloroform	245	245
Cyclohexane	210	
Ethanol	219	
Isooctane	220	210
Isopropanol	218	210
Methanol	218	210
Methylethyl ketone	327	
Nitric Acid — 6N	334	
Sulfuric Acid — 6N	215	
Trichloroethylene	287	
Tetrachloroethylene	292	290
Toluene	285	285
Water	212	

"Ultraviolet spectrophotometric and Fluorescence Data", J. A. Houghton and George Lee.
Am. Ind. Hyg. J. Vol. 22, No. 4, page 296, 301, 1961.

When the material of interest does not absorb in the ultraviolet region, it is sometimes possible to couple it with an absorbing compound to give an absorbing complex.

The sensitivity of ultraviolet methods is much greater than that found with either visible or infrared methods. Frequently, it is necessary to dilute an absorbing compound to the range of 1 microgram per milliliter to read the absorbance. A solution of styrene in cyclohexane as a concentration of 2 micrograms per milliliter gives an absorbance of 0.300 at a wavelength of 247 nanometers.

Much information can be obtained from the absorbance spectrum. If the system is essentially transparent in the region from 210 to 800 nanometers, it contains no conjugated unsaturated or benzenoid system, no aldehyde or keto groups, no nitro group and no bromine or iodine. When absorbance bands do appear, their wavelength will give some indication as to the identity of the group causing the absorbance. Several tables of chromophoric groups and their wavelengths have been published and can be used for identification.[1]

Instrumentation

Many types of ultraviolet spectrophotometers are available. They can be generally classed as single or double beam with either a prism or grating for the refraction or diffraction, respectively, of the spectrum. In the single-beam instruments, the unit is balanced against the solvent to read zero absorbance, then the sample is moved into the beam and the absorbance measured. Several of the visible range spectrophotometers can be converted to ultraviolet spectrophotometers by a suitable attachment.

In some double-beam instruments the beam is chopped by a rotating disc containing an open and a mirrored segment. The beam is sent through the sample cell and then through the reference cell. In other instruments the beam is split and sent through both the reference and the sample cells.

Initially, with the solvent in both the sample and the reference light paths the instrument is adjusted to give a zero absorbance at the wavelength to be used. When the sample is placed in the sample beam, the absorbance due to the cells and the solvent is cancelled and the instrument measures the absorbance of the solute only. An example of a double beam instrument is shown in Figure 19-9.

The single-beam instruments are much lower in price than the double-beam instruments. While it is not necessary to have a recording instrument, it is very convenient to have a recorded spectrum produced by the instrument. If not, the spectrum must be determined by a point-to-point scan and plotting of the points. This is a time-consuming operation.

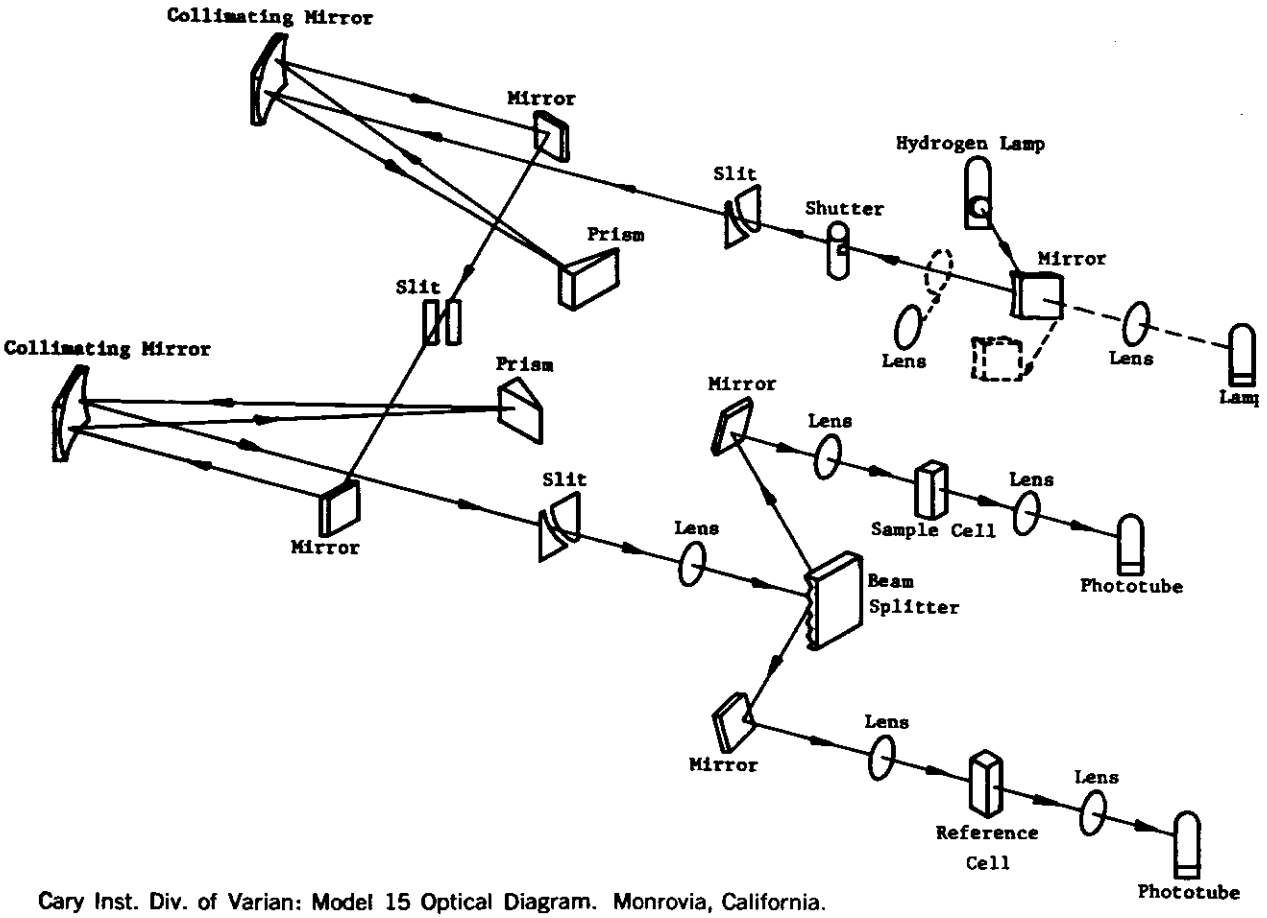
In ultraviolet spectrophotometry the cells used must be transparent to ultraviolet light. This requires that the cells usually be constructed of pure silica because the ordinary glass cells used in visible work absorb much of the ultraviolet. Silica cells are expensive and must be handled with care to prevent scratching and etching. Light sources used are generally either hydrogen, deuterium or xenon lamps. These sources all require special power supplies and in many cases auxiliary cooling systems. Many types of detector tubes may be used. All these tubes have their specific properties. In the less expensive instruments the 1P21 phototube is used as the detector. More expensive photomultiplier tubes are available which are more sensitive in specific regions.

In summary the ultraviolet spectrophotometer is a valuable tool for both the qualitative and quantitative analyses required in environmental work. It offers many possibilities for the analysis of air pollutants. At times it may be the only method available for the analysis of trace amounts of organic pollutants.

Applications

While it is not possible to discuss all the possible determinations that can be made with ultraviolet light, a few will be briefly discussed to illustrate possible procedures. If long-path gas cells are available for the spectrophotometer, it is possible to determine benzene, toluene or xylene directly in air samples. A standard curve is first prepared in the ppm (parts per million) range using the absorbance measurements obtained with known concentrations of the aromatic hydrocarbon vapor at the wavelength giving the maximum absorbance. The absorbance of the air samples are then determined under the same conditions. From the calibration curve prepared earlier it is possible to estimate the concentration of the aromatic hydrocarbon, directly in the air sample.

Where a suitable gas handling system or gas



Cary Inst. Div. of Varian: Model 15 Optical Diagram. Monrovia, California.

Figure 19-9. Schematic Diagram of Double Beam Spectrophotometer.

cells are not available, it is possible to absorb the aromatic hydrocarbon in a transparent solvent and determine it in this solvent as was shown earlier in Figure 19-8 of this section. Calibration curves must be prepared to compensate for incomplete absorption in the solvent.

It is possible to determine phenols and cresols in dilute sodium hydroxide solutions directly in the absorbing solution. The phenolic-type compounds are absorbed in a dilute caustic solution and after dilution the absorbance is measured at the wavelength of maximum absorbance. From previously prepared calibration curves the amount of phenolic compounds can be determined. It must be emphasized that it is not possible to identify the specific phenolic compound present. The results will give only total phenolic content.

The use of the ultraviolet spectrophotometer for the identification of polynuclear aromatic hydrocarbons was developed by Sawicki.[2] Benzene extracts of air samples are dissolved in a chlorinated solvent and passed through a chromatographic column. Specific sections of the column are extracted with a suitable solvent and the ultraviolet spectrum is determined. The polynuclear aromatic hydrocarbons can be identified and quantitated by the ultraviolet spectra using previously prepared calibration curves. More recent work has utilized the trapping of gas chromatographic peaks and the identification and quantitation of the material present by ultraviolet spectrophotometry.

With all the recent emphasis on mercury pollution, the ultraviolet absorbance spectrum of mercury vapor has been utilized for the quantitative determination of this element. Mercury strongly absorbs ultraviolet light of wavelength of 253.6 nanometers. Many direct reading instruments are available for the determination of mercury in air. Recent development of the mercury meter for water analysis also utilizes this property. The sample is ashed with potassium permanganate, sulfuric and nitric acids and reduced to elemental mercury with stannous chloride. The mercury is vaporized from the liquid by a stream of filtered air and passed through a cell and the absorbance of 253.6 nanometer ultraviolet light is measured. From previously prepared calibration curves the mercury content of the sample can be determined. It should be emphasized that any organic compound that absorbs at this wavelength and can be vaporized along with the mercury vapor into the gas cell will be determined as mercury and will cause high results. Acetone, for example, can cause serious interference in the determination of mercury.

While the above examples are only a few of the possibilities for the use of ultraviolet spectrometry, experience in the technique will open up many other uses. It is a powerful tool for the solution of many analytical problems.

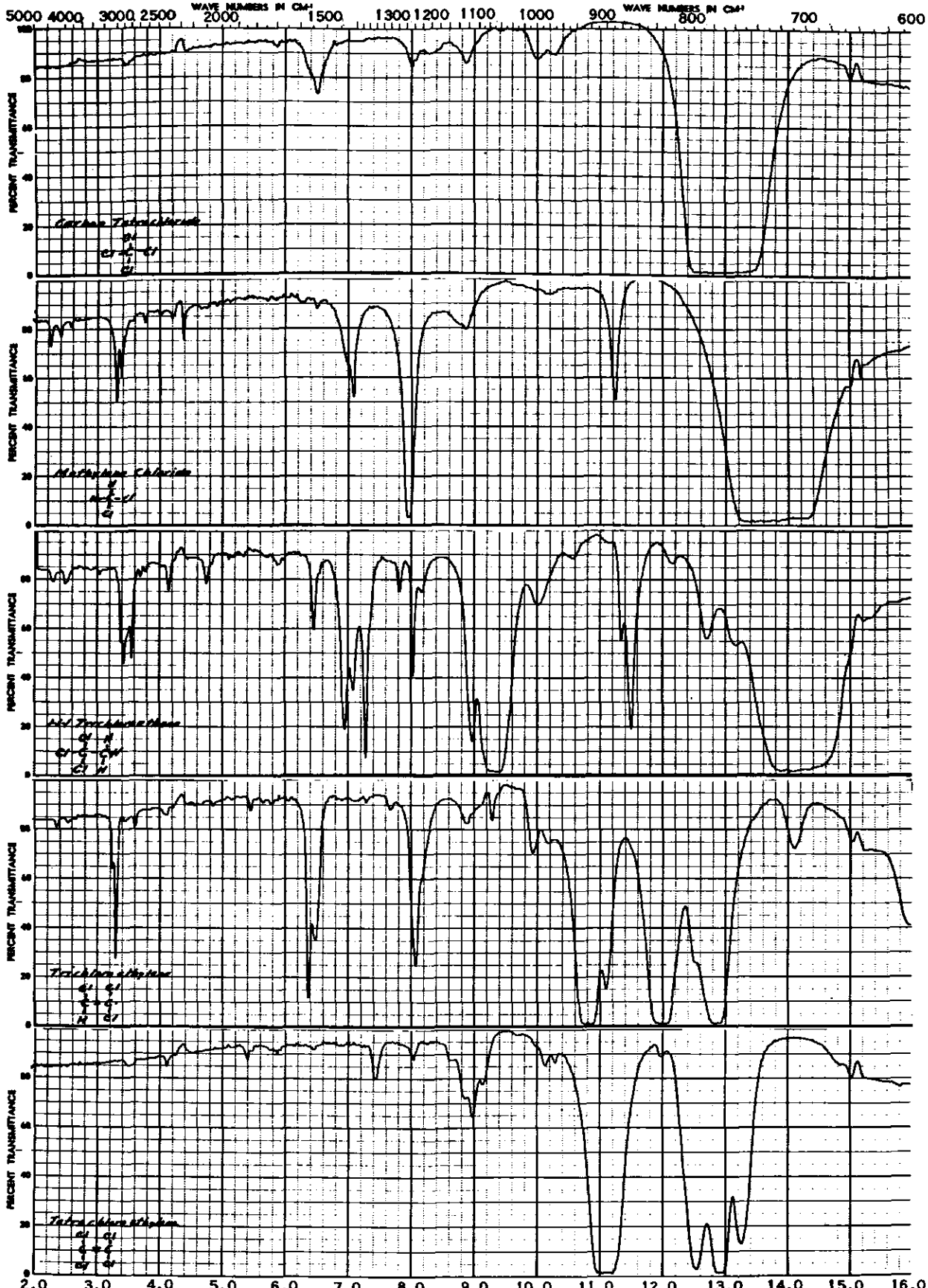


Figure 19-10. Infrared Spectra of Chlorinated Hydrocarbons — Wavelength in Micrometers.

INFRARED SPECTROPHOTOMETRY

Introduction

The section of the electromagnetic spectrum extending from 0.8 to 200 micrometers is classed as the infrared region. However, most of the analytical uses of this energy fall in the range of 0.8 to 50 micrometers, which can be explored with commercially available instruments. Absorption of energy in this section of the spectrum results from the vibrational-rotational stretching and bending modes in the molecule. The infrared absorption spectrum of a compound can be characterized as a fingerprint of that compound. The absorbance bands are so definite that it is possible to identify stereoisomers from their spectra. It is possible to define the structure of complex molecules, such as penicillin, from study of its infrared spectrum.

The infrared region is divided into three primary sections, the rock salt (sodium chloride) or fundamental region from 2 to 16 micrometers, the potassium bromide region from 10 to 25 micrometers and the cesium iodide region from 10 to 38 micrometers. These regions are so named because of the material used for the prisms and cell windows. Silica and glass cannot be used in infrared equipment since they absorb any energy with a wavelength above 4 micrometers.

Much valuable information can be gained from the absorbance bands found in the fundamental region. This section is usually divided into the "group frequency" region from 2.5 to 8 micrometers and the "fingerprint region" 8 to 16 micrometers.

In the group frequency region the principal absorption bands are primarily due to vibration of units consisting of only two atoms of the molecule, units which are more or less dependent only on the functional group giving the absorption and not on the complete molecule structure. Structural influences may cause small shifts in absorption bands from their normal position.

In the region from 2.5 to 4.0 micrometers the absorption is due to hydrogen stretching vibrations with elements of mass of less than 20. The center range from 4 to 6.5 micrometers is termed the unsaturated region. Primarily, triple bonds cause absorption from 4.0 to 5.0 micrometers. Double bonds frequently absorb in the region from 5.0 to 6.5 micrometers. Careful study of the absorption bands can help to identify and distinguish between C=O, C=C, C=N, and N=O bonds.

Absorptions in the region from 8.0 to 16.0 micrometers are single-bond stretching frequencies and bending vibrations of poly-atomic systems involving motions of bonds linking a substituent group to the remainder of the molecule. This is the fingerprint region. While too many absorption bands appear in this region to allow for specific identification it is possible to determine much information about the molecule. Ortho, meta and para substitutions are easily identified.

Chlorinated molecules absorb strongly in this region. Figure 19-10 shows the infrared spectra of five of the chlorinated hydrocarbons. Carbon tetrachloride and tetrachloroethylene show a complete absence of any absorption below 6 micrometers. As hydrogen is added to the molecule the bands at 3.3, 3.4, and 3.6 micrometers appear in the spectra. The intense absorption bands above 11 micrometers are typical of chlorinated compounds. From these spectra it is quite evident that there is little problem identifying the specific compound present. Generally for liquid work the light path is relatively short. These spectra were prepared using a cell with a light path of 0.25 mm.

Inorganic molecules also have characteristic absorption bands in the rock salt region. The inorganic material is generally ground to a very small particle size in a clear mineral oil and a mull prepared or it can be dispersed in potassium bromide powder and pressed into a pellet. As will be discussed later, methods are available to determine small amounts of alpha quartz in respirable dust by the pellet technique.

Some of the specific absorption bands of interest in environmental control work are shown in Table 19-4. Many more complete tabulations of absorption bands are available in the literature. One of the most useful is the COLTHUP chart.[5] From the data in Table 19-4 it is evident that the bands tend to overlap in some areas. For example the esters, acids, ketones and aldehydes all show strong absorption bands in the same region. Bands in the fingerprint region may make it possible to identify the particular type of compound present.

Table 19-4. Specific Infrared Absorption Bands

Grouping	Absorption Band Micrometers
Alkanes, CH_3-C,-CH_2=	3.35 to 3.65
Alkenes CH=CH_2	3.25 to 3.45
Alkyne C=C	3.05 to 3.25
Aromatic Hydrocarbons	3.25 to 3.35
Aromatic (Subst, benzenes)	6.15 to 6.35
Alcoholic (OH)	2.80 to 3.10
Acids (COOH)	5.75 to 6.00
Aldehydes (COH)	5.60 to 5.90
Ketones (C=O)	5.60 to 5.90
Esters (COOR)	5.75 to 6.00
Chlorinated (C-Cl)	12.80 to 15.50

It should be pointed out that while infrared is a very valuable analytical tool it does have its deficiencies. The sensitivity of infrared methods is much less than that for the ultraviolet methods.

As an example, in the determination of mineral oil using the 3 micrometer bands, the minimum concentration that can be determined with conventional cells is 1 milligram per milliliter of solvent.

Water and lower molecular weight alcohols cannot be used as solvents as they damage the cell windows. Moisture condensation on cell windows will also cause severe damage to the cell windows. In addition, water absorbs infrared energy strongly.

A solvent, to be of value in infrared work, should have as few absorption bands as possible and none in the region of interest. No organic solvent is completely transparent to infrared radiation. Carbon disulfide and carbon tetrachloride

are the common solvents. Carbon tetrachloride absorbs strongly from 12.5 to 13.5 micrometers while carbon disulfide absorbs strongly at 4.5 and 6.5 micrometers. Tetrachloroethylene is transparent except in the region from 10 to 16 micrometers. The high-boiling liquid Freons also are useful as solvents. The solvent may influence the spectrum of the solute. Particular care should be exercised in the selection of a solvent for compounds which are susceptible to hydrogen bonding effects. All solvents must be free of water.

Instrumentation

Basically, an infrared spectrophotometer consists of a source to produce the radiation, a monochromator to disperse the radiation, a sample compartment, a detector and a recorder. The equipment may be classified as either a single- or a double-beam system. In the single-beam system the beam passes through a single sample cell and to the detector. In this system it is necessary to determine the spectrum of the solvent, the combined spectrum of the solvent-solute mixture and then subtract the spectra to determine the net spectrum of the solute.

In the double-beam system the incident beam is chopped and sent alternately through the sample cell and then through the reference cell. The beams are then brought to the same detector. The detector balances the signal it receives from both cells by driving a comb in and out of the reference beam to alter the intensity of the reference beam to equal that of the sample beam. The position of the comb is transmitted to the recorder. An example of a double beam instrument is shown in Figure 19-11.

The source in most infrared spectrophotometers is either a Nernst glower or a Globar. The Nernst glower consists of a mixture of zirconium and yttrium oxides which is formed into a hollow rod 2 millimeters in diameter and 30 millimeters long. The surface temperature is between 1500° and 2000°C. The glower furnishes a wide range of infrared wavelengths, with maximum emission at 1.4 micrometers. A secondary heating source is necessary to light the glower since it is nonconducting when cold. It must be protected from drafts but still must be ventilated to remove the vaporized oxides and binders from the glower.

The Globar source is a solid rod of sintered silicon carbide. It is self-starting and is heated to 1300° to 1700°C. Maximum intensity occurs at 1.9 micrometers. Although it is less intense than the Nernst glower, it is more suitable for work beyond 15 micrometers, since its radiant energy output decreases less rapidly with increasing wavelength.

The monochromator is generally a Littrow mount. The beam from the collimating mirror is focused on the entrance slit. Either a grating or a prism may be used to disperse the incident beam. The prisms are made from single crystals of either sodium chloride or potassium bromide, depending upon the required working range of the instrument.

The grating provides better dispersion and thus better resolution but is usable over only a limited range. Two gratings are generally used to cover the entire range of the instrument so that each is used only in the first order.

The detectors are of a thermal type. Photoconductors are not applicable except in the near infrared region. A special type thermocouple is the most widely used. Quartz fibers are used to support a blackened gold foil receiver less than one micron in thickness to which is fastened the hot junction made by welding two different semiconductors together at one end. The semiconductors must have a high thermoelectric efficiency. The cold junction is maintained at a constant temperature and kept darkened. The pair is housed in an evacuated steel casing with a potassium bromide or cesium iodide window.

A second type of thermal detector is the bolometer. It produces an electrical signal as the result of a change in resistance of a metallic conductor with temperature as the infrared energy is absorbed.

Cells for infrared use consist of polished, optically flat discs of sodium chloride or potassium bromide separated by an amalgamated lead spacer and held liquid-tight by a holder. Liquid cell path lengths range usually from 0.1 mm down so that only a thin layer of the sample is exposed to the beam. Gas cells with path lengths of 1 meter are available for analysis of air pollutants. Infrared cells are very expensive and must be protected from any contact with moisture either in the sample or by exterior condensation to prevent etching of the crystals.

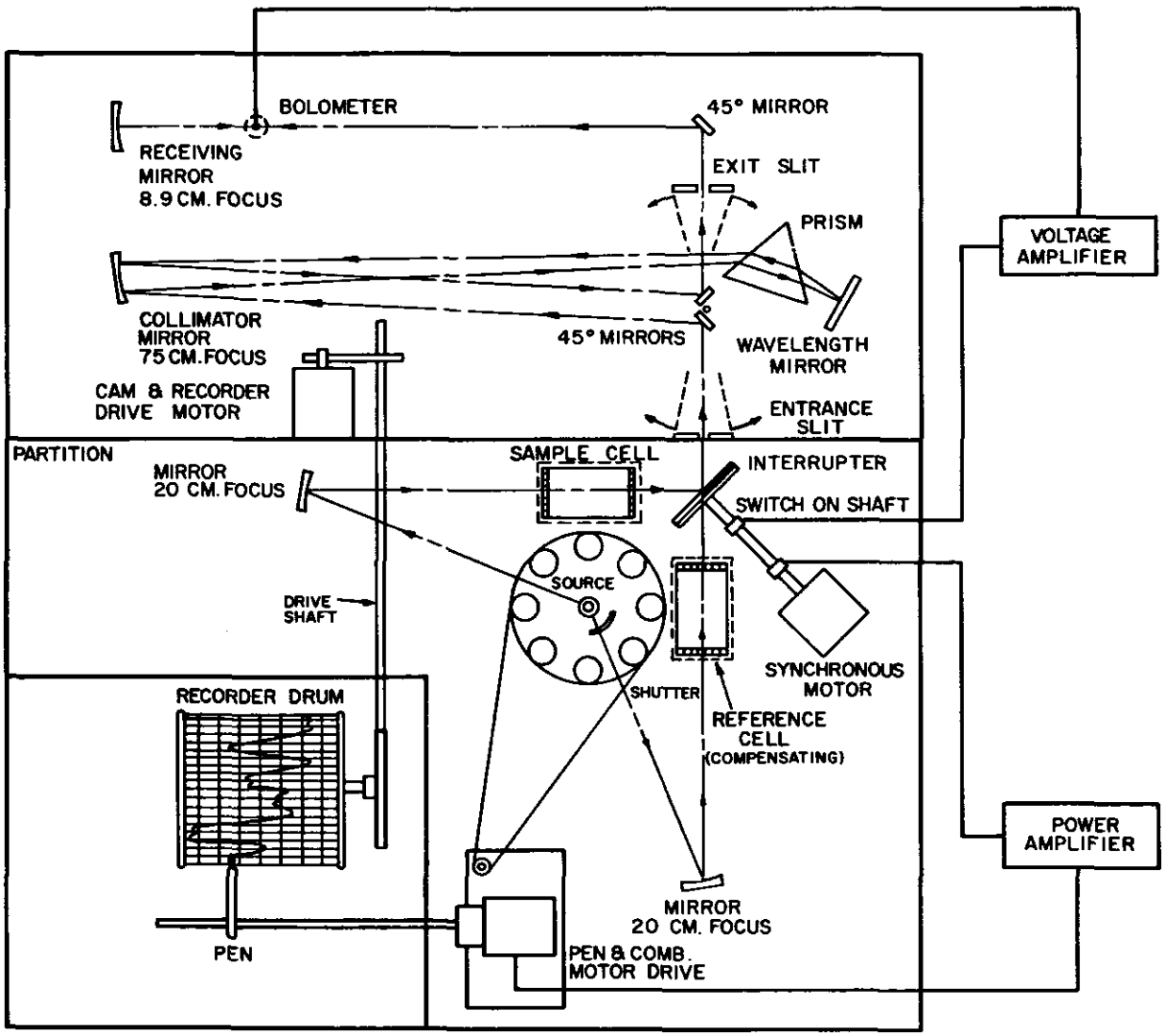
The potassium bromide pellet technique was developed to handle materials that could not be dissolved in a suitable solvent. The material to be examined is reduced to a fine powder, dispersed in high-purity potassium bromide powder and formed into a pellet under high pressure. The pellet is placed in the cell compartment and the spectrum determined. Instruments to be used for pellet analysis should be equipped with a beam condensing system to concentrate the incident beam to a small size. Using the condensing system the pellet size can be kept small and the dilution of the sample by the potassium bromide is reduced.

Applications

The use of infrared spectrophotometers in environmental control analysis has been quite limited in the past probably due to the cost of the equipment and a limited knowledge of its possibilities. It is extremely valuable for the qualitative and quantitative analysis of solvents. With experience in the technique, simple solvent mixtures can be analyzed qualitatively from one spectrum. An estimate of the quantitative analysis can usually be made from the same spectrum. A known synthetic can be prepared at the approximate concentration and its spectrum determined. By comparing the spectra of the unknown and known mixtures it is possible to get an estimate of the actual concentration.

For complex solvents, it is usually advisable to fractionate the mixture and examine the fractions by infrared analysis.

The determination of airborne mineral oil on filters used to collect particulate material is pos-



Baird Associates: Bulletin XXXIII. Cambridge, Massachusetts.

Figure 19-11. Schematic Diagram of a Double Beam Infrared Spectrophotometer.

sible using infrared. The oil is first extracted from the filter with ether or hexane. The solvent is allowed to evaporate at room temperature. The oil is then redissolved in a known volume of carbon tetrachloride. The absorbance of the sample is determined over the range of 3 to 4 micrometers. The mineral oil content is calculated from a previously prepared calibration curve, using a comparable oil.

The fixed gases such as carbon monoxide, sulfur dioxide and ammonia can be determined directly in air by use of gas cells with a one meter light path. The gases have definite spectra and can easily be identified. The technique requires relatively large volume air samples.

One recent development is the determination of alpha quartz in respirable air samples as is required by both the Coal Mine Safety and Health Act and the Occupational Safety and Health Act. The dust sample and filter are ashed or the dust removed from the filter by ultrasonic means and ashed. The ashed sample is mixed with potassium bromide and formed into a pellet under high pressure. The infrared absorbance is measured at 13.1 micrometers. Using a previously prepared calibration curve, the alpha quartz content is then determined. Under carefully controlled conditions it is possible to measure 10 micrograms of quartz by this technique.

FLUORESCENCE SPECTROPHOTOMETRY

Introduction

Use of the fluorescence properties of certain compounds as an analytical tool has become important in the environmental control field only in the last few years. The analysis of beryllium in particulate material collected in air samples is based on the flourescence of a beryllium morin complex. Later work on the polynuclear aromatic hydrocarbons has developed additional interest in this technique.

Fluorescence spectrometry is a highly sensitive analytical tool which can be used to measure concentrations as low as 10^{-8} to 10^{-10} grams per milliliter. Few colorimetric procedures are of value at concentrations below 10^{-7} grams per milliliter.

Fluorescence is essentially an electronic phenomenon and is primarily concerned with light of wavelengths in the region of 200 to 800 nanometers. When light in this region strikes some compounds they absorb energy at specific wavelengths which are characteristic of the compound. This is called the absorption spectrum of the compound. As a result of this absorption of energy, some of the molecules are raised from the ground state to a higher energy level called a singlet or excited state. Since this excited state is unstable, the molecule tends to return to the ground state by emitting the absorbed energy as fluorescence. As some of the released energy is lost by other means, the energy released as fluorescence is always less than the absorbed energy. Therefore, the wavelength of the fluorescence is longer than that of the absorbed energy. The absorption and release of energy as fluorescence takes place in 10^{-8} seconds.

In some instances the absorbed energy may be released in two steps. First a small amount of energy is lost, allowing the molecule to reach the triplet or metastable state. It then returns to the ground state by releasing energy slowly. The energy lost from the triplet to the ground state is called phosphorescence. As in fluorescence, the energy lost by phosphorescence is less than the total absorbed energy and thus, the wavelength of the phosphorescence is longer than that of the absorbed energy. Since the energy release is slower, phosphorescence is more persistent than fluorescence.

Not all organic compounds exhibit fluoresence. In general, the compounds which fluoresce are aromatic or contain conjugated double bonds (i.e., alternating single and double bonds). Those compounds containing electrons which undergo energy transformations readily should fluoresce. Any radical, which when added to the molecule increases the freedom of these electrons, will enhance the fluorescence. Conversely, any radical which tends to restrict the electrons' ability to absorb energy will decrease the fluorescence.

Two different spectra are generally shown for compounds showing fluorescence. The excitation spectrum is obtained by measuring the variation in intensity of a strong emission wavelength as the wavelength of the excitation energy is changed. Conversely, when the wavelength and intensity are measured over the emission range using a strong excitation wavelength, an emission spectrum is obtained.

The effects of substitution upon fluorescence can be illustrated with benzene, aniline and nitrobenzene. In dilute solutions, aniline is 40 to 50 times more fluorescent than benzene whereas nitrobenzene does not fluoresce. The $-NH_2$ group increases the freedom of the electrons while the $-NO_2$ group tends to decrease the freedom.

Many factors may affect the intensity of fluorescence. Among the more important are instrumental parameters, concentration, solvent, pH, temperature and the stability of the compound to light. Instrumental slit widths and light intensity can affect the intensity and all instrumental parameters must be kept constant during any set of determinations.

Concentration plays a very important role in the intensity of emitted light. Generally the fluorescence is viewed at right angles to the incident light. The fluorescence emitted must pass through the cell and some is reabsorbed by the solution. The higher the concentration of the compound, the greater the energy which is reabsorbed and lost. Consequently, a linear relationship between concentration and fluorescence exists only in a very dilute solution.

Solvents used in fluorescence measurements may affect the results radically. Many solvents may contain impurities which will fluoresce and need extensive purification to make them usable. Solvents such as water, simple alcohols, ether or hexane can be used. The fluorescence wavelength may shift rather widely as the solvent is varied.

Ionization of the fluorescing compound may change or eliminate fluorescence. Thus, pH may become an important factor in any measurement. Aqueous buffer solutions are frequently used to control pH.

Fluorescence intensity tends to increase as the temperature is lowered and decreases as the temperature is raised. The fluorescence may change by as much as 5 percent per degree of temperature change.

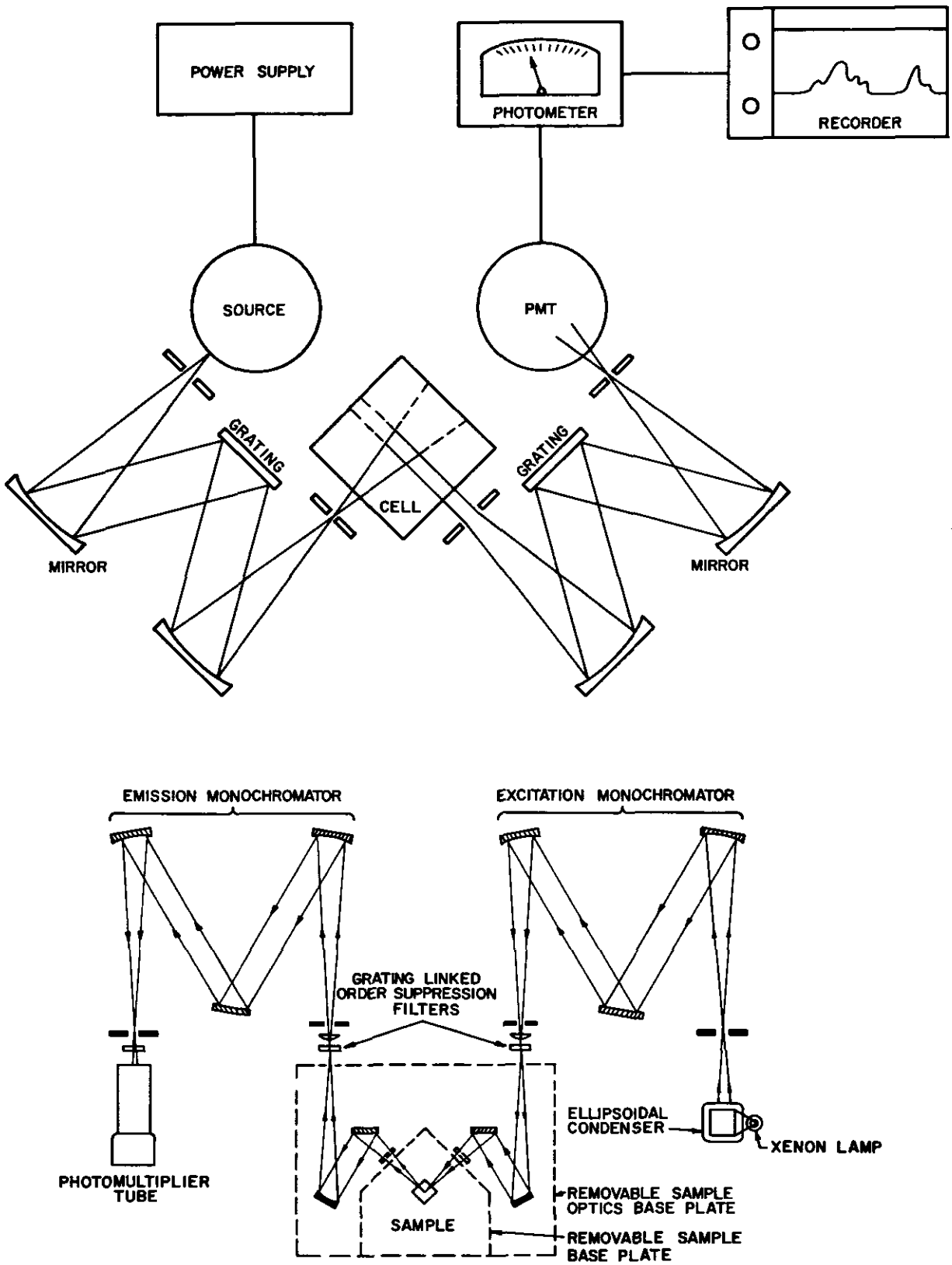
Some compounds tend to decompose under the influence of ultraviolet light. Thus, as the concentration of the solute decreases the intensity of fluorescence decreases, except in those cases where the decomposition products may fluoresce.

Quenching is the term applied to the loss of fluorescence. As mentioned earlier, the compound itself may cause concentration quenching. Some compounds can reduce or eliminate fluorescence. Quenching can be caused by inner filter effects, energy degradation, chemical change, absorption and/or intersystem transfer.

It should be pointed out that all glassware must be kept very clean. Many detergents fluoresce and should not be used to clean cuvettes (sample cells). Chromic acid absorbs ultraviolet light and should not be used for cleaning glassware for fluorescent procedures. Concentrated nitric acid is frequently used to clean cuvettes.

Instrumentation

There are several types of instruments available on the market. Many of the UV-visible light spectrophotometers have fluorescence attachments.



American Instruments Co.: Bulletins 2392H and 2423-1. Silver Springs, Maryland.

Figure 19-12. Schematic Diagram of Two Types of Spectrophotofluorometers.

In these the excitation is caused by the entire spectrum of the ultraviolet region. The emitted radiation is measured at a right angle to the incident beam. The emitted radiation is analyzed by filters, gratings or prisms. With these instruments the excitation spectrum cannot be determined. These units are generally suitable for routine analysis.

The second general type of instrumentation involves units with two monochromators. With these spectrophotofluorometers a specific wavelength can be used for excitation and the fluorescence spectrum can be determined. Since both the excitation and emission spectra are valuable for identification purposes, these systems are very valuable for the identification of unknown samples. These instruments can be either direct reading or equipped with a recorder.

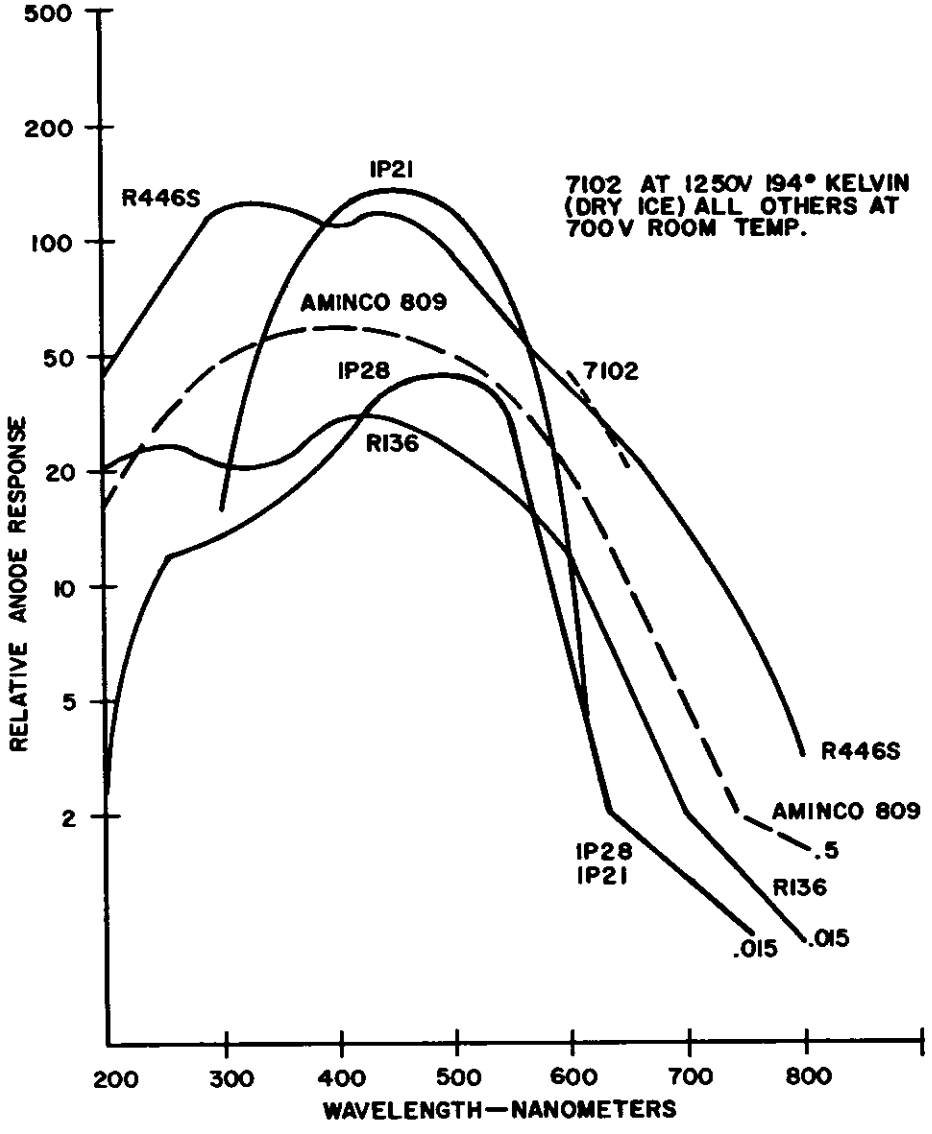
Schematic diagrams of two different spectrophotofluorometers are shown in Figure 19-12.

The light source is an important part of any spectrophotofluorometer. The xenon-arc lamp is commonly used. It produces a continuous spectrum from 200 to 800 nanometers and has a greater intensity in the ultraviolet region than does the tungsten lamp. The xenon-arc lamp produces large amounts of ozone and should be locally exhausted to remove the toxic gas.

Mercury lamps give a discontinuous spectrum consisting of high intensity lines at 365, 405, 436 and 546 nanometers. If the compound to be studied is excited in this region, the mercury lamp is very satisfactory. It is not satisfactory for studying compounds excited by other wavelengths.

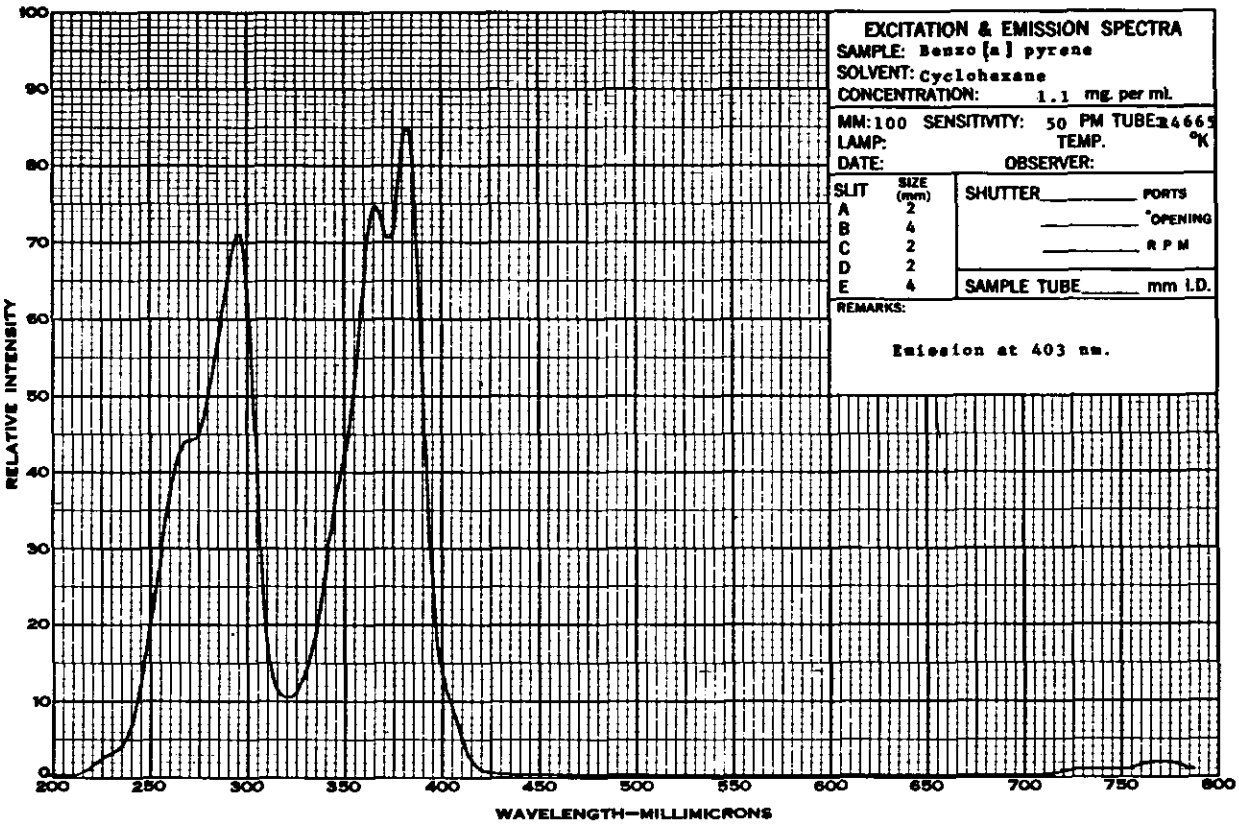
The cells used are of either glass or silica. If the exciting wavelength is above 320 nanometers the cheaper glass cells can be used. Below this wavelength silica cells are required. All cells shoud be checked for fluorescence.

The detectors are photomultiplier tubes. Since



American Instrument Co.: Aminco Lab. News 26 (182):8, 1970.

Figure 19-13. Spectral Response of Some Photomultiplier Tubes.



Tracing made at U.S. Steel Research.

Figure 19-14. Excitation Spectrum of Benzo (a) Pyrene.

the spectral response of the tube varies from type to type, consideration should be given to specifying a tube with the maximum response in the region of interest. Figure 19-13 shows the spectral response of several tubes. The 446 S gives the greatest response over the widest range but is the most expensive.

Applications

The use of fluorescence in environmental control has developed only in the last few years. Its use will probably increase rapidly as new organic compounds are added to the hazardous chemicals list and permissible levels are promulgated.

The analysis of airborne particulate material for beryllium is one example of fluorescence analysis. Under proper conditions beryllium can be reacted with morin to produce a compound with strongly fluorescent properties. This reaction has been the basis for many published methods for the determination of microgram quantities of beryllium.

As interest in the polynuclear aromatic hydrocarbon content of the effluent from combustion processes has increased, the use of the spectrophotofluorometer has increased rapidly. Most of these compounds have specific excitation and emission spectra, and very minute quantities of these compounds can be determined in complex mixtures after separation by column chromatography, gas chromatography or thin layer chromatography. Procedures for the quantitative measurement of such compounds as benzo (a) pyrene directly from thin layer chromatograph plates have been published. The excitation and emission spectra of benzo (a) pyrene are shown in Figures 19-14 and 19-15. These spectra are specific for this compound.

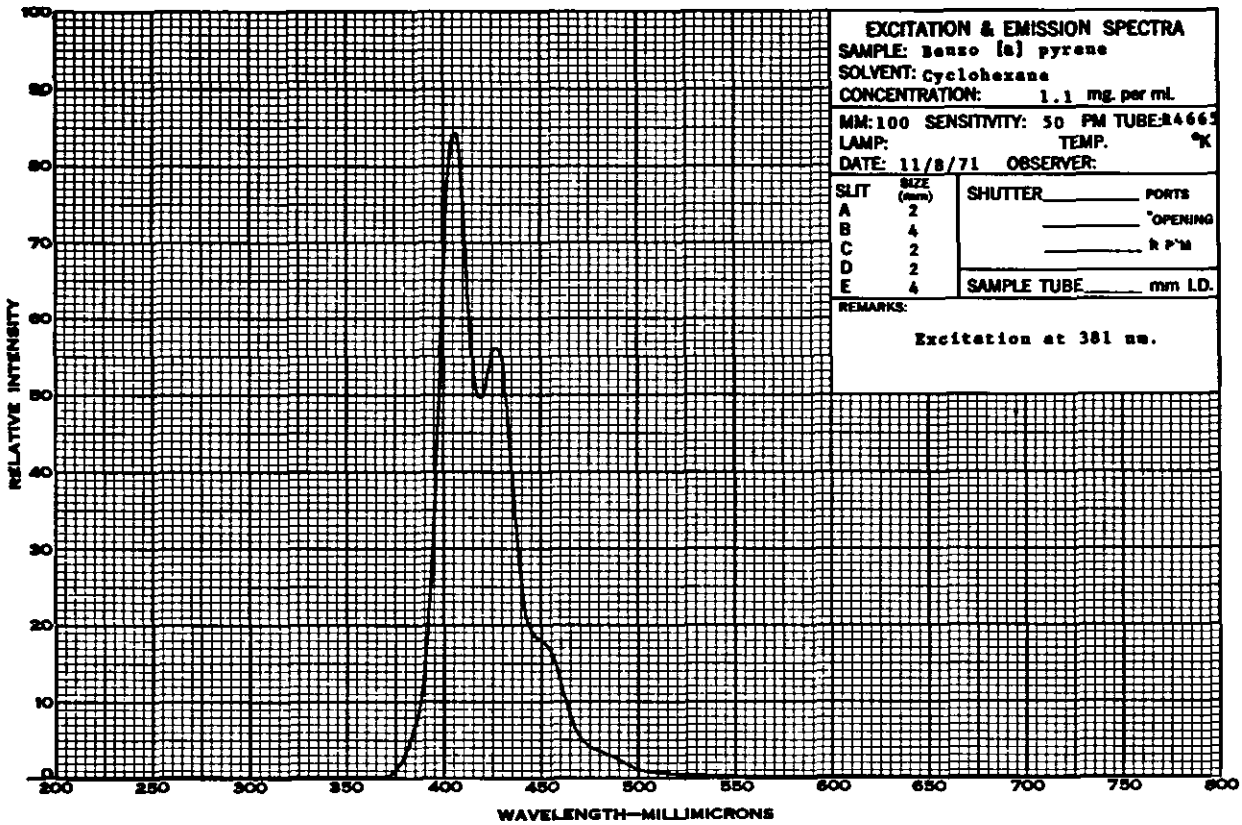
The extended use of these fluorescent techniques is expected to lead to a greater number of analytical methods in this phase of spectrophotometry.

ATOMIC ABSORPTION SPECTROPHOTOMETRY

Introduction

The basic principle of atomic absorption has been known for over one hundred and fifty years, but its application to analytical chemistry started with the work of Walsh, first published in 1955. Since that time the field has developed rapidly, producing new and improved instrumentation and many new and improved analytical techniques.

The basic principle of atomic absorption can be considered to be the inverse of that of the emission method of analysis. In the thermal excitation of atomic vapor, only a small proportion of the atoms are raised to an energy state where they emit characteristic radiation of specific wavelengths. This property is the basis of emission spectrography. Atomic absorption utilizes



Tracing made at U.S. Steel Research.

Figure 19-15. Emission Spectrum of Benzo (a) Pyrene.

the ability of the remaining atoms, which are in the ground state, to absorb energy at specific wavelengths as this energy passes through the atomic cloud.

At the temperature of a gas-air or gas-oxygen flame, a few atoms are excited but the remainder are in the ground or unexcited state. Thus, because of the higher population of ground state atoms, it would be advantageous, from the analytical viewpoint, to measure the ground state atoms. While it is possible to measure the absorption of a continuum at any specific wavelength, the width of the absorption line at the temperatures of 2000° to 3000°K is of the order of 0.002Å and a spectrometer with a very high resolving power would be required. With the advent of the hollow cathode lamp it has become possible to produce a source emitting specific wavelengths. Thus, a lamp with a lead cathode will produce radiation of specific wavelengths characteristic of lead. If the physical properties of the metal prevent its use as the cathode, a salt of that metal may be packed in a cathode made of some other suitable metal.

The formation of an atomic vapor cloud requires (1) the dispersion of the solution in the form of small droplets, (2) evaporation of the solvent to form dry particulate material in the flame, (3) the decomposition of the salt or oxide, and (4) the formation of the atomic cloud.

Much information has been published on the detection limits of atomic absorption techniques. The detection limits should be used only as a general guide, as they will vary from instrument to instrument. The data in Table 19-5 will give some estimate of the detection limits of the technique for various elements. The detection limit is generally defined as the concentration in aqueous solution which will produce a signal that is twice the standard deviation of the random noise or baseline signal.

"Sensitivity" is another term often employed in the atomic absorption literature. It refers to the concentration in aqueous solution which will generate a signal of 1 percent absorption or 0.0044 absorbance units. The sensitivity will vary from instrument to instrument as it is a function of the lamp, burner head and the nebulizer.

As with other analytical systems, chemical interferences may occur for many elements in atomic absorption. A chemical interference may be defined as any reaction which affects the analyte or element to be determined. Any cation or anion present in the sample which will form compounds with the analyte that are not easily dissociated will reduce the absorption. Aluminum, phosphate, titanium and silica interfere with the determination of alkaline earth metals. Setting all parameters such as burner type, oxidant, fuel, flame temper-

Table 19-5. Detection Limits (ug/ml)

Element	D.L.	Element	D.L.	Element	D.L.
Ag	0.002	Ho(a)	0.1	Ru	0.3
Al(a)	0.03	In	0.05	Sb	0.1
As(b)	0.1	Ir	2.	Sc(a)	0.1
Au	0.02	K	0.005	Se(b)	0.1
B(a)	2.5	La(a)	2.	Si(a)	0.08
Ba(a)	0.02	Li	0.0006	Sm(a)	2.
Be(a)	0.001	Lu(a)	3.	Sn(c)	0.02
Bi	0.04	Mg	0.0001	Sr(a)	0.01
Ca	0.001	Mn	0.002	Ta(a)	2.
Cd	0.001	Mo(a)	0.03	Tb(a)	3.
Co	0.01	Na	0.002	Tc	0.9
Cr	0.003	Nb(a)	1.	Te	0.09
Cs	0.05	Nd(a)	2.	Ti(a)	0.09
Cu	0.002	Ni	0.01	Tl	0.03
Dy(a)	0.2	Os(a)	0.5	Tm(a)	0.2
Er	0.1	P(a)	100.	U(a)	30.
Eu	0.04	Pb	0.02	V(a)	0.06
Fe	0.01	Pd	0.02	W(a)	3.
Ga	0.1	Pr(a)	10.	Y(a)	0.3
Gd(a)	4.	Pt	0.1	Yb(a)	0.04
Ge(a)	1.	Rb	0.005	Zn	0.002
Hf(a)	8.	Re(a)	1.	Zr(a)	5.
Hg	0.5	Rh	0.03		

(a) Nitrous oxide-acetylene flame used.
(b) Argon-hydrogen-entrained air flame used.
(c) Air-hydrogen flame used.

Taken from Analytical Methods for Atomic Absorption Spectrophotometry
Perkin Elmer — General Information Section
Page 32.

ature, flame region and sample medium at the optimal values will reduce this interference. The addition of releasing or chelating agents to the sample will also reduce this interference. Lanthanum is used as a releasing agent and EDTA (ethylenediamine tetraacetic acid) is a common chelating agent.

Use of an organic solvent to extract the analyte from its solution will concentrate the analyte and improve the sensitivity and detection limit. Ammonium pyrrolidine dithiocarbamate (APDC), diethylammonium diethyldithiocarbamate (DDDC), thenoyltrifluoroacetone (TTA) and dithizone are used extensively as chelating agents. They can be made fairly selective by control of pH and the use of masking agents. Methyl isobutyl ketone (MIBK) is the most common organic extractant because it produces a stable flame. MIBK is somewhat soluble in acid aqueous solutions which makes the accurate control of the solvent volume difficult. A mixture of MIBK-cyclohexane (3:1) does not have as great a solubility in aqueous solutions and yet is reported to have as good extractive powers as MIBK. Also the MIBK can be saturated with an aqueous solution at the same pH as the sample solution to control this problem. If the standards are treated in exactly the same manner as the samples, the solubility effect will be cancelled. Carbon tetrachloride or chloroform can be used, but the enhancement is less than that with MIBK and their combustion produces phosgene which is very toxic. Benzene and kerosene produce a smoky luminous flame and thus are not satisfactory.

While burners are the most common source of the vapor cloud, some new methods of forming the cloud have been developed for specific determinations. Flameless atomic absorption has been used to determine mercury, by the reduction of the mercury ions to elemental mercury and vaporization of the mercury in a gas stream into a gas cell substituted for the burner. Carbon rod and tantalum strips, electrically heated, to vaporize the sample have been developed. These special techniques will be discussed later.

Figure 19-16 is a modified atomic chart illustrating the elements that can be determined by atomic absorption methods at this time. Some elements can be determined by either flame photometry or atomic absorption, while others can be determined by one or the other technique.

Instrumentation

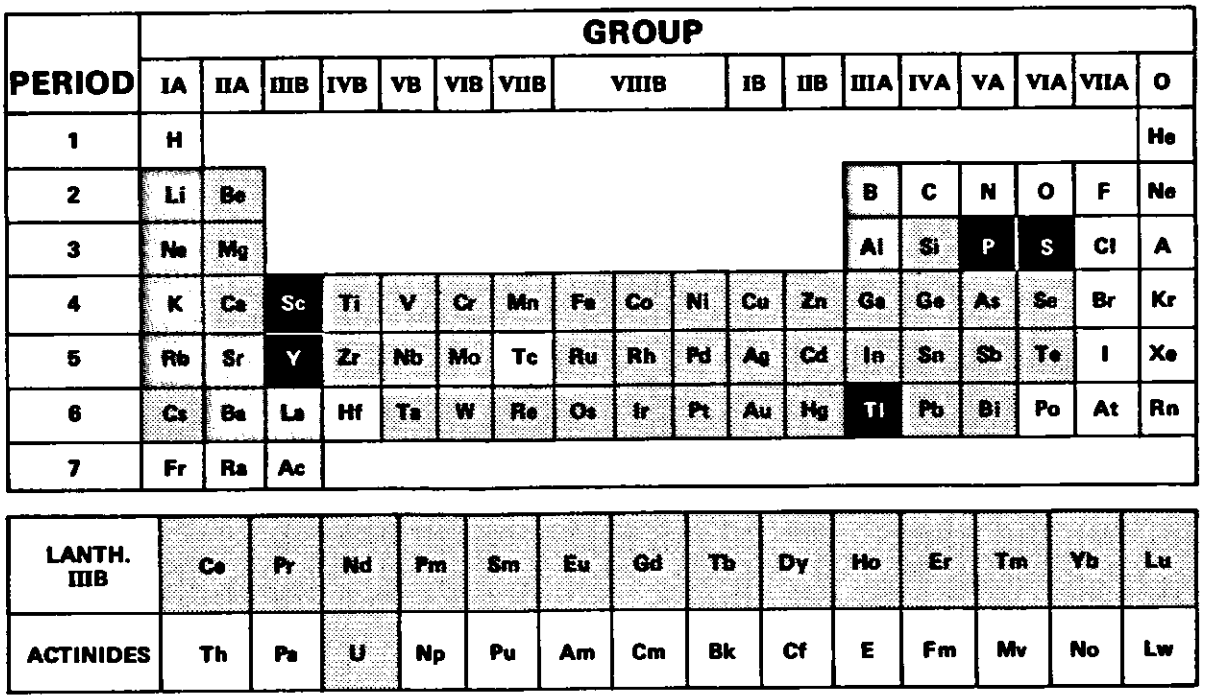
As discussed in the preceding section, the basic instrument consists of a light source, a burner or other producer of a ground-state vapor cloud, a monochromator and a detector. The basic equipment is shown in Figure 19-17. Many modifications of this basic design are available commercially. Single and double-beam spectrophotometers have been developed and each has specific advantages and disadvantages. Single-beam instruments require very stable electronic and burner components to prevent instrumental variations. In some types of double-beam instruments the beam is chopped so that part of the beam bypasses the burner. The detector balances the two beams to produce a signal. Other double-beam units pass two beams of light directly through the flame. The double-beam principle is used to eliminate instrumental variations.

The choice of the proper type of commercial instrumentation is a complex problem and is beyond the scope of this chapter. Either system is adequate for most of the determinations required of an environmental control laboratory. Personal preference of the user enters into the choice.

At the present time there are hollow cathode lamps to cover approximately 70 elements. These are available as single element lamps. The current requirements vary from 5 to 40 milliamperes. To reduce the number of lamps required some lamps are made with multiple element cathodes. The cathodes may contain from two to six elements in a single lamp.

The type of monochromator used varies from manufacturer to manufacturer and each is designed to give adequate performance with the remaining components of the system. Generally 0.25 to 0.5 meter monochromators are used. The shorter lightpath units have less energy loss in the system thus producing higher signals while the longer light-path gives better resolution.

A photomultiplier tube is generally used as the detector. Because the detection limit is di-



Jarrell Ash Div., Fisher Scientific Co.: Bulletin No. 6B (Elements Suitable for Analysis by Atomic Absorption and Flame Photometry). Waltham, Massachusetts.

Figure 19-16. Elements Suitable for Analysis by Atomic Absorption and Flame Photometry.

rectly affected by the stability of the measuring circuit, the phototube, power supply, amplifier and readout system must be sensitive and stable. Some of the phototubes and their spectral range are shown in Table 19-6. It should be pointed out that while a tube can respond over the entire range, its response will vary widely. The spectral response of the tube to the region of interest will dictate the choice of the tube.

A small galvanometer or meter is supplied with the instrument to indicate the absorbance. The addition of a recorder or digital readout system greatly facilitates the handling of the data. Some of the higher priced models now have digital readout and averaging circuits built into the instrument.

Applications

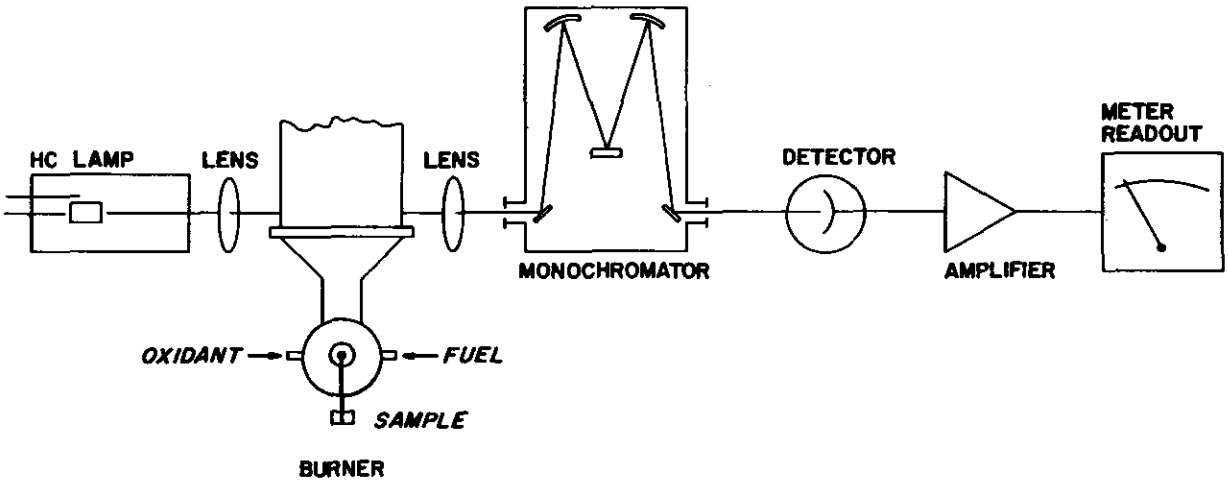
The atomic absorption spectrophotometer offers a very sensitive method of analysis for many of the metals of interest in environmental control analysis. The sensitivity is sufficient to determine the metallic elements in particulate material collected on air filters.

Several attachments for the atomic absorption spectrophotometer have been developed for the determination of mercury in particulate material, water sewage, and biological specimens. The material to be analyzed is ashed to convert all mercury to the inorganic form. The mercury ion is reduced to metallic mercury. Air or some other fixed gas is bubbled through the solution, vapor-

Table 19-6. Photomultiplier Tubes and Their Spectral Ranges

Tube	Range — Å
106	1600 to 6500
1P21	3000 to 6000
1P28	2000 to 6500
6217	3300 to 8000
1102	4000 to 10000
6256	1650 to 6700
9558 Q	1650 to 8500
118	3200 to 10000
6291	3200 to 6000

Source: Atomic Absorption Spectroscopy ASTM Technical Publication #443 Page 26.



Jarrell Ash. Div., Fisher Scientific Co.: Bulletin 160A. Waltham, Massachusetts, p. 5.

Figure 19-17. Schematic Diagram of a Basic Atomic Absorption Spectrophotometer.

izing the metallic mercury and carrying it to a sample cell mounted in the light-path in place of the burner. The absorption of light of a wavelength of 253.7 nanometers is measured. The mercury content is determined from a calibration curve prepared under identical conditions.

The use of atomic absorption for the determination of lead in biological speciments has been studied and many procedures have been published. The analysis of urine directly for lead has been unsuccessful with commercial instruments due to the heavy salt concentrations. The large amount of solids in the urine causes clogging of the nebulizer and variations in the atomization rate and the presence of large amounts of sodium causes interference. The detection limit for lead is approximately the same concentration as that found in normal urines.

Extraction of the urine or blood, after ashing, with APDC in methylisobutyl ketone has been reported. Co-precipitation of the lead with bismuth and solution of the precipitate has also been suggested.

Several modifications of equipment have been suggested for the analysis of urine or blood for lead directly. The tantalum boat has been suggested. An acidified solution of the urine or blood is placed in the boat, the boat is advanced to the edge of the flame to dry the sample. Then the boat is placed directly in the flame to ash the sample and vaporize the lead. The Delves cup procedure for blood has been suggested. The blood sample (0.1 ml) is placed in a small nickel cup and dried at the edge of the flame. After drying, the cup is pushed into the flame directly under a nickel tube. The lead is vaporized and passes into the tube where it can be held in the light-path for a longer period of time to enhance the sensitivity.

The tantalum strip or carbon rod furnace has been suggested as a technique for the analysis of blood. A few microliters of blood are placed in a depression in the tantalum strip or in a cavity in the carbon rod. An electrical current is passed through the strip or the rod to dry the blood. The current is then increased to vaporize the lead.

In its present state of development atomic absorption spectrophotometry is a very valuable tool for an environmental control laboratory.

SUMMARY

Spectrophotometry is a valuable tool for the solution of many of the analytical problems of an environmental health laboratory. This chapter has presented a discussion of the principles of the techniques of spectrophotometry and their application to this type of analysis. The methods were presented as illustrations of the principles, and specific details can be found in any of the standard texts on analytical chemistry. The actual choice of the method will depend upon the equipment available in the laboratory, and the type of sample submitted for analysis.

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CHAPTER 20

EMISSION SPECTROSCOPY

C. L. Grant

INTRODUCTION

Emission spectroscopy is that method of analysis that depends on the fact that energized atoms, ions, and molecules emit electromagnetic radiation when they lose energy. The characteristic line spectra emitted in flames, arcs, sparks, and related sources are highly specific for each element. Further, spectral line intensities are functionally related to element concentrations in the excitation source. Thus, when samples are introduced to these sources, both qualitative and quantitative analyses can be accomplished.

As a survey method, probably no other analytical technique provides so much information for a given amount of effort. A wide variety of sample types and forms can be analyzed, usually with a minimal amount of pretreatment. Up to 70 elements may be detected and estimated simultaneously, although procedures of this breadth are not generally attempted. The detection capability varies widely with elements, but it is generally best for the lower atomic number elements. Absolute detection limits are often below 10 nanograms which makes the technique quite useful for the analysis of small samples; i.e., one milligram or less. When sample size is not limiting, large spectrographs with high resolution readily provide detection capabilities as low as a few parts per million; and, in ideal cases, the limits may be as low as a few parts per billion.

In view of the above, it is not surprising that some of the earliest applications of emission spectroscopy were in the field of occupational health. Industrial workers are exposed to many substances containing toxic elements. Some elements tend to accumulate in body tissues and, therefore, very low concentrations of these elements in drinking water and/or air can be hazardous. For the safety of workers, it is necessary to monitor concentrations of toxic elements in the working environment and in body samples such as blood, urine and feces. The enormity of this analytical problem requires techniques of broad scope, high selectivity and detection capability, and adequate precision and accuracy. Emission spectroscopy is such a technique.

PRINCIPLES

Atomic line spectra are produced when energy is added to atoms in the ground state in an amount sufficient to cause some electrons to move from their normal energy levels to higher energy levels. In this form, the atom is said to be "excited." When the electrons return to their normal energy levels, they release energy stepwise in the form of radiation. Each step accounts for a definite amount of energy. The radiation produced has specific frequencies corresponding to the energies associated with the various steps, as indicated by the relationship $E = h\nu$.

where: E = energy of the radiation (photon)
h = Planck's constant
ν = frequency of radiation

An element is characterized by as many different spectra as the atom has electrons. Lines originating from the electron transitions of the neutral atom are called arc lines, whereas those from the singly ionized atom are called the first spark spectra. Although greater degrees of ionization do occur to a limited extent in conventional spectroscopic sources, the lines originating from neutral atoms and singly ionized atoms are the ones of major analytical interest.

The number of spectral lines produced for any element depends on the atomic structure. Elements with comparatively few valence electrons produce relatively simple spectra (e.g., alkalis and alkaline earth metals). In contrast, an element such as uranium produces thousands of discrete lines, none of which are very intense. The spectrographic determination of elements with very complex spectra is less attractive than for elements with simple spectra. This is due to possible incomplete resolution of lines of very similar wavelengths and also because elements with very complex, less intense spectra exhibit only fair detection limits.

While all the elements can be excited, gaseous elements, bromine and iodine are only infrequently determined this way. These elements can occasionally be determined with conventional excitation procedures by measuring the band spectrum of a compound such as calcium fluoride. However, this approach is not widely used. Carbon, phosphorus, and sulfur have their most sensitive lines below 2000Å and, therefore, require the availability of a vacuum spectrograph to overcome air absorption. Fortunately, most elements that are readily studied by optical emission spectroscopy produce useful lines between 2000 and 8000Å. In this wavelength region, simple optical, photographic and electronic equipment can be used to isolate and record spectral lines.

Qualitative analysis by emission spectroscopy is based on the fact that the atomic structure for each element is different. Therefore, a unique set of spectral lines is produced for each element; and these lines serve as a fingerprint. Line identification is usually accomplished by comparison of lines in the unknown spectrum with lines in a

series of standard spectra prepared from pure elements.

Quantitative analysis is based on the fact that the intensity of a spectral line depends on the amount of parent element present in the excitation source. When the spectra are photographically recorded, relative intensities are estimated by measuring the optical density with a densitometer. Density values are then converted to relative intensities by means of an emulsion calibration curve relating these two variables for the particular emulsion involved, at the wavelength of interest. Alternately, line intensity can be recorded photoelectrically, thereby eliminating many of the errors inherent in photographic procedures.

SYSTEM COMPONENTS

Analysis by optical emission spectroscopy involves four main steps: 1) vaporization and excitation, 2) resolution of emitted radiation into constituent wavelengths, 3) recording spectral lines, and 4) interpretation.

Vaporization and Excitation

The total radiation output of a spectroscopic source is dependent on the aggregate of the processes of volatilization and excitation. Often, a clear distinction between these two processes is not made, probably because both are occurring simultaneously in most sources. For optimum reproducibility in the production of spectra, it is important to know which process is most important in a particular situation so that it may be properly controlled. The comprehensive treatise by Boumans[1] is an excellent source of information on this topic.

Vaporization can be accomplished by thermal means as exemplified by flames, direct current arcs, ohmic heating and laser evaporation. The commonly used high voltage spark discharge promotes vaporization by bombardment with positive ions and high velocity electrons. Of course, the vaporization process is seldom entirely thermal or entirely bombardment; and, therefore, no sharp separation between these sources should be assumed.

Flames. When a solution is aspirated into a flame in the form of minute droplets, desolvation occurs leaving small residue particles. Decomposition and vaporization of these particles produce a vapor containing atoms and molecules which are then excited via inelastic collisions with high velocity molecules liberated by chemical reaction between the fuel gases. Since the energy available in a flame is relatively limited, the spectra obtained are quite simple. As a consequence, spectral interferences are uncommon, thereby making it possible to employ comparatively inexpensive spectrometers of low dispersion.

Because of the comparatively low temperature of common flames such as air-acetylene, this source was traditionally considered suitable only for the determination of easily vaporized and excited elements such as the alkalis and alkaline earths. However, Fassel et al.[2,3] showed that many elements that tend to form stable oxides in normal stoichiometric flames can be dissociated to produce analytically useful atomic populations in a fuel rich oxygen-acetylene flame. Pickett and Koirtyohann[4] reported the successful use of the nitrous oxide-acetylene flame for the emission determination of many elements previously considered to be too refractory for analysis by this method. The development of a system for studying desolvation and vaporization processes of single droplets by Hieftje and Malmstadt[5] gives further promise for the development of optimized systems of flame vaporization and excitation. These developments coupled with the comparative simplicity of spectra produced by flame excitation and the inherent reproducibility of flames for quantitative work, suggest a bright future for flames in the field of environmental analysis.

Arcs. The direct current arc is usually considered to be the most sensitive source for trace element analysis by emission spectroscopy. One reason for this high detection capability resides in the fact that comparatively large samples (100 milligrams or more) can be employed. Most of the current in this type of discharge is carried by the electrons which impact on the anode and quickly elevate it to very high temperatures. This promotes rapid sample vaporization with an attendant high concentration of atoms in the analytical gap.

Typically, nonconducting powders, solution residues, and similar samples are placed in the crater of a supporting graphite or carbon electrode. Three typical electrode geometries for direct current arc analyses are shown in Figure 20-1

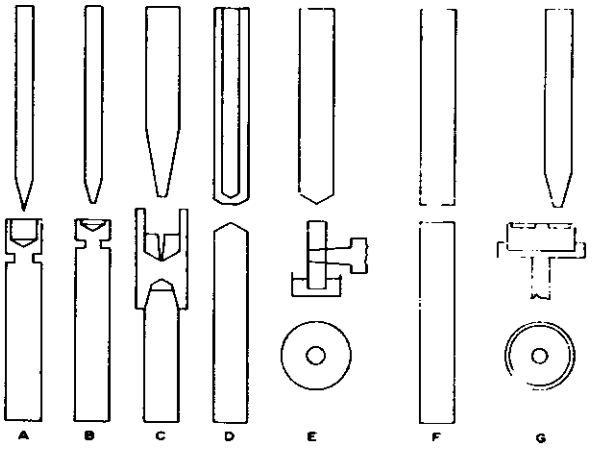

Figure 20-1. Some Typical Electrode Geometries.

(A), (B) and (C). Many other electrode shapes have been employed in direct current arc work.[6] These variations in electrode configuration influence volatilization rates of elements and related aspects of the volatilization-excitation process. The choice of graphite and carbon as materials of construction for electrodes is based on the fact that (1) they are electrically conductive, readily manufactured in high purity, and easily shaped and (2) the carbon vapor produced during use does not depress the excitation characteristics of

the arc since the ionization potential of carbon is greater than that of most elements determined by this method.

One of the major objections to the use of the direct current arc is its tendency toward poor reproducibility in quantitative analysis. If we remember that the sample-containing crater is analogous to a miniature distillation pot, any fixation of the arc at one or a few spots on the anode results in temperature gradients that will promote selective volatilization of the sample. This type of behavior is generally nonreproducible from one burn to the next. The use of "spectroscopic buffers," extensively discussed by Boumans,[1] can greatly improve this situation if the buffer is carefully selected. Such buffers are usually compounds containing elements having low ionization potentials which tend to reduce the effective excitation temperature of the arc plasma and increase the population of neutral atoms. Using various buffers in a free-burning 10-ampere direct current arc in air at atmospheric pressure, Boumans reported a range of temperatures from 5,000 to 6,200°K.

Many of the objectionable features of the direct current arc can be reduced by directing an annular stream of gas upwards around the sample as it is arced. This system, first proposed by Stallwood,[7] reduces arc wander. In addition, it has a tendency to reduce selective volatilization, thus oftentimes improving both precision and accuracy. If an inert gas is employed, the cyanogen bands are eliminated, thereby opening up a region of the spectrum which contains a number of sensitive lines.

Although we frequently go to great lengths to eliminate selective volatilization, sometimes it is possible to take advantage of this phenomena. In this approach, the shutter of the spectrograph is opened only while elements of interest are in the analytical gap. With the overall exposure level reduced, very large samples may be used; and this may produce a significant gain in detection capability. Many examples of this procedure have been described by Ahrens and Taylor.[8]

Occasionally, the addition of certain compounds to the sample can be used to induce chemical reactions which will either promote or reduce selective volatilization. One of the best known examples of this procedure is the carrier-distillation method first described by Scribner and Mullin.[9] A comparatively large sample (100 mg.) is mixed with a carrier such as gallium oxide or silver chloride and then packed into a deep-cratered electrode of the type shown in Figure 20-1 (C). Many investigators also like to put a vent hole in the center of the sample to permit a smooth evolution of gases after the arc is struck. The electrode geometry is especially chosen to reduce heat loss from the sample-containing anode. Volatile impurities are transported into the excitation zone with the carrier while the refractory matrix is left behind.

Spark Discharges. Spark discharges are those in which the energy flow between the electrodes varies in a cyclic fashion, usually with a change of polarity each time the energy flow drops to zero. Because the discharge is being constantly reignited, there is improved random sampling of electrode surfaces. Consequently, this discharge is generally considered to provide better precision but less sensitivity than arc discharges. The poorer sensitivity compared to a direct current arc is largely because the electrodes remain relatively cool, and considerably less sample is consumed. Despite this restriction, spark discharges have been employed with increasing frequency for trace analysis due to their excellent reproducibility. A comprehensive review of the characteristics of spark discharges was published by Walters and Malmstadt.[10]

The most extensive application of spark discharges is for the analysis of metals as self-electrodes. However, powders have been analyzed by blending and compressing with graphite to form conductive briquets. Solutions have been analyzed by a variety of innovative procedures. In the porous cup technique developed by Feldman,[11] a solution slowly percolates through the porous bottom of a hollow electrode [Figure 20-1 (D)]. As the liquid sample seeps through the porous bottom of the electrode, the spark discharge vaporizes and excites the residue to produce an emission spectrum. Another very versatile solution method involves the use of a rotating graphite disc that dips into the solution to be analyzed and transports fresh solution on its periphery into the spark excitation zone [Figue 20-1 (E)].

For very small samples, some solution methods are impractical because of the volume of sample required for analysis. In such situations, residues from the evaporation of these solutions can be analyzed. One of the best known solution residue methods is the copper-spark technique of Fred, Nachtrieb, and Tomkins.[12] A hydrochloric acid solution containing a very small amount of sample (<0.2 mg) is applied to the end of high purity copper rods and dried. The copper rods are then mounted, and the sample is subjected to spark excitation. Excellent detection capabilities are realized with this procedure; but, of course, copper cannot be determined and solvents that react with copper cannot be used. In an attempt to circumvent this limitation, Morris and Pink[13] employed flat-topped graphite electrodes [Figure 20-1 (F)] which had been treated with Apiezon-N grease to render them impervious to solutions. This procedure, called the graphite-spark technique, has exhibited detection capabilities in the nanogram range for several elements. A variation of this approach employs the rotating "platrode" developed by Rozsa and Zeeb[14] in which a graphite disc is substituted as the bottom electrode so that solution volumes up to 0.5 ml can be evaporated [Figure 20-1 (G)].

Plasmas. The plasma jet, sometimes called the gas-stabilized direct current arc, is an excitation source which has been used advantageously for the analysis of solutions.[15,16] In this procedure, the solution is aspirated into a chamber by an inert gas under pressure and then swept through a small orifice into a direct current arc discharge. When the gas flow is increased through the orifice, the electrical conductivity of the jet rises, resulting in a high temperature at the core of the discharge.

The special advantages of this system are the excellent detection capability and the high degree of reproducibility. Precision values are generally much improved over conventional direct current arc excitation.

Another plasma excitation system of special interest is the induction-coupled plasma. In the system described by Dickinson and Fassel,[17] the solution is converted into an aerosol by an ultrasonic generator. A condenser system is employed to desolvate the aerosol particles which are then introduced to the center of a donut-shaped argon plasma in a clear quartz tube. Power is supplied by a high frequency generator operating at 30 MHz. With this system, solutions were introduced at a rate of 0.3 ml/min. For many elements, detection capabilities were in the nanogram/ml range. Since the temperature of this source is on the order of 10,000°K, even the most refractory species are dissociated; and chemical interferences from matrix elements should be drastically reduced. The induction-coupled plasma deserves considerable attention in future methodological development.

Another very unique and potentially useful plasma source has been described by Kleinmann and Svoboda.[18] In this system, the sample is evaporated from a graphite disc which is resistively heated. The evolved vapors are excited by a low voltage, high frequency, induction-coupled discharge in argon. Moderately good detection capabilities were obtained. Since the ideal of separating vaporization and excitation is attained in this source, there is an excellent opportunity to control interferences from elements which make up the bulk of a sample.

Laser Excitation. The analysis of very small areas of samples can be accomplished by emission spectroscopy using a laser to vaporize the sample. This system, first described by Brech and Cross,[19] employs a high intensity pulsed laser beam focused on a spot that may be as small as 10 microns in diameter. With biological and geological specimens, it provides a means of gaining much greater insight into compositional variations than can be attained by bulk analytical systems.

A major problem with the system is the difficulty in obtaining precise quantitative results. Reasons for this difficulty relate to problems in controlling the laser output energy, and the fact that it is extremely difficult to prepare standards for the establishment of calibration curves. Recently, however, Scott and Strasheim[20] have described the use of a Q-switched neodymium laser without an auxiliary excitation system. They obtained promising results in the analysis of aluminum alloys with relative standard deviations in the range of 2% to 4%. Clearly, the ultimate potentialities of laser excitation for emission spectrographic analysis have not been realized.

Resolution of Emitted Radiation. Prisms and diffraction gratings are used to resolve the emitted light from a spectroscopic source into its component wavelengths. A variety of commercial instruments are available with differing geometric arrangements of the necessary optical components. For additional details, the interested reader can consult the recent text by Slavin.[21] Only a few points of special interest in the analysis of industrial hygiene samples will be considered here.

One of the most important properties of a spectrograph is dispersion, usually expressed as the reciprocal linear dispersion in Å/mm. According to Mitchell,[22] "It is in general not the elements to be determined, but the source to be used and the composition of the material to be examined, insofar as its major constituents are concerned, which decide the instrument to be used." When the number of lines produced by the major component is large, the reciprocal linear dispersion of the spectrograph must be small so that lines from the matrix material will not interfere with lines of elements to be determined. Fortunately, the major elements in most biological samples yield relatively simple spectra; and so, a spectrograph of only moderate dispersion is adequate. Of course, the industrial hygienist also encounters samples whose major components yield very complex spectra. For example, a plant processing heavy metals could yield dust samples which would produce extremely complex spectra requiring a large instrument with small reciprocal linear dispersion.

Detection capability can be a very important consideration in the analysis of typical industrial hygiene samples. In those cases where sample size is not limited, dispersion is the most critical factor governing detection limits. In a large spectrograph with low reciprocal linear dispersion, a given amount of background radiation is spread over a large area while the line remains unaffected. Jarrell[23] emphasized that this increase in line-to-background ratio occurs only up to the critical dispersion, i.e., the point at which the slit and line widths are equal.

When the industrial hygienist is interested in analyzing extremely small samples, the absolute quantity of an element is more important than its relative concentration. Mitteldorf[24] has emphasized that the critical consideration here is the speed of the spectrograph (approximately defined as light yield). Thus, we would normally use a small spectrograph of high speed (typically f/10) for micro samples. In contrast, a large spectrograph with optical speed on the order of f/30 might be employed where sample size is not limiting.

Recording Spectral Lines. Spectral lines are recorded either photographically on films and plates or photoelectrically. Emulsions of widely varying speed and resolving power are available to photograph the various wavelength regions of interest. When the investigator wishes to determine elements over a wide concentration range with moderate precision and accuracy, an emulsion of moderate contrast and high speed is selected. For precise quantitative analysis, an emulsion of high contrast is usually preferred.

Direct photoelectric recording of spectral line intensities is considerably more precise than photographic recording and is, therefore, often used for quantitative work. However, direct readers are expensive and sometimes lack the flexibility required for survey analyses. There-

fore, it is unlikely that photographic recording will become obsolete in the immediate future.

Quantitative Measurements. In order to do quantitative analysis by emission spectroscopy, it is necessary to estimate the intensity of spectral lines. When lines are recorded photographically, their optical densities can be measured by a microphotometer. In this system, a narrow slit of light is scanned through the spectral line. A photoelectric detector records the decrease in light transmission associated with the blackening on the photographic plate. The photoelectric detector output is recorded as optical density of the spectral line.

Next, optical densities must be translated into relative intensities by means of an emulsion calibration curve. To prepare an emulsion calibration curve, a variety of procedures are used including the use of rotating step sectors and filters with known light transmittances. Several accepted calibration procedures have been carefully described along with worked examples in ASTM Designation E 116.[25]

For high volume repetitive quantitative determinations, direct reading spectrometers are generally employed to reduce the manual effort required. Photoelectric detectors are mounted behind exit slits located in the proper positions to record selected spectral lines. Calculation procedures have also been automated by using computers to handle data reduction.[26,27] Even when spectra are recorded photographically, data reduction can be greatly facilitated via computer handling. Besides speeding up computations, errors are minimized, and precision is frequently improved.

SCOPE

Sample Types

Considering the variety of excitation sources which have been devised, it should be clear that emission spectroscopy provides the capability for detecting traces of most elements in solids, liquids, or gaseous samples. Solid metal specimens can be analyzed directly as self-electrodes, or they may be converted to other forms such as solutions. Inorganic powders can be directly analyzed; or they, too, may be converted to solution form. Solid specimens which contain large amounts of organic matter are usually either wet digested or dry ashed prior to analysis. Otherwise, the rapid combustion of organics may cause vaporization losses and inefficient excitation of the elements to be determined. Despite this fact, some methods have been devised for the direct analysis of organic solids and fluids without ashing. A wide variety of solutions such as natural water and acid digests of samples can be analyzed either by direct solution aspiration or by analysis of the solution residues. Gaseous samples can be analyzed by excitation in hollow cathode discharge tubes or in flowing systems. In short, the diversity of sample types that can be analyzed by emission spectroscopy is one of the great strengths of the method.

Sensitivity

Throughout much of the literature, the terms, sensitivity and detection limit, have been used interchangeably. In this discussion, sensitivity is defined as the ability to discern a small change in concentration of analyte at some specified concentration. Thus, sensitivity is directly correlated with the slope of the analytical curve relating line intensity to analyte concentration. Sensitivity is also inversely related to the reproducibility of line intensity measurement. For high sensitivity, we require a large slope for the analytical curve and a small value for the standard deviation of line intensity.

It should be apparent from the foregoing sections that sensitivity is controlled by a multiplicity of factors in the total analytical procedure. Sample treatments such as selective preconcentration can improve sensitivity. Choice of excitation source, the fundamental properties of the spectrograph and the extent to which its performance is optimized, choice of detector, and the method of data manipulation all affect sensitivity.

Concentration Ranges

Although there is no hard and fast rule which precludes using emission spectroscopy for the determination of high concentrations of elements, the most advantageous concentration range lies below 10 percent. In fact, the preponderance of applications deal with determinations between 1 percent and the limit of detection.

Limit of detection is defined here as the smallest quantity or concentration of analyte that can be detected "with certainty." Apparently, then, limit of detection is merely a special case of sensitivity which depends on an ability to distinguish a difference in line intensity for a small increment of analyte in comparison to the blank signal. Thus, the limit of detection is inversely correlated with sensitivity. The limit of detection also depends on the definition of "with certainty." Although it is widely accepted that a statistical definition should be used, there is little agreement on the proper confidence level. To assume that a single value for the confidence level is correct seems naive. It is much more realistic to allow each investigator to choose a probability level that suits the particular requirements of the problem at hand.

Some specific procedures necessary to achieve improved detection limits have been discussed by DeKalb et al.[28] for both photographic and photoelectric sensors. It must be emphasized, however, that many of these detection limits are reported for ideal situations in which there is no matrix interference or other limiting characteristic. In addition, many different definitions of the detection limit have been employed by various investigators, thereby making direct comparisons very difficult. It should also be remembered that limits of detection do not represent concentrations that can be determined with the same quantitative precision and accuracy expected for higher concentrations. In general, the lower limits for good quantitative precision are approximately 10 times the detection limits.

While much can be accomplished through the selection of optimum instrumental conditions, sensitivity and detection limits can also be enhanced by sample preparation procedures that provide an enrichment of the analyte. Some

typical procedures are described in Chapter 18 of this syllabus. Aside from the extra effort required in sample preparation, a major precaution with enrichment procedures is the possible introduction of impurities from chemical reagents which obscure the true pattern of variation present in the original samples.

Precision and Accuracy

Precision is defined here as the extent of agreement of a series of measurements with their average, frequently measured by the standard deviation. It is essential to express the conditions under which the data have been obtained. Commonly, precision is expressed as the percent relative standard deviation (sometimes called coefficient of variation).

If we accept that a realistic value for the precision of a total analytical method applied to a given material should include components from the sampling step, the sample preparation step, and the measurement step, then the difficulty in making general statements for different methods is obvious. Nonetheless, it is generally conceded that the precision of emission spectroscopic techniques is superior to chemical methods at very low concentrations and inferior to chemical methods at concentrations much above 1 percent.

Usually, methods that employ solutions for the final intensity measurement give better precision than direct methods with solids. One reason for this difference is that larger samples are usually employed for solution methods. Under ideal conditions, percent relative standard deviations of $\pm$ 1% can be obtained with solutions except when impurity concentrations approach their detection limits. Good reproducibility is also attained for the analysis of metals as self-electrodes. With the direct excitation of powdered solids, sample heterogeneity becomes more important; and percent relative standard deviations of $\pm$ 5 to 10% are more typical. For survey procedures which cannot be optimized for each element determined, percent relative standard deviations of $\pm$ 25% or larger might be considered acceptable.

Accuracy is defined as a quantitative measure of the variability associated with the relating of an analytical result with what is assumed to be the true value. Strictly speaking, accuracy can never be exactly measured because true values are never known. However, when primary standards are available, the accuracy can be specified within acceptable limits.

Since quantitative emission spectroscopy requires the establishment of an analytical calibration curve based on standard samples, the accuracy is limited by the quality of the available standards. The precision of a method is sometimes thought to be an estimate of accuracy. However, a method can easily yield very precise but highly inaccurate results if systematic error is present. For example, a common misconception is that synthetic solution standards obviate the need for primary standards in the analysis of miscellaneous materials which can be converted to solution form. Unfortunately, this concept overlooks all of the systematic errors that can occur while converting samples to solutions.[29] The recent activity of the National Bureau of Standards in the preparation of standard reference materials certified for trace amounts of different elements in matrices such as orchard leaves, beef liver, and serum will be of tremendous help to the emission spectroscopist concerned with the accuracy of his procedures.[30]

STEPS OF A QUANTITATIVE METHOD

The first step in any quantitative analysis by emission spectroscopy is to clearly define the problem by designating the elements to be determined and the expected concentration ranges. Any special aspects of the sample such as its major element composition, its quantity, and its physical form should also be noted. Reference standards are required which are similar, both chemically and physically, to the samples to be analyzed. For example, if we wish to determine the concentration of several trace metals in water residues in which the matrix is a mixture of calcium, potassium, magnesium, and sodium salts, we normally require standards with a matrix of these same salts to match volatilization-excitation behavior. It may even be necessary to alter the ratios of the salts in the standards to match particular water samples. Lacking standards of similar physical and chemical composition, the samples must be modified to correspond with standards that are available. This may entail conversion to solutions, inorganic powders, or some related operation.

Once the physical form of samples and standards has been decided and the analytical requirements specified, it should be possible to make an optimum choice of the excitation system, assuming that several are available. Similarly, we should attempt to insure that the spectrograph will provide sufficient resolving power and adequate speed for the detection and estimation of the elements to be determined. This means that we must be able to locate lines of the analyte elements which are free from interferences by lines of the matrix elements. Further, these lines must be sufficiently sensitive to provide measurable optical densities down to the concentration levels required by the analysis.

In prior discussions, we have inferred that quantitative analysis involves only the construction of an analytical calibration curve relating intensity of the line of an element to be determined to the known amount of that element in a series of standards. However, because of the multitude of factors that affect the total amount of light emitted by a given weight of an element, this direct approach has usually not provided adequate precision and accuracy. To circumvent this difficulty, the principle of internal standardization is employed. In this procedure, concentration of the element to be determined is measured in terms of the ratio of the intensity of the analysis line to the intensity of a "homologous" line of another element present in fixed concentration in all samples and standards. The internal standard element may be a major component of the matrix which is present in invariant concentration. Alternatively, it may be an element which is absent in the sam-

ples and which has been added in constant amount from an external source. In this manner, uncontrollable fluctuations such as variations in excitation efficiency that affect both lines to a similar extent will not alter the intensity ratio between the lines. Unfortunately, it is usually impossible to find line pairs whose intensity ratios are completely insensitive to changes in chemical and physical composition of the sample. However, the literature provides references to many line pairs which are sufficiently insensitive to extraneous influences to permit excellent precision in quantitative work.

After preparation of samples and standards, they are excited in random order and recorded on a photographic plate or photoelectrically. If the lines are recorded photographically, their optical densities are measured and converted to relative intensities by means of an emulsion calibration curve. The intensity ratios of analytical lines relative to the selected internal standard lines are calculated and plotted on log-log paper versus the respective concentrations of the elements in the standards. Intensity ratios for the unknown concentrations in the samples are then read from these calibration curves.

Analytical calibration curves must be frequently checked. Day-to-day variations in atmospheric conditions will exert sufficient influence on excitation processes and photographic emulsions to cause significant curve shifts. The extent to which an investigator must check for curve shifts and recalibrate is partially dependent on the required precision and accuracy of the analyses being performed. For a summary of recommendations, the ASTM Designation E305-67 titled "Establishing and Controlling Spectrochemical Analytical Curves" should be consulted.[31]

APPLICATIONS IN INDUSTRIAL HYGIENE

Analysis of Biological Tissues and Fluids

The work of Tipton and Stewart[32] is a typical example of a dry ash-direct current arc excitation procedure for surveying trace element contents of biological tissues and fluids. Samples of food, urine, and feces are dried and ashed at 550°C after treatment with double distilled sulfuric acid. This treatment produces a clean ash of mixed sulfates and oxides. The ash is combined with a graphite buffer containing 2,000 parts per million of palladium which serves as the internal standard. Synthetic standards of similar composition are prepared from inorganic materials. The elements Ag, Al, B, Ba, Be, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sn, Sr, Ti, V, Zn, and Zr are determined at concentrations down to 1 part per million or less in a few cases with typical percent relative standard deviations on the order of ± 10%.

A typical procedure employing wet ashing of biological fluids prior to spectroscopic analysis is described by Niedermeier et al.[33] In this procedure, 2 ml of blood serum are digested with high purity nitric and perchloric acid. A battery of samples is treated simultaneously using a number of digestion tubes in a constant temperature block which can be maintained at 130°C. After ashing, the excess acid is evaporated; and the residue is dissolved in ammonium chloride solution which serves as a spectroscopic buffer. An aliquot of the solution is transferred to a graphite electrode and evaporated to dryness in a vacuum desiccator. Synthetic standards are prepared in a matrix solution with composition closely approximating that of normal human blood serum. Excitation is accomplished by a 10-ampere direct current arc. Selected lines of Cu, Fe, Al, Ba, Mn, Ni, Cs, Sn, Sr, Cr, Zn, Pb, Mo and Cd are monitored with a direct reading emission spectrometer. Data analysis is accomplished by an IBM 7040 computer.

For rapid survey purposes, the procedure described by Bedrosian et al.[34] is especially attractive because ashing of the sample is not required. Materials such as animal tissue, blood serum, stool, bone, and plant leaves are dried. Twenty-five mg of samples are blended with graphite containing lutetium and yttrium as internal standards. The blended mixture is formed into a 3/16 inch diameter pellet using a small hand press. The pellet is placed in a graphite electrode, and a 1 mm diameter vent hole is placed in the center of the pellet. Electrodes are mounted in a Stallwood jet, and a gas mixture of 20% oxygen and 80% helium is used while the samples are excited by a 25-ampere direct current arc. They detected 26 elements in these various matrices at 1 part per million or less. Quantitation is accomplished using standards containing known concentrations of the trace metals in a matrix of p-nitrobenzene-azo-resorcinol which serves to simulate the organic matrix of samples being analyzed. The authors reported percent relative standard deviations of ± 15% or less.

Water Samples

The determination of trace metals in natural waters is of great interest from a toxicological point of view. An excellent survey procedure for the determination of 19 minor elements in water has been described by Kopp and Kroner.[35] In this procedure water samples are filtered through a 0.45-micron membrane filter. Total dissolved solids are determined, and a volume of sample is selected to contain 100 mg of solids when concentrated to 5 ml. A portion of this concentrate is placed in a porcelain combustion boat and analyzed in triplicate using a rotating disc electrode and high voltage spark excitation. Standard solutions for construction of the analytical calibration curves are prepared using known amounts of the elements to be determined in a matrix of sodium, potassium, calcium and magnesium in proportions approximating the average composition of U. S. waters. Line intensities are recorded photoelectrically, and background is used as an internal standard. The concentration range is from 0.01 to 100 parts per million in the concentrated solution. Thus, the lower limits for the original samples depend on the degree of concentration employed to get 100 mg of dissolved solids. Precision expressed as the percent relative standard deviation was on the order of ± 5%. Recoveries from known additions varied from 80% to 113%.

Analysis of Air Samples

Not all spectrochemical analytical procedures are devised to determine multiple elements simul-

taneously. For example, O'Neil[36] described a procedure for the determination of beryllium in airborne dust. A high volume air sampler is used, and the sample is collected on Whatman No. 41 filter paper. The filter is ignited in a platinum crucible, and the ash weighed and mixed in definite proportions with an internal standard (lutetium) and graphite. The mixture is excited in an 11-ampere direct current arc and burned to completion. The author reported being able to detect 0.1 nanogram of beryllium in an electrode. Acceptable precision and accuracy was reported.

Of course, procedures have also been described for determining a large number of trace elements in air particulates. For example, the procedure by Keenan and Byers[37] permits the determination of 20 elements collected on paper filters.

SUMMARY

Emission spectroscopy provides an effective analytical technique as both a survey method and as a quantitative analysis tool. The applicability of this technique is being continually extended in the industrial hygiene field.

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