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PB82-147604

INSTRUCTOR MANUAL
INDUSTRIAL HYGIENE CHEMISTRY COURSE

LESSON NUMBER 6

April 1975

Prepared for:

National Institute for Occupational Safety and Health
Rockville, Maryland

Contract Number CDC-99-74-72

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ACKNOWLEDGEMENTS

Dunlap and Associates, Inc., of Darien, Connecticut wishes to acknowledge with sincere appreciation the support received from the National Institute for Occupational Safety and Health in the administration and conduct of this project. In particular, we would like to express our gratitude to Dr. Thomas Purcell, the Project Officer.

We wish also to thank our consultants who contributed directly to the preparation of course materials: Mr. J. D. Johnson of Spectrogram Corporation, Dr. C. L. Grant of the Center for Industrial and Institutional Development - University of New Hampshire, Dr. Melvin W. First of the Harvard School of Public Health, and Mr. Adrian L. Linch, private consultant.

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INTRODUCTION

This Instructor Manual has been prepared for industrial hygienists and analytical chemists participating in the National Institute for Occupational Safety and Health's Regional Training Program. The purpose of this Manual is to assist these professionally qualified, but possibly inexperienced, instructors in the preparation and conduct of a one-week "Industrial Hygiene Chemistry" course. This Manual will guide instructors through both lecture and laboratory lessons. It is complemented by a matching Student Manual. The course is recommended for students having, as a minimum, an undergraduate degree in chemistry (or its equivalent) along with at least one year's experience in instrumental analysis.

It is not necessary for instructors to have had prior teaching experience although such experience would be desirable. All instructors should be thoroughly familiar with industrial hygiene chemistry procedures, instruments and equipment relevant to the subject areas they will teach. In addition, each instructor should attend the course director's orientation seminar presented before the start of each one-week "Industrial Hygiene Chemistry" course.

The remainder of this introduction describes the course objectives, lessons, and the organization and format of the documentation in each lesson, including lecture and laboratory lesson plans.

Course Objectives

The following course objectives will be attained by graduates of this program:

- Given a particular chemical health hazard commonly found in the occupational environment, the trainee will be able to select an appropriate sampling strategy using available sampling techniques and to select a corresponding appropriate analytical method for quantitative characterization of the sample by using his knowledge gained from the course and technical information referenced in the course.

- Trainee will be able to apply his knowledge of wet chemical and/or instrumental analysis in employment of current methodologies for evaluating the typical work environment.

- Trainee will be able to perform and evaluate quantitative analytical determinations for four classes (types) of hazardous substances using a correspondingly different method for each class or type.

- Given the analytical results obtained through proper measurement procedures, the trainee will be able to define the data in terms of actual environmental concentration levels and to interpret the results in light of existing exposure standards.

Lessons

18 lessons are presented in this course:

- Introduction to Course
- Introductory Topics
- Direct Reading Instruments
- Air Flow Calibration and Sampling
- Ion Selective Electrode Laboratory
- Introduction to Spectrophotometry
- Instrumentation and Application of Spectrophotometry
- Colorimetric Determination of Free Silica (Quartz) Laboratory
- Introduction to Spectroscopy
- Atomic Absorption Spectrometry
- Atomic Absorption Spectrometry Laboratory
- Introduction to Chromatography
- Insturmentation and Application of Chromatography
- Gas Chromatography of Organic Solvents Laboratory
- Titrametric Determination of SO_2 Laboratory
- Colorimetric Determination of SO_2 Laboratory
- Biological Monitoring
- Related Topics

Lectures

Each lesson that is to be presented as a lecture is documented in a standardized format.

A. Lecture Cover Sheet

A cover sheet for each lecture presents the following information:

- Lesson title
- Lesson number and length
- Behavioral objective
- Scope of the lesson
- List of visuals
- List of exhibits
- List of equipment needed for the lesson

B. References

After the cover sheet, there is a list of references. These references are keyed to the paragraphs within each lesson. The number in parenthesis following each paragraph is the reference number. These references are included so that the instructor, if he wishes, may further research specific instructional subject matter.

C. Additional Readings

Following the reference list, in most lessons, is another listing called "Additional Reading." This bibliography contains books and articles which are generally pertinent to the subject area covered in this lesson. These are considered as important secondary reference sources.

D. Expanded Outline (left-hand page)

On the left-hand page, beginning after the Additional Readings section, is an expanded outline. This outline indicates the information that should be emphasized and covered during the lecture. The sequence of the outline should be followed during

teaching. The expanded outline gives sufficient information to explain the brief outline which is on the right-hand page. All test questions (both self tests and course evaluator) come from the expanded outline. Additionally, there are descriptions of the visuals within the outline.

E. Brief Outline (right-hand page)

This page consists of a notes column and the outline.

1. Notes Column - times (both elapsed and projected) are indicated in this column. The elapsed time designates the time it should take the instructor to reach this point in the lecture starting from 0 at the beginning of each lecture. The elapsed time is in parentheses. The projected time designates the time it should take the instructor to reach the next major portion of the outline. A major portion of an outline is designated by a capital letter in the outline. In addition, transitional phrases connecting the major outline portions are included in the notes column. These phrases are to assist the instructor in bridging from one section of the outline to the next. Notations of what visual, exercise, table, etc., should be introduced at a given point in a lesson and miscellaneous notes to the instructor are contained also in this column.

2. Outline - this is a brief outline corresponding to the expanded outline on the facing page. Words and phrases in the brief outline key the instructor to the lesson's subject content and to the expanded outline on the left-hand page. There is sufficient space between the key words in the brief outline for the instructor to write his own additional notes when he is preparing his lecture.

F. Exercises and Problems

In some lessons, exercises and problems are included. These are given during class time. The answers to the problems are worked out with students after they have had an initial try at completing them on their own. Answers are provided in the Instructor Manual.

G. Self Tests

Self tests are included after most lessons. The Instructor Manual contains the correct answers, whereas the Student Manual does not. The students should first answer the questions, and then the instructor should review the answers, explaining fully the reasons for the correct answers. The self tests are not scored by the instructor and no records are kept of the individual student's performance. The instructor should use the information from the discussion of self tests to remove student misunderstandings or lack of understanding.

H. Copies of Visuals

Copies of visuals that are to be shown in a lecture are included at the end of that lesson documentation. These can be useful in preparing for the lecture presentation.

I. Homework

No specific homework assignments are included within the lesson documentation. However, there is a great quantity of information for the students to absorb during this one-week course. Therefore, students should be urged to review nightly all lessons covered during the day and all lessons to be presented on the following day. In particular, they should become familiar with the laboratory procedures for the following day. There is much to be accomplished in every laboratory and little time to do it. If the students are familiar with the procedure, the laboratory experiments will progress much more smoothly.

Laboratories

Each lesson that is to be presented as a laboratory is documented in standardized format consisting of four elements.

A. Laboratory Cover Sheet

A cover sheet for each laboratory presents the following information:

- Lesson title
- Lesson number and length
- Behavioral objective
- Scope of the lesson
- List of equipment, apparatus and forms

B. Special Preparation Section

This section will follow the laboratory cover sheet, and includes specific directions that must be followed prior to actual class time. These instructions are concerned with the preparation of apparatus, facilities, chemicals and materials that are necessary for the laboratory session.

C. Laboratory Procedures

The procedures for performing each laboratory are fully documented on the left-hand page. The elapsed and projected times are indicated for some lessons with the elapsed times appearing in parentheses. The right-hand page is a blank page for notes on specifics of the laboratory to aid the individual instructor in giving an efficient lesson.

D. Figures and Forms

Equipment figures and student forms are included after the procedures. The figures are presented to aid the instructor in setting up the experimental equipment. The forms are to be used by the students during the laboratory to assist them in recording, calculating and analyzing data.

LESSON TITLE	LESSON NUMBER	LESSON LENGTH
Introduction to Spectrophotometry	6	2:00

BEHAVIORAL OBJECTIVE

The student will be able to list the basis, uses, conditions, and sampling constraints for visible light, ultraviolet, infrared and fluorescence spectrophotometry.

SCOPE

Definition and description
Molecular structure and energy levels
Basic structure of the spectrophotometer
Beer's Law, transmittance, and absorbance
Visible light spectrophotometry
Ultraviolet spectrophotometry
Fluorescence spectrophotometry
Infrared absorption spectrophotometry
Sampling considerations
Sample preparation

VISUALS	EXHIBITS
6-1 through 6-10	6-1

EQUIPMENT

Overhead projector
Screen
Blackboard
Chalk

REFERENCES

LESSON TITLE

Introduction to Spectrophotometry

1. American Society for Testing and Materials, Committee E-13. Manual on Recommended Practices in Spectrophotometry, Method E 131-68, 30-34, Philadelphia, Penn. (1969).

2. Cheng, K. L. Spectrophotometry and Fluorometry, Ch. 5, Trace Analysis, G. L. Morrison, (Ed.), Wiley Interscience, New York (1971).

3. Conley, R. T. Infrared Spectrophotometry, 221-238, Allyn and Bacon, Inc. Boston, 2nd ed. (1972).

4. Bumsted, H. F. Spectrophotometry, Ch. 19, 232, U. S. Dept. of Health, Education and Welfare, Public Health Service, National Institute for Occupational Safety and Health. The Industrial Environment--Its Evaluation and Control, U. S. Government Printing Office, Washington, D.C. (1973).

5. Harrison, G. R., et al. Practical Spectroscopy, Ch. 11, Prentice-Hall, New York (1948).

6. Conley, R. T. Infrared Spectrophotometry, 26, Allyon and Bacon, Inc. Boston, 2nd ed. (1972).

7. Van Duuren, B. L., and Chan, T. L. Fluorescence Spectrometry, Ch. 7, Spectrochemical Methods of Analysis, J. D. Winefordmer, (Ed.) in Vol. 9, Advances in Analytical Chemistry and Instrumentation, Wiley Interscience, New York (1971).

8. Cheng, K. L. Absorptiometry, Ch. 6, Spectrochemical Methods of Analysis, J. D. Winefordner, (Ed.) in Vol. 9, Advances in Analytical Chemistry and Instrumentation, Wiley Interscience, New York (1971).

9. Jacobs, M. B. The Analytical Toxicology of Industrial Inorganic Poisons Ch. 2, 9, Wiley Interscience, New York (1967).

10. U. S. Department of Health, Education and Welfare, Public Health Service, National Institute for Occupational Safety and Health. NIOSH Manual of Analytical Methods, Government Printing Office (1974).

ADDITIONAL READINGS

LESSON TITLE

Introduction to Spectrophotometry

1. Herzberg, G., Molecular Spectra and Molecular Structure I Spectra of Diatomic Molecules, Van Nostrand Reinhold Co., New York, 2nd ed. (1950).

2. Jaffe, H.H., and Orchin, M., Theory and Application of Ultraviolet Spectroscopy, John Wiley and Sons, New York (1962).

3. Mavrodineanu, R., Shultz, J. I., and Menis, O., (Eds.) Accuracy in Spectrophotometry and Lummescence Measurements, NBS Special Publication 378, Library of Congress #73-600066 (May 1973).

4. Morrison, G.H. (Ed.) Trace Analysis, Interscience Publishers (1955).

5. Whiffen, D.H., Spectroscopy, John Wiley & Sons, New York, 2nd ed. (1972).

6. Yoe, J.H., Koch, H.J., (Eds.) Trace Analysis, John Wiley & Sons, New York (1957).

TITLE	LESSON NUMBER
Introduction to Spectrophotometry	6

A. Definition and Description

1. Spectrophotometry is a measurement technique which furnishes the ratio of the radiant power of two beams as a function of spectral wavelength. The two beams may be separated in space or time or both. Spectrophotometry quantifies the interaction of electromagnetic radiation with matter. The matter may be atomic or molecular, and the radiation may extend from x-rays through ultraviolet, visible, and infrared radiation and also through the microwave region. In this lesson spectrophotometry will be limited to the visible, ultraviolet, and infrared regions of the spectrum and with the interactions with molecular species. (1)

2. Spectrophotometric measurements are made for two purposes; to quantitatively measure, at one or more wavelengths, the amount of a substance present or to measure, at many wavelengths, the response of a substance to determine its composition and to elucidate its structure. The majority of the measurements for industrial hygiene chemistry are made to determine the amount of the substance present. Also, most of the measurements are based on the specific absorption of a molecular species and in some instances emitted energy from a stimulated molecular species is measured as fluorescence. (2)

3. The absorbing or stimulated molecular species is often produced chemically, during the progess of the analytical method, for the purpose of measurement of an analyte with high specificity. Therefore, a measurement by spectrophotometry is often a part of an analytical procedure which involves sampling, combustion, dissolution, chemical reaction, extraction or filtration before the spectrophotometric measurement is made. Such a colorimetric method need not finish with a spectrophotometric measurement. The measurement may be made visually, with a photometer, with a filter photometer or with a spectrophotometer. (2)

4. Visual 6-1 and exhibit 6-1 shows the electromagnetic spectrum.

NOTES	Times (elapsed) projected	LESSON OUTLINE
	(---) 0:10	A. Definition and Description 1. Definition of spectrophotometry 2. Spectrophotometric measurements--quantitative and qualitative 3. Spectrophotometry part of total analytical procedure
Visual 6-1 Exhibit 6-1		4. Electromagnetic spectrums

5. Analytical methods in the visible region without specific wavelength selection have been used since 1852, when Beer first proposed a general law for the absorption of radiation. Instrumental developments since 1940 have extended photometric measurements through the ultraviolet region to approximately 190 nanometers and into the near infrared region to 1000 nanometers. These developments include improvements in detectors, sources, gratings, optics and electronics. These same developments have also extended the applications of fluorescence analysis in trace analysis since 1935. (8)

6. While these and similar developments have increased the sensitivity of measurements in the infrared region of 2000 nm to 40,000 nm., the infrared region has been used mainly to determine the structure of molecules. However, recent applications, particularly for gases, make quantitative use of the infrared region. (3)

7. Due to the historic development of visual colorimetric analysis, most determinations in spectrophotometric trace analysis involve a chemical reaction in which a color is developed. However, ultraviolet and infrared measurements without color development are increasing in the number of applications, particularly where physical separation or selection of the sample is involved. (4)

B. Molecular Structure and Energy Levels

1. Atomic spectra result from varying energy levels of orbiting shell electrons in association with a single positively charged nucleus. The motion of the whole atom does not greatly influence the atomic spectra because all the components of the atom move in close association along a straight path. The Doppler effect, for instance, is relatively minor in that it produces a broadening of a spectral line when some of the emitting atoms move away from the measuring instrument and some move towards it. If all the emitting atoms move away, the observed wavelength would be longer than normal and the spectra is said to be shifted to the red. The term comes from astronomical spectroscopy, where significant red shifts are used to determine the velocity of an astronomical object moving away from the earth. (5)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		5. Historial developments
		6. Uses in infrared region
		7. Color development in trace analysis related to spectrophotometry
(Transition A.-B.) From a general definition to details of molecular structure.	(0:10) 0:20	B. Molecular Structure and Energy Levels
		1. Atomic spectra and Doppler effect

2. In a molecule the electrons move with varying energy levels about two or more atoms. In addition, the group of atoms has linear translational energy, rotational energy and periodic vibrational energy around one or more centers within the molecule. These vibrational energies oscillate harmonically, providing the extremes of the stretching and bending of the atomic bonds are not severe. Generally, harmonic oscillations occur within ten percent of the average bond length between two atoms and the average bond angle between three atoms. Vibrational energies, which are observed mainly in the infrared region of the spectrum, also affect the electron energy levels, which are observed from the vacuum ultraviolet region through the near infrared region of the spectrum. (5)

3. The total energy of a molecule in dynamic equilibrium may be taken as the sum of its translational, rotational, vibrational and electronic energies. (Visual 6-2) (5)

4. The translational energy, H_{trans}, has no significance in molecular spectra as applied here to analytical spectrophotometry. (5)

5. The rotational energy, H_{rot}, is directly related to the angular velocity with which a molecule rotates. According to quantum mechanics, these velocities result in discrete moments of inertia which depend upon the geometry of the molecule. All molecules may be classed as linear rotators, spherical tops, symmetrical tops or asymmetrical tops which rotate about their x, y and z axis. For example, acetylene has four atoms in a straight line and is a linear rotator. Methane, in the shape of a tetrahedron, is a spherical top. Ammonia as a trigonal pyramid and benzene as a plane hexagon are symmetrical tops. All polyatomic molecules, which do not have a threefold or more axis of symmetry, such as vinyl chloride, are asymmetrical. Since the molecule only freely rotates in the gaseous state, rotational spectra are not distinctly observed in liquids and solids. This plus the relatively low energy involved at the long wavelength of the far infrared and microwave regions, precludes the analytical use of rotational spectra, particularly for trace analysis. (5)

NOTES	Times (elapsed) projected	LESSON OUTLINE
A brief consideration of these energies will illustrate how the structure of the molecule is related to the analytical application of spectrophotometric measurement.		2. Linear translational, rotational and periodic vibrational energy
Visual 6-2		3. $H_{mol} = H_{trans} + H_{rot} + H_{vib} + H_{el}$
		4. Translational energy
		5. Rotational energy--linear, spherical, symetrical, and asymetrical rotation

6. The vibrational energy, H_{vib}, results from the interaction of atoms within a molecule. A molecule which has N number of atoms exhibits 3 N kinds of motion. Three are simple translations and an additional three are rotational. (Only two are rotational if the molecule is linear.) Therefore there are 3N-6 remaining kinds of motion and these are all vibrational. (For linear molecules there are 3N-5 remaining motions.) Each of these remaining vibrational motions is associated with a particular frequency. These frequencies are the 3N-6 fundamental frequencies of the molecule. These fundamentals may all be different or they may be grouped in pairs or triplets at the same frequency. (5)

7. While it is theoretically possible to calculate all the fundamental frequencies of a molecule, it becomes complicated with increasing numbers of atoms and with greater asymmetrical configurations. For instance, the solution for water requires a third degree equation. Benzene has 12 atoms, but great symmetry and its solution only requires a fourth degree equation. However, the asymmetrical orthocholorophenol molecules require the solution of a thirty-third degree equation. Therefore, an empirical approach has been used, for the most part, to relate the observed absorptions in the spectrum to the structure of the molecule. (6)

8. For example, in the case of the molecule chloroform, which has a single C-H bond, the higher masses of the chlorine atoms allow the stretching and bending frequencies of the C-H bond to appear at 3.44 micrometers and 6.89 micrometers, practically independently of of the rest of the molecule. Therefore, chloroform in the presence of carbon tetracholoride could be determined quantitatively at these wavelengths. However, its determination in the presence of other chlorinated hydrocarbons would require a prior separation such as with gas chromotography. This is because the C-H bonds would no longer be specific for chloroform. (3)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		6. Vibrational energy--different kinds of motions
		7. Fundamental frequencies of molecules
		8. Chloroform--C-H bond, 3.44 μm and 6.89 μ m

9. The electronic energy, H_{el}, arises from the electrons in a molecule which are shared by two or more atoms. An electron, which is shared by two atoms, has four designated quantum numbers, n, l, λ and s which are similar to the four atomic quantum numbers. These quantum numbers have restrictions on them, as in the case of atoms, according to Pauli's principle. This principle states that no two electrons can have the same energy, or combination of quantum numbers. There is a correlation of the diatomic quantum numbers with the atomic quantum numbers of the outer electrons in each of the two atoms of the molecule. The electronic energy associated with two molecular states, as in a free diatomic molecule, is illustrated in Visual 6-3. Two separate electronic states are shown and within each state the energy levels are seen to vary with the distance between atoms. As this distance becomes exponentially large, a limiting value is reached and this is the dissociation energy. At higher levels of the quantum number, n, the separate multiple states will converge mainly in the vacuum UV region in the form of the Rydberg series. The limit of this series is then the ionization potential of the molecule, the same as it is in atoms. (5)

10. Electrons, which are associated with three or more atoms, are often at energy levels peculiar to a small group of atoms within the molecule and largely unaffected by neighboring groups of various kinds. These electrons have less energy than those associated with one or two atoms, and they generate characteristic absorptions in the ultraviolet, visible and near infrared spectral regions. For example, a C=C double bond will have a maximum absorption at about 190 nanometers; conjugated double bonds, C=C=C will be at 220 nanometers; three conjugated double bonds $(C=C)_3$ will be at 260 nanometers; and finally, four $(C=C)_4$ will be at 290 nanometers. (8)

11. Absorption in the visible region by a group of atoms, largely independent of the rest of the molecule was first noted in the dye chemistry industry. Such a group of atoms is termed a chromophore, which means color carrier. Visual analytical colorimetry methods were developed from these specific chromophores. These are generally associated with the reagent which combines with the analyte in an analytical colorimetric method. With the use of instruments above and below the narrow visible range, the term chromophore remains, though the chemistry does not produce a visible color. (8)

NOTES	Times (elapsed) projected	LESSON OUTLINE
Visual 6-3		9. Electronic energy, quantum numbers, Pauli's principle
		10. Energy of electrons associated with 3 or more atoms
		11. Chromophores--analytical colorimetric methods

12. Whether the electronic energy levels are associated with a subgroup of atoms or the molecule as a whole, the energy levels and the modes of transition can be represented schematically as in Visual 6-4. The absorption of ultraviolet energy will cause the molecules to move from the ground state to one or more excited states. In the excited singlet state all the electrons are paired. There is a rapid exchange of energy between levels of one state by relaxation and between different energy states by internal conversion. In many cases there may be a complete return to the ground state by a radiationless vibrational relaxation. This involves rotational, vibrational or kinetic energy and is seen as heat or chemical energy, such as in a photochemical reaction. However, many molecules, such as aromatic compounds, containing specific chromophoric groups may rapidly return to the ground state by the emission of a lower energy fluorescence radiation. The fluorescence emission is generally rapid and takes place in one nanosecond or less. (7)

13. In other instances, all but two of the molecular electrons are paired and the molecule may exist in a metastable or triplet state for a longer period of time. Again, the return to the ground state may be by vibrational relaxation or by a further lower energy release of radiation. This radiation is known as phosphorescence. Depending upon the lifetime of the excited singlet state and the energy difference between the singlet and triplet states, the time required for the intersystem or level crossing transition is 10 to 100 nanoseconds. However, the time required for the decay of the triplet state by phosphorescence emission may vary widely from microseconds to several seconds. (7)

14. Whereas many compounds may have analytically useful absorption frequencies, a few of these will also fluoresce and even a fewer number will exhibit a delayed phosphorescence. Each of these steps increases the selectivity of an analytical procedure, as instrumental discrimination is made with respect to the wavelength and time period in the analytical measurement. (7)

NOTES	Times (elapsed) projected	LESSON OUTLINE
Visual 6-4		12. Energy levels and modes of transition
		13. Phosphorescence--triplet and singlet states
		14. Problems with fluorescence and dealyed phosphorescence

C. Basic Structure of the Spectrophotometer

1. Visual 6-5 shows a schematic representation of a photometer. A complete instrument needs only a light source, light concentrating lens, wavelength filter, sample cell, photocell detector and meter.

2. The addition of a monochromator, with its superior wavelength selection, makes a simple photometer a spectrophotometer. (Visual 6-6)

3. A monochromator is an instrument or device which will select, from a suitable energy source, a continuous calibrated series of electromagnetic radiant energy bands of determinable wavelength or frequency. Most often, it consists of a narrow entrance slit to produce a confined beam of polychromatic radiation and one to three optical elements. This includes a prism or grating which spreads the polychromatic radiation in a wavelength spectrum. The exit slit is narrow so that it will pass only a few selected wavelengths. The monochromator also contains a wavelength drive mechanism, with an indicator to register the wavelength position. Slit widths may be continuously variable or fixed. In some instances, a single slit may serve both as entrance and exit slit. With a continuum source, the selected wavelength becomes more nearly monochromatic as the slit width is reduced. With a line source, such as a hollow cathode discharge lamp, the selected wavelength becomes monochromatic as soon as the reducing width of the slit rejects any spectral line other than the desired one. (1)

4. A double beam absorption spectrophotometer, shown in Visual 6-7, greatly increases the precision and analytical flexibility in comparison with the single beam instrument. In the dual beam mode, the selected wavelength beam is most often directed by a rotating mirrored chopper alternately through a sample cell and a reference cell. The two beams are then combined to impinge on a single detector. The alternating signal from the detector, when amplified and displayed, indicates the percent absorption or the absorbance directly. In other instances, the beam is separated into two of equal intensity by an optical splitter and then two separate detectors observe the sample and reference beams to obtain the difference signal. In the single beam mode, the instrument is adjusted for zero absorbance with the reference cell in place and then the sample cell is mechanically moved into place to obtain the absorbance. The greater precision of the double beam instrument is

NOTES	Times (elapsed) projected	LESSON OUTLINE
(Transition B.-C.) Molecular structure to the structure of a spectrophotometer	(0:30)	C. Basic Structure of the Spectrophotometer
Visual 6-5 This section is presented just to introduce the spectrophotometer briefly.	0:15	1. Schematic of photometer
		2. Schematic of spectrophotometer
Visual 6-6		3. Monochromator
Visual 6-7		4. Dual beam absorption spectrophotometer--different possible configurations and advantages of instrument

due to the cancellation of source fluctuation, and also alternating current amplifiers are more stable than direct current amplifiers. However, the singular advantage of the double beam mode is the ability to record a spectrum of the analyte or solute as the monochromator is driven through a range of wavelengths. The double beam method compensates for variations in the source, the solvent transmission, the solvent purity and the detector response are greatly reduced. Thus the double beam mode provides a convenient, rapid and precise way to obtain quantitative measurement for many wavelengths, instead of a single point measurement. (4)

5. The measurement of fluorescence intensity can be made with a filter fluorometer which is comparable to the filter photometer. Normally there is a 90 degree angle between the beams from the source and to detector. Since the majority of fluorescence methods rely upon the ultraviolet irradiation of the analyte in dilute solutions, a quartz cell is required. Some UV-visible spectrophotometers have fluorescence attachments with which the sample is excited with a band of ultraviolet radiation and a monochromator analyzes the fluorescence spectra emitted from the sample. The discriminating instrument is shown schematically in Visual 6-8. One monochromator is used to select a specific wavelength for efficient excitation and the second monochromator selects the most intense or interference free emission wavelength of the analyte for measurement. (7)

D. Beer's Law, Transmittance and Absorbance

1. The absorption of energy by a solution is covered by two laws. The first is the Bouguer or Lambert law which states that if a beam of parallel monochromatic light penetrates a medium at right angles to its surface, then the decrease in intensity through the medium is proportional to the intensity of the beam.

2. Visual 6-9 shows Lambert's law.

NOTES	Times (elapsed) projected	LESSON OUTLINE
Visual 6-8		5. Filter Fluorometer schematic--uses and advantages
(Transition C.-D.) From a brief description of the spectrophotometer to a theoretic basis for the principles used.	(0:45)	D. Beer's Law, transmittance, and absorbance
	0:25	1. Lambert's law
Visual 6-9		2. Shows Lambert's law

3. The ratio I/I_o is the internal transmittance through the cell system. When the cell walls do not appreciably absorb or scatter the incident intensity, there is little difference from the total transmittance. Usually a pair of matched and calibrated cells are used in analysis. Alternatively, a blank cell is used to correct the differences to yield a net transmittance. Thus:

$$\ln T = \ln \frac{I}{I_o} = -Kb \qquad (4)$$

4. The Bernard or Beer law states that the same beam of parallel monochromatic light is decreased in intensity exponentially with the increase in concentration, c, of the solute in solution. (4)

5. Thus:

$$\ln \frac{I}{I_o} = -K'c$$

Combination of these two laws with integration over the cell length, b, yields

$$\ln \frac{I}{I_o} = -Kbc$$

or more generally

$$-\log \frac{I}{I_o} = -\log T = A = \epsilon bc$$

where A is the absorbance and ϵ is the molar absorptivity when the concentration, c is expressed in moles per liter and b is in centimeters. This is the Beer-Lambert law, or more simply, Beer's law. (Visual 6-10) (1)

NOTES	Times (elapsed) projected	LESSON OUTLINE
Write equation on the blackboard		3. Internal and net transmittance
		4. Beer's law
Write derivation of Beer's law on blackboard. Visual 6-10		5. Derivation of Beer's law

6. When the concentration is expressed in weight per volume, the term a is used for absorptivity. This expression (i.e. A = abc) is derived by assuming that the incident radiation is monochromatic, that the absorption takes place in a volume of uniform cross section and that other absorbing species do not interact in the absorption process. (4)

7. In the case of several absorbing species the total absorbance is then

$$A_{total} = \epsilon_1 bc_1 + \epsilon_2 bc_2 + \ldots.$$

or

$$A_{total} = b(\epsilon_1 c_1 + \epsilon_2 c_2 + \ldots.) \quad (8)$$

8. In dilute solutions of 10^{-2} moles or less, there is generally a linear relationship between the absorbance and the concentration in conformity with Beer's law. Deviations resulting in nonlinearity are derived from instrumental and chemical variables in the analytical method. (8)

9. The instrumental variables are:

- Polychromatic radiation causes deviations from Beer's law since the edges of a wide wavelength band are absorbed proportionately less than the central peak absorption region of the analyte.

- The slit width controls the width of the band pass and also the total radiant power that reaches the detector. Narrow slit widths are best for resolution. However, a compromise must often be made between accuracy, sensitivity and detector noise which ultimately defines a detection limit in trace analysis.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		6. Basis of absorptivity
		7. Total absorbance
		8. Deviations of Beer's law
Careful attention to instrumental and chemical variables will allow the deviations from Beer's law to be confined and controlled so that precise and accurate spectrophotometric measurements may be made.		9. Instrumental variables: . Polychromatic radiation . Slit width

- Stray light refers to wavelengths, most often scattered within the monochromator, which are not absorbed by the analyte and which cause negative deviations from Beer's law. That is, at a particular wavelength selected by the monochromator, some proportion of the emerging radiation passed by the exit slit is random with respect to the calculated or desired band pass defined by the slit widths. Stray light errors are usually most extreme near the wavelength limits of the spectrometer. Refined instruments, in which the stray light is less than 0.005 percent, often employ two monochromators, which sequentially pass the chosen wavelength with almost total rejection of stray or scattered wavelengths. (8)

10. The chemical variables which cause deviations are:

- The equilibrium of the chemical chromophoric species may be upset with variations in concentration due to the effects of association, complex formation, polymerization or dissociation. For example, the dichromate ion, $Cr_2O_7^{-2}$, has an absorption maximum at 350 nm. and the chromate ion, CrO_4^{-2} has a maximum at 372 nm. In dilute aqueous solution they are in equilibrium thus:

$$Cr_2O_7^{-2} + H_2O \rightleftharpoons Cr_2O_4^{-2} + 2H^+$$

A strong acid solution is required to assure that all the chromium being measured at 350 nm. exists as the dichromate. Otherwise Beer's law will not be followed.

- Temperature variations may shift the peak wavelength of the absorbing species, with a higher temperature often producing a shift to longer wavelengths (bathochromic).

NOTES	Times (elapsed) projected	LESSON OUTLINE
		. Stray light
		10. Chemical variables:
		. Variations in chemical concentration due to association, complex formation, polymerization, or dissociation
		. Temperature variation

- The spectrum of a vapor is often shifted to longer wavelengths when the substance is dissolved in a solvent. Solvation causes large shifts in the infrared region and lesser ones in the ultraviolet region. This occurs particularly when polar solvents or other highly ionized additives are added to nonpolar solvents. Unless wavelength compensation is made, there will be an apparent deviation from Beer's law.

- Many photo processes may cause deviations due to fluorescence, colloidal light scattering, polarization and photochemical reaction. These effects, though relatively small, will produce errors in spectrophotometric measurement that are not immediately obvious to the analyst. (8)

E. Visible Light Spectrophotometry

1. Analytical methods based upon the chemical formation of a visible chromophore are most often known as colorimeteric procedures. Many methods for the determination of metals in solution at trace concentrations require the generation of a colored complex with one of several possible organic reagents. For maximum analytical use, the reaction should feature the following:

   - The reaction should be stoichiometric.

   - The reaction should be specific for the element.

   - The complex should have a well isolated absorption band.

   - The complex and its reagent should be stable and the color development should be rapid.

   - The complex should be soluble in a solvent free of spectral interference.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		. Vapor dissolved in solvent
		. Photo processes cause deviation
(Transition D. -E.) From general theory to visible light spectrophotometry.	(1:10) 0:10	E. Visible Light Spectrophotometry 1. Colorimetric procedures
		. Stoichiometric
		. Specified element
		. Isolated absorption band
		. Complex reagent stable
		. Soluble in solvent free of spectral interference

- The complex should be little affected by such variables as temperature, pH, photolysis, etc. (4)

2. Thirty-nine analytical methods were published by NIOSH of the U.S. Department of H.E.W. in 1974. Fourteen of these are colorimetric and eight of the fourteen are for the determination of nonmetallic substances. (10)

3. Colorimetric procedures designed for the determination of a single analyte may involve a single visible chromophore developed from a colorless reagent, or depletion of a chromophore in a colored reagent as the complex increases with increasing concentration of the analyte. This is generally true for the metals that react with dithizone. Some compensation must be made for the variable amount of excess reagent remaining in solution. (8)

4. In other cases a fixed concentration of a metallo-organic complex is established first in a procedure. The effect of the analyte is to dissociate the complex and decrease the chromophore intensity in a reverse reaction. (8)

5. In many instances the analyte reacts with the colorimetric agent in solution for a definite period and at a controlled temperature. The complex is then extracted in a separate organic solvent. Additional concentration of the analyte may be attained in this way. (8)

6. Visible spectrophotometric or colorimetric procedures are one of the most useful to the industrial hygiene chemist. (4)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		. Little affect by temperature, pH, etc. 2. Determination of metals small part 3. Amount of reagent 4. Fixed concentration of complex 5. Obtaining additional concentration of analyte 6. Visible spectrophotometry--useful

F. Ultraviolet Spectrophotometry

1. The useful spectral region for ultraviolet spectrophotometry extends from 380 nanometers down to 200 nanometers. Applications at lower wavelengths are not common due to the absorption bands of the oxygen molecule in the atmosphere. However, in the useful region many organic compounds, in particular the aromatic compounds, exhibit absorptions with high molar absorptivities and trace determinations are made. (8)

2. Whereas colorimetric methods of analysis are largely restricted to solutions, the vapor phase of volatile aromatic compounds will produce many highly resolved bands, such as the five distinct band groups of benzene in the region of 250 namometers. When benzene spectra are recorded with cyclohexane as the solvent, these bands are broadened and shifted slightly toward a longer wavelength. Although the vapor state of aromatics in the ultraviolet has not been used in routine analysis, the identification of small samples of 0.1 to 5 microliters is feasible. (4)

3. Commonly the analyte is dissolved in a solvent which transmits at a sufficiently low wavelength in the ultraviolet region, so as not to interfere with the spectrum of the analyte. (4)

G. Fluorescence Spectrophotometry

1. Fluorometric determinations are finding increased application with the industrial hygiene chemist because of greater interest in aromatics and polynuclear aromatic compounds. When applicable, fluorescence measurements often are more sensitive than ultraviolet absorption by two to three orders of magnitude. Most work is performed with the analyte in solution at 10^{-4} to 10^{-9} molar. (7)

NOTES	Times (elapsed) projected	LESSON OUTLINE
(Transition E.-F.) From visible to ultraviolet spectrophotometry.	(1:20) 0:05	F. Ultraviolet spectrophotometry 1. Usefulness in 380 nm - 200 nm 2. Vapor phase of volatile aromatic compounds produce many bands 3. Analyte dissolved in low wavelength type solvent
(Transition H.-G.) From ultraviolet to fluorescence spectrophotometry. Describe relevant experience in this area.	(1:25) 0:03	G. Fluorescence Spectrophotometry 1. Application of fluorescence spectrophotometry

2. As indicated in the discussion on electronic molecular spectra, fluorescence spectra can be very specific. For instance, in dilute solutions aniline and benzene produce fluorescence spectra but nitrobenzene does not. In addition, the degree of fluorescence of similar compounds can vary widely. For instance, in equal concentrations aniline is 40 to 50 times more sensitive than benzene.

H. Infrared Absorption Spectrophotometry

1. Although the infrared energies of vibration and rotation are low, the information power of infrared absorption is very great. Although the sensitivity is not as great as UV absorption and fluorescence, very specific determination may be made among homologous compounds. Most of the infrared work is performed with dual beam instruments with the analyte in non-polar solvents. Small samples are analyzed on optical surfaces by internal reflection spectrometry. For the direct analysis of gases and vapors one to twenty meter cell path-lengths are used effectively with an infrared filter photometer. (6)

I. Sampling Considerations

1. Since industrial hygiene determinations are usually made at trace levels, the analyst must include sampling as an integral part of the analytical procedure. Without detailing the wide variety of sampling techniques, some general principles can be established. (8)

2. The sampling procedure must collect the analyte to be determined over a sufficient time to represent the time weighted average exposure to the worker, the rate of contamination of the work area and the general level of contamination. Often peak exposure rates are also determined. When the rate of contamination varies rapidly and widely, the sampling frequency must be higher. (9)

3. At the same time, enough analyte must be collected to satisfy the analytical procedure whether it be by the collection of a volume of air or by the collection of the analyte from the atmosphere. (9)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		2. Can be very specific
	(1:28)	H. Infrared Absorption Spectrophotometry
(Transition G. -H.) From fluorescence to infrared spectrophotometry	0:02	1. Application and limitation
	(1:30)	I. Sampling Considerations
(Transition H. -I.) From specific spectrophotometric applications to general sampling considerations.	0:10	1. Integral part of analysis
Discuss relevant experience to enhance importance of sampling considerations.		2. Proper temporal sample
		3. Enough sample for analysis

4. Analysts are careful to include blank determinations in the analytical procedure, so that residual amounts of the analyte in solvents and reagents may be corrected for. The same principle must be applied to sampling. For instance, when sampling of aerosols and particulates, blank filters and filter holders must be included with the samples. The same is true for aqueous solutions and solvents used in the collection of gases and vapors. (9)

5. It is equally important to assure that the analyte is not lost during sample transfer and treatment prior to the analysis. For example, arsine might be sampled in a solution of sodium hydroxide in a bubbler, transported to the laboratory in the glass container, digested in acid and finally determined colorimetrically. Without control measures taken at each step, it would not be clear whether arsenic was lost as arsine through inefficient conversion in the samples, as arsenate in aerosol, particles due to an excessive sampling rate, as arsenate ion absorbed in the glass wall surface of the container, or as a volatile compound formed during the acid digestion. (9)

6. Those procedures which reduce the sample handling and even perform the analysis in the field are preferred when the analyte may be lost or contaminated. The spectrophotometric techniques in the visible, ultraviolet and infrared regions can often be done in the field with a minimum of effort. (8)

J. Sample Preparation

1. Sample preparation for spectrophotometric measurements vary widely with the type of analysis and with the technique employed. As one example, quantitative analysis utilizing visible spectrophotometry is most often accomplished with a single beam instrument at a single wavelength. However, the limitations of many colorimetric procedures are the chemical reactions for which the procedures are based, rather than in the instrumentation. Although few reactions are specific for a particular analyte, many reactions are quite selective or can be made selective through the control of pH, change of oxidation state, the introduction of masking agents, the use of solvent extraction, or the removal of an interfering substance prior to the analysis. Thus, the instrumental measurement may constitute only five percent of the total effort in a colorimetric procedure. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		4. Blanks necessary
		5. Care in transfer of sample
		6. Do analysis in field if possible
(Transition I.-J.) From general sampling considerations to the preparation of samples.	(1:40)	J. Sample Preparation
	0:10	1. Role of sample preparation in visible light spectrophotometry

2. Following the sampling as outlined in Section I above, a typical colorimetric procedure for the determination of a metal might include the following steps in sample preparation:

- The sample is digested with one or more mineral acids to render the analyte soluble.

- Insoluble material is filtered from the sample and washed to remove all the soluble analyte.

- The sample is diluted to a known volume in dilute acid - 50 milliliters, for example.

- Twenty ml. may be then taken and the pH adjusted to a value of possibly $4.5 \pm .2$.

- A volume of a buffer solution might then be added along with a solution of an organic reagent.

- The mixture might then be heated in a covered plastic beaker to 70°C for 15 minutes to complete the reaction of the metal analyte with the organic reagent.

- The mixture could then be placed in a separatory funnel and extracted with three successive 3 ml. portions of carbon tetrachloride.

- The combined extracts might then be diluted to 10 ml. and the spectrophotometric measurement made within the next five minutes.

- The sample extract is used to fill the sample cell, and the reference cell is filled with a blank extract. This blank is a synthetic sample which includes all the acid, buffers and reagents used with the real sample.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		2. Typical sample preparation for determination of metals: . Digested with mineral acids . Insoluble-filtered . Diluted in acid . Small amount taken and pH adjusted . Buffer and organic reagent added . Mixture heated . Placed in separatory funnel and extracted with CCl_4 . Extracts diluted . Sample and blank cells

- Two or three standards should have also been prepared exactly as the samples have been.

- The absorbance of the blank is subtracted from that of the samples and standards. The concentration of the analyte is calculated from the comparison with the standards. (2)

3. The sample preparation for a determination in the ultraviolet region may be similar to the procedure illustrated above for the visible region. However, if the measurement were to be made at 240 nanometers, carbon tetrachloride could not be used since it does not have appreciable transmission below 265 nm. The extracting solvent could be cyclohexane which transmits down to at least 210 nm. Transmission below 200 nm. would be limited by the instrumentation, by the quartz absorption cell and by the oxygen in the air in the light path. (9)

4. The ultraviolet procedure might utilize a simple sample preparation technique. This could consist of the extraction of an organic analyte from the air into cyclohexane during the sampling stage, the distillation of the sample, the dilution to a fixed volume, and the spectrophotometric measurement in a double beam instrument. Blank cyclohexane would be placed in the reference cell, and the absorption spectra might be recorded from 220 to 340 nm. The shape of the absorption spectra would confirm the identity of the analyte. The amount present would be determined by comparison with known standards. (9)

5. If the organic analyte has an appreciable molecular fluorescence, this same sample preparation in cyclohexane could be used for a quantitative determination with a spectrophotofluorometer. The excitation monochromator would be set at the wavelength of maximum ultraviolet absorption, and the fluorescence would be measured against standards at the peak of the emission spectrum. The fluorescence measurement is often more selective and sensitive than UV absorption, but precuations must be taken so that foreign substances do not quench or inhibit the fluorescence.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		• Standards prepared • Determination of concentration 3. Sample preparation for UV -- C_6H_{12} instead of CCl_4 4. UV procedure in specific 5. Same sample preparation for measurement of fluorescence

6. Although most sample preparations for UV and visible spectrophotometry depend upon the generation of solutions, the sample preparations for infrared analysis may be much more varied. Gases, liquids and solids may be examined directly. Although quantitative analysis by IR has not been widely used until recently, sample preparation of an analyte in solution similar to UV absorption may be used. The choice of solvents is more limited because a given solvent may have transmission in one region and not in another. Also the solvent should not attack the cell windows. Dilute solutions with non-polar solvents are used to eliminate or reduce intermolecular reactions. (3)

7. Solid samples can be analyzed by IR spectrophotometry by placing the suspension or mulling of particulates in a viscous liquid, by dispersing particles in an inorganic halide pressed disc, such as KBr, by using fine particulates on a supporting surface,or by dissolution in several solvents. All these methods suffer from solvent interferences and contamination, such as the inclusion of water in a KBr pressed pellet. The method of attenuated total reflectance (ATR) offers many advantages. In this method a beam of radiation passes into a prism from which it is reflected after two or more internal reflections. When a finely dispersed solid or a liquid sample is placed in intimate contact with one of the prism surfaces where internal reflection occurs, the absorption that is recorded is similar to a normal transmitted absorption spectra, but no solvent is involved, and the sensitivity is greatly increased. The use of ATR has greatly increased in recent years, and it has good potential in industrial hygiene when a trace analyte can be concentrated at the surface of an ATR crystal or prism. (3)

K. Self Test

1. Test instructions and review of questions are presented.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		6. Sample preparation for IR analysis -- more varied than UV or visible choice limits
		7. Solid samples analyzed by IR; method and use of ATR
	(1:50)	
Self Test		K. Self Test
	0:10	
	(2:00)	1. Instructions and review

LESSON TITLE	LESSON NUMBER
Introduction to Spectrophotometry	6

1. The total energy of a molecule in dynamic equilibrium is the sum of what energies?

   Translational, Rotational, Vibrational and Electronic

2. What is the purpose of a monochromator?

See C. 3

3. Which of the following are correct statements? (Circle True or False)

   T (F) a. Absorptivity is the term in Beer's law for the concentration expressed in moles of absorber per liter.

   (T) F b. Beer's law is expressed by:

$$-\log \frac{I}{I_o} = -\log T = A = \epsilon bc$$

4. Check those of the following instrumental and chemical variables that produce nonlinear deviations in the relationship between absorbance and concentration.

   ( ) Spectral variations

   (✓) Photo processes

   ( ) Vapor pressure

   (✓) Slit width

   (✓) Temperature variations

LESSON TITLE	LESSON NUMBER
Introduction to Spectrophotometry	6

5. Which technique uses the low energies of vibration and rotation, can make specific determination among homologous compounds, and uses dual beam instruments with the analyte in non-polar solvents?

   a. Visible light spectrophotometry

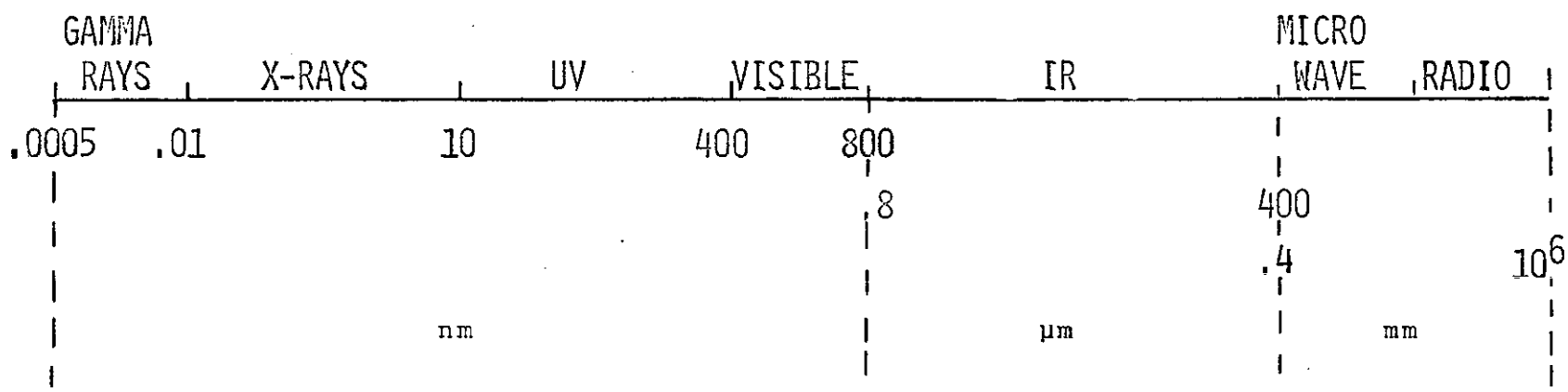
   (b.) Infrared absorption spectrophotometry

   c. Ultraviolet spectrophotometry

   d. Fluorescence spectrophotometry

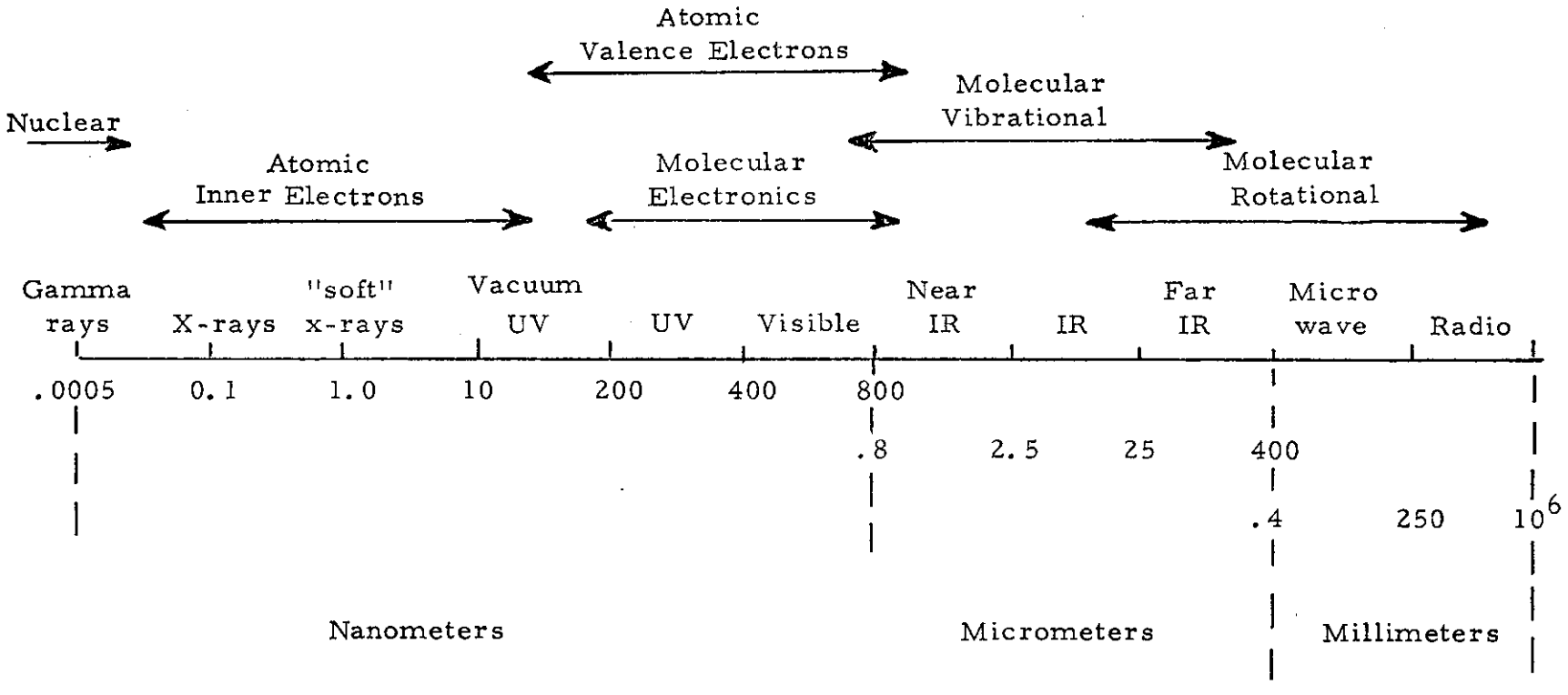
VISUALS, TABLES, FIGURES AND EXHIBITS

SH-9



ELECTROMAGNETIC SPECTRUM WITH RANGES OF ENERGY LEVELS

NOTE: WAVELENGTH NOT TO SCALE



Electronmagnetic Spectrum with Ranges of Energy Levels

Note: Wavelength is not to scale

Exhibit 6 - 1

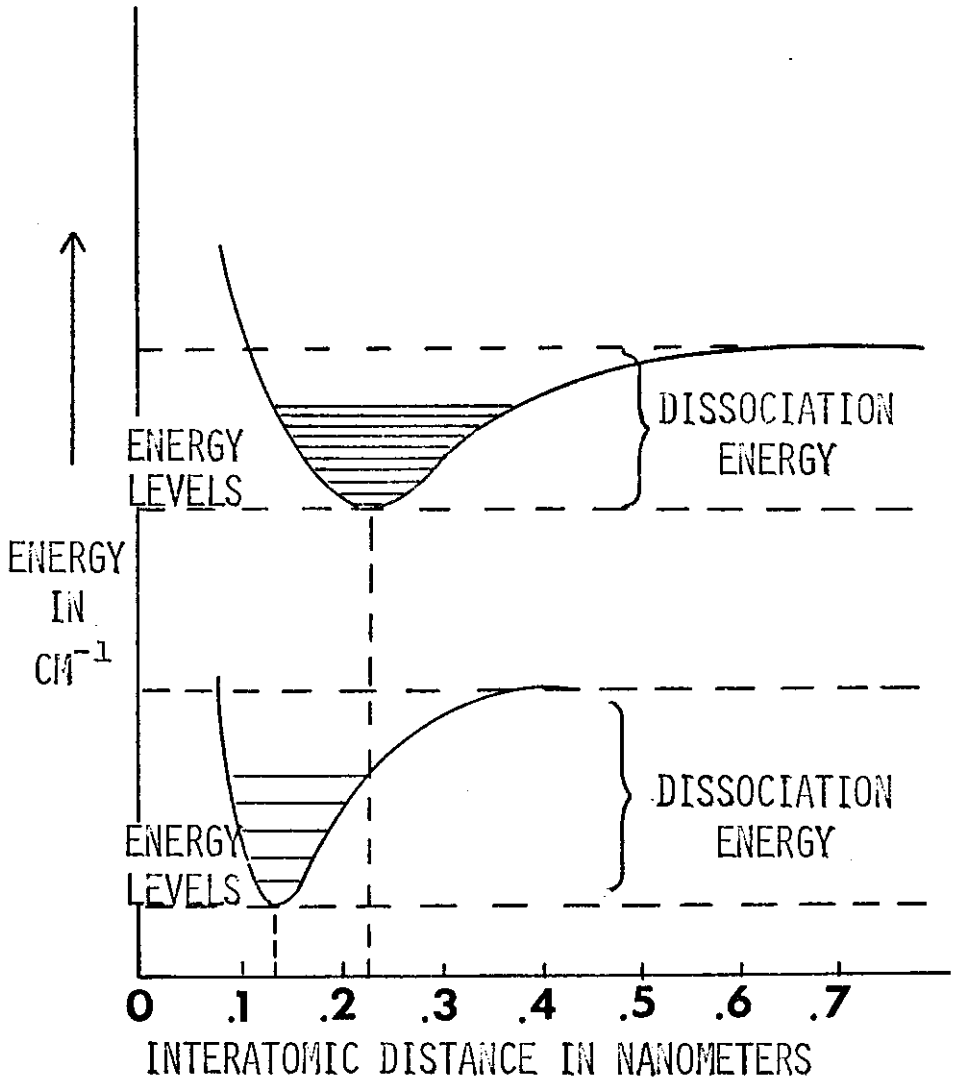
TOTAL MOLECULAR ENERGY

$$H_{MOL} = H_{TRANS} + H_{ROT} + H_{VIB} + H_{EL}$$

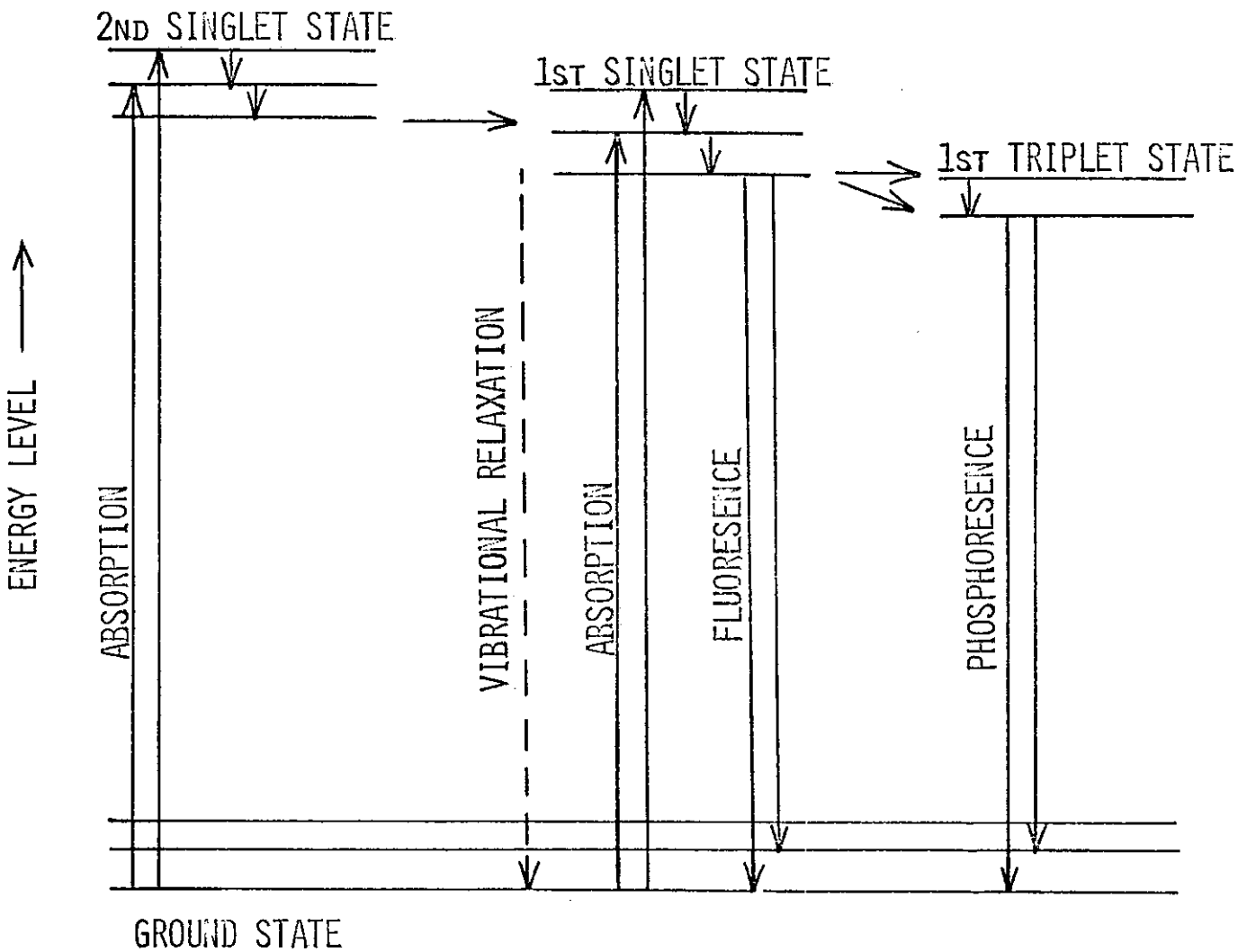
WHERE:

H_{MOL}	=	TOTAL MOLECULAR ENERGY
H_{TRANS}	=	TRANSLATIONAL ENERGY
H_{ROT}	=	ROTATIONAL ENERGY
H_{VIB}	=	VIBRATIONAL ENERGY
H_{EL}	=	ELECTRONIC ENERGY

Visual 6 - 2

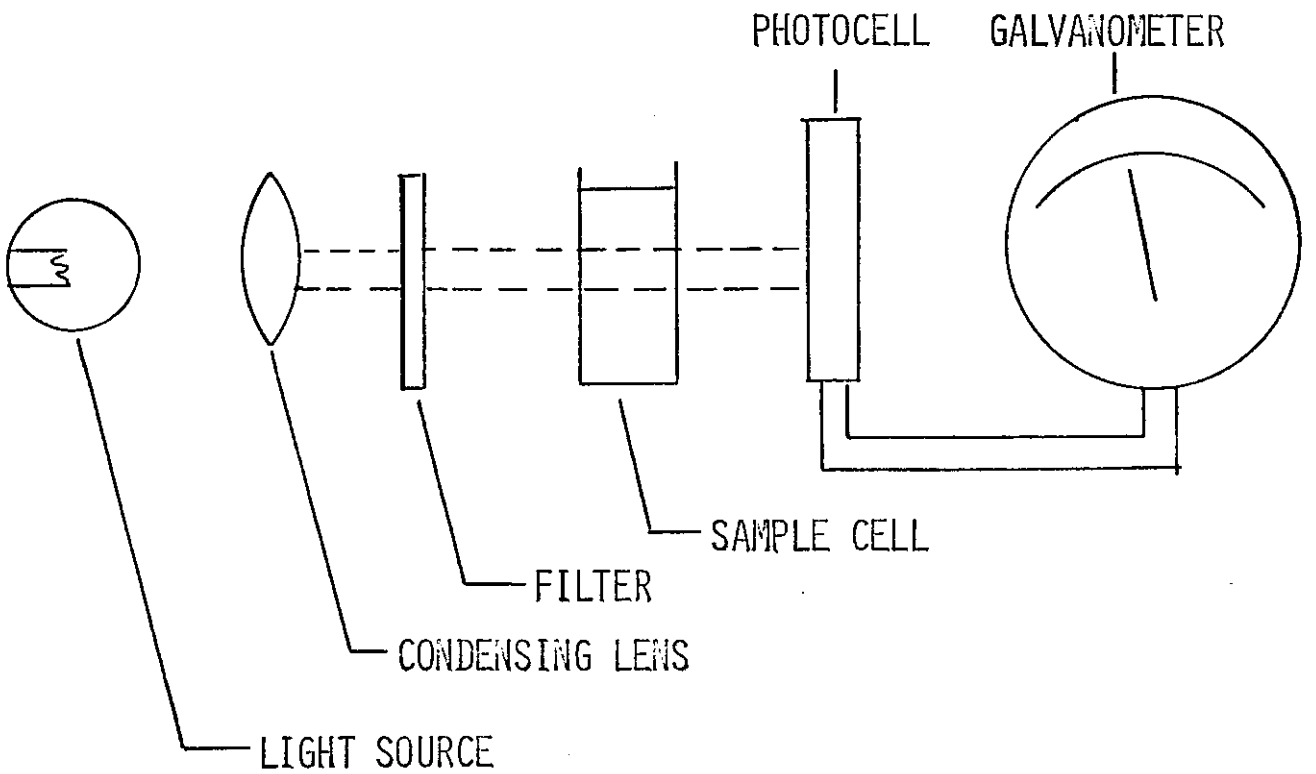


POTENTIAL CURVES FOR THE TWO STATES
OF A DIATOMIC MOLECULE



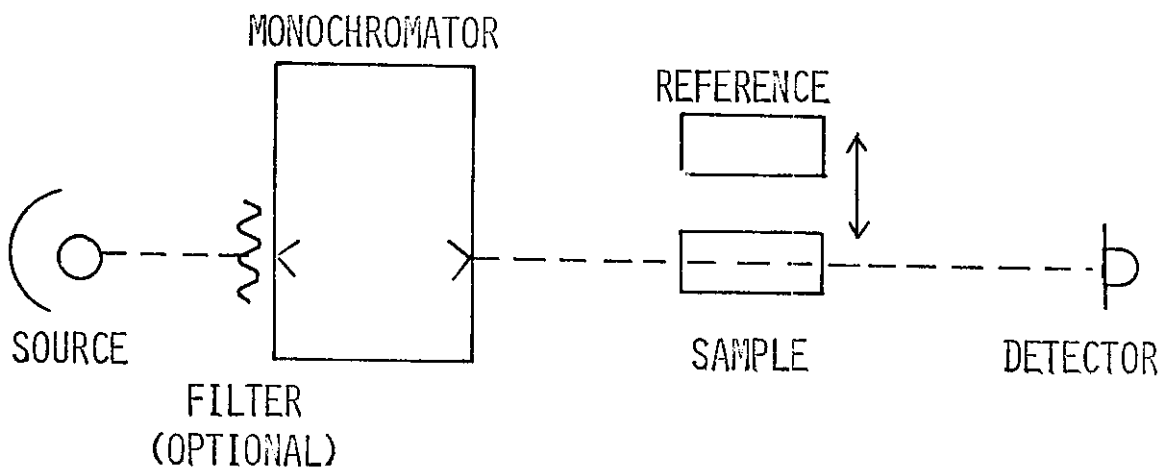
SCHEMATIC OF MOLECULAR ELECTRONIC ENERGY LEVELS
AND MODES OF TRANSITION

Visual 6 - 4



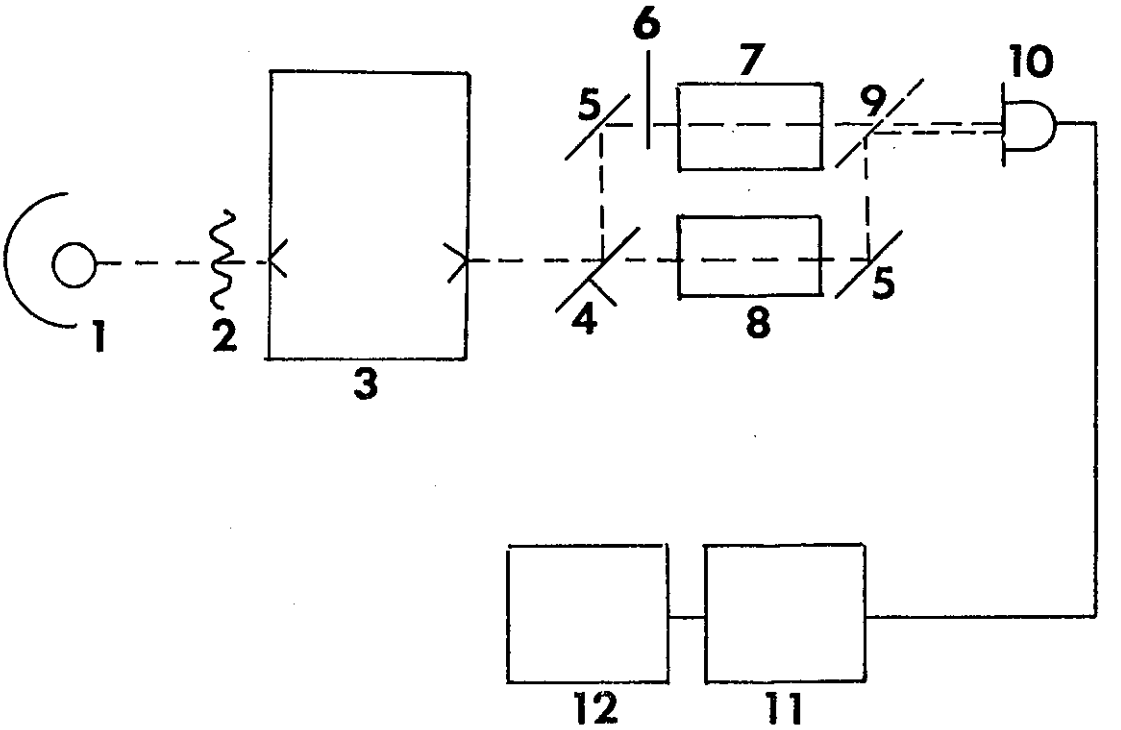
SCHEMATIC DIAGRAM OF FILTER PHOTOMETER

Visual 6 - 5



SINGLE BEAM ABSORPTION SPECTROPHOTOMETER

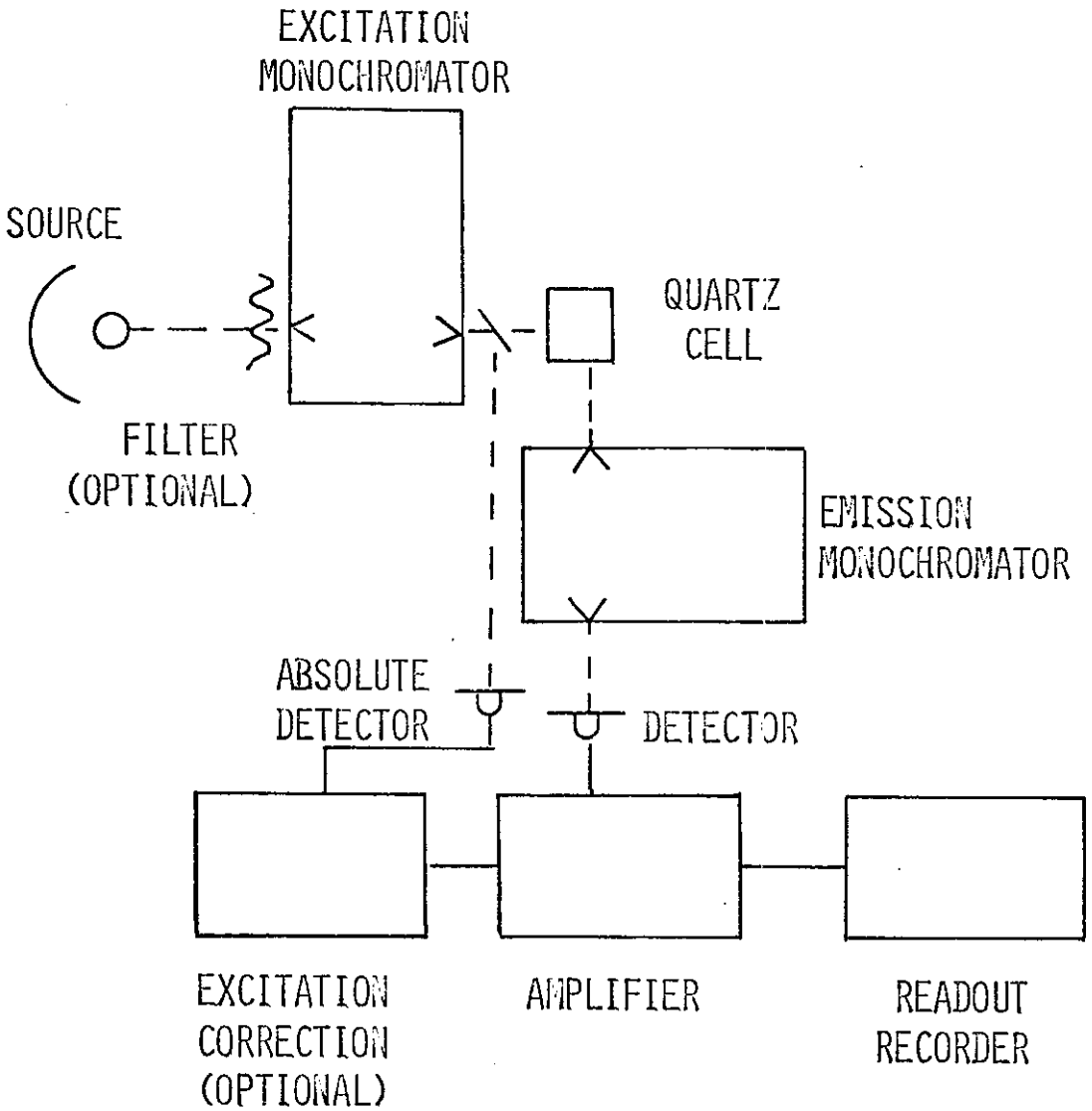
Visual 6 - 6



1.	SOURCE	7.	REFERENCE
2.	FILTER (OPTIONAL)	8.	SAMPLE
3.	MONOCHROMATOR	9.	BEAM COMBINER
4.	CHOPPER	10.	DETECTOR
5.	MIRROR	11.	A.C. AMPLIFIER
6.	VARIABLE ATTENUATOR	12.	READOUT/RECORDER

DOUBLE BEAM ABSORPTION SPECTROPHOTOMETER

Visual 6 - 7



SPECTROPHOTOFLUOROMETER

WITH ALTERNATIVE EXCITATION SPECTRA CORRECTION

Visual 6 - 8

LAMBERT'S LAW

$$\frac{I}{I_O} = e^{-Kb}$$

WHERE:

I	=	ABSORBED INTENSITY
I_O	=	INCIDENT INTENSITY
K	=	A CONSTANT
b	=	PATH LENGTH

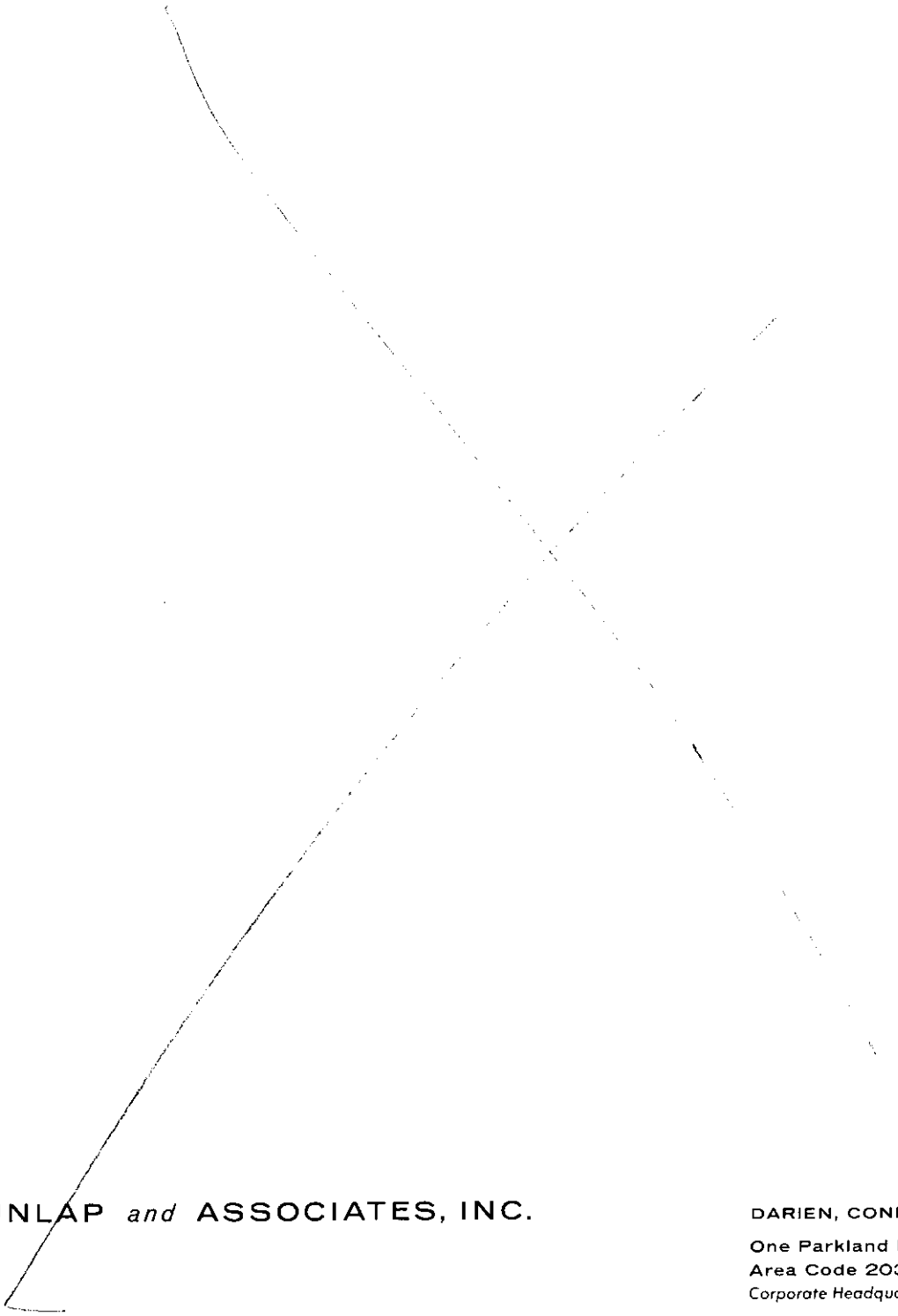
Visual 6 - 9

BEER'S LAW

$$-\text{LOG}\,\frac{I}{I_O} = -\text{LOG}\;T = A = \epsilon bc$$

WHERE:

I	=	TRANSMITTED INTENSITY
I_O	=	INCIDENT INTENSITY
T	=	INTERNAL TRANSMITTANCE
A	=	ABSORBANCE
ϵ	=	MOLAR ABSORBTIVITY
b	=	PATHLENGTH
c	=	CONCENTRATION



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