INDUSTRIAL HYGIENE CHEMISTRY COURSE

LESSON NUMBER 12

Prepared for:

National Institute for Occupational Safety and Health
Rockville, Maryland

Contract Number CDC-99-74-72

Prepared by:

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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 Chromatography	12	1:20

BEHAVIORAL OBJECTIVE

The student will be able to list the basis, the variety, the conditions and the sampling constraints for chromatography.

SCOPE

Development of chromatography
Basis for chromatographic separations
Partition chromatography theory
Column parameters in gas chromatography
Column parameters in liquid chromatography
Sample preparation in chromatography

VISUALS	EXHIBITS
12-1 through 12-7	None

EQUIPMENT

Overhead projector
Screen
Blackboard
Chalk

REFERENCES

LESSON TITLE

Introduction to Chromatography

1. Ettre, L. S. The Development of Chromatography, Analytical Chemistry, 43, 21A, (1971).

2. Rosenthal, I., Weiss, A. R., and Usdin, V. R. Chromatography: General Principles, in Kolthoff, I. M., and Elving, P. J. (Eds.) Part I, Vol. 3, Section C, Ch. 13, Treatise on Analytical Chemistry, Wiley-Interscience, New York (1961).

3. Kirkland, J. J. Modern Practice of Liquid Chromatography, Wiley Interscience, New York (1971).

4. Ewing, G. W. Instrumental Methods of Chemical Analysis, 3rd ed. Ch. 20, 21, McGraw Hill, New York (1969).

5. Willard, H. H., Merritt, L. L., and Dean, J. A. Instrumental Methods of Analysis, 5th ed. Ch. 18, D. Van Nostrand Co., New York (1974).

6. Leathard, D. A., and Shurlock, B. C. Identification Techniques in Gas Chromatography, Ch. 2, Wiley-Interscience, New York (1970).

7. Harris, W. E., and Habgood, H. W. Programmed Temperature Gas Chromatography, Wiley-Interscience, New York (1966).

8. Brewer, L. W. Gas Chromatography, Ch. 21, U. S. Department 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).

9. Rohrschneider, L. The Polarity of Stationary Liquid Phases in Gas Chromatography, in Vol. 4, 333, Advances in Chromatography, Marcel Dekker, New York (1967).

10. Franken, J. J. et al. Gas-Solid Chromatographic Analysis of Aromatic Amines, Pyridine, Picolines and Lutidines on Cobalt Phthalocyanine with Porous-Layer Open-Tube Columns, Analytical Chemistry, 43, 2034, (1971).

11. Kirkland, J. J. Columns for Modern Analytical Liquid Chromatography, Analytical Chemistry, 43, 36A, (1971).

ADDITIONAL READINGS

LESSON TITLE

Introduction to Chromatography

1. Bobbitt, J. M., Schwarting, A. E., and Gritter, R. J. Introduction to Chromatography, D. Van Nostrand, New York (1968).

2. Jones, R. A. An Introduction to Gas Liquid Chromatography, Academic Press, New York (1970).

3. Moshier, R. W., and Silvers, R. C. Gas Chromatography of Metal Chelates, Pergamon Press, New York (1965).

4. Perry, S. G., Amos, R., and Brewer, P. I. Practical Liquid Chromatography, Plenum Press, New York (1972).

5. Purnell, H. Gas Chromatography, Wiley-Interscience, New York (1968).

6. Samuelson, O. Ion Exchange Separations in Analytical Chemistry, John Wiley and Sons, New York (1963).

TITLE	LESSON NUMBER
Introduction to Chromatography	12

A. Development of Chromatography

1. Techniques in analytical chemistry are a blend of separation and measurement methods which, together, yield the specific determination of one or more components in a sample. The selectivity of the determination may rest in the measurement method, as in atomic absorption; or it may rest wholly in the separation method, as in the case of silver when precipitated as the chloride and weighed. The selectivity is shared in the case of a metal complexed with an organic reagent and then determined spectrophotometrically. Many chromatographic techniques are so selective in their separation capability that specific detectors are not used. The high sensitivity of many chromatographic techniques, particularly gas chromatography, is very useful in industrial hygiene chemistry. (4)

2. Chromatography started around 1906 with Tswett who extracted pigments from plant leaves and resolved them as colored bands on an adsorbent column of calcium chloride. He was the first to recognize, to correctly interpret the chromatographic process and to apply it analytically. He used the word "chromatographic," but did not specifically state that it was taken from Greek to mean "color-writing." In further detailed investigations of the role of various adsorbents and solvents, Tswett continued to develop his technique, i.e., the separation of substances by retardation through selective adsorption. His work was largely ignored until 1931 when it was revived to resolve several components of carotene and egg yolk xanthophyll. Adsorption chromatography continued in development from this time. (1)

3. Since the advent of adsorption chromatography, four major advances have been introduced into the field: ion exchange chromatography, partition chromatography on paper and columns, gas chromatography and high pressure liquid chromatography. Ion exchange chromatography was developed analytically in a chromatographic system in 1935 using organic ion exchanges although inorganic exchanges, such as the zeolites, had been used since 1917. In ion exchange chromatography, both inorganic and organic ions are firmly held on active sites in the resinous exchange material and are removed by displacement with another ion which has a stronger attraction. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
	(---) 0:10	A. Development of Chromatography 1. Chromatography--separation and measurement methods 2. Start of chromatography--Tswett 3. Ion exchange chromatography--history

4. In 1941, Martin and Synge described a liquid-liquid partition chromatographic system in which one liquid (water) was held stationary on a silica gel support while the other liquid (chloroform with a small amount of water) was mobile. This system replaced a complicated countercurrent extraction apparatus, and they were able to separate the monoamino monocarboxylic acids. In partition chromatography an equilibrium concentration is established in the two phases at each active site in the dynamic column. Subsequent development of descriptions of the efficiency of the chromatographic column borrowed terms long established in distillation theory (such as the number of theoretical plates in a column). (1)

5. In 1944, Consden, Gordon and Martin substituted paper for the silica gel support in the separation of the dicarboxylic and basic amino acids. The paper support was used in circular form in Petri dishes, in strip form in a saturated atmosphere and finally in sheet form, when the paper was run in two dimensions. This was the beginning of paper and thin layer chromatography. Paper chromatographic applications grew rapidly, because of their simplicity, low cost, versatility and their capacity to accommodate micro-samples. All these forms of chromatography, in particular, completely revolutionized biochemical analysis. (1)

6. In 1941, it was suggested that the mobile phase in partition chromatography could be a gas into which the stationary liquid phase was not volatile. This was not attempted until 1952 when gas-liquid chromatography was first applied to fatty acid analyses. Concurrently, during the period 1942-1947, gas-solid chromatography was developed with hydrogen gas through a silica gel column for the analysis of acetylene-ethylene mixtures. A thermal conductivity detector was used in this work for the first time to measure the gases as removed from the end of the column by elution. Subsequently others in the petroleum industry applied the thermal conductivity detector to gas-liquid chromatographic columns for the analysis of hydrocarbon components. Commercial instrumentation appeared in 1955 and a rapid growth in instrumentation and applications began at that time. Currently chromatography is the most commonly used physical separation method in analytical chemistry. (1)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		4. Liquid-liquid partition chromatography--history
		5. Paper and thin layer chromatography--history
		6. Gas-liquid and gas-solid chromatography--history

7. There has been a recent revival of liquid chromatography, chiefly due to the addition of high pressure pumps at the inlet of long, narrow-bore columns. This produces a chromatographic separation with high analytical speed. The technique is referred to as high pressure, high speed or high performance liquid chromotography. It is 100 to 1000 times faster than the classical liquid column technique and 10 to 100 times faster than thin-layer chromatography. With the addition of a wide range detector at the column outlet, high speed liquid chromatography is comparable to gas chromatography. Although gas chromatography is faster and technically more convenient than liquid chromatography, only 15 to 20 percent of organic compounds are suitable for gas chromatography. This is due to insufficient volatility or thermal instability of the compounds. (3)

B. Basis for Chromatographic Separations

1. These diverse developments in chromatography can be generalized to illustrate a chromatographic separation on a column as shown in Visual 12-1. For purposes of discussion, paper and thin layer chromatograms can be considered open or planar columns, not necessarily physically confined in a tubular form. Initially, the column which contains a stationary phase is flushed with, or equilibrated with, the mobile phase, i.e., a gas or liquid solvent. If a detector is located at the column outlet, a stable baseline with time is attained. The sample, with or without its own solvent, is then injected at the inlet position A of the column in the shortest possible time. The three component (in this case) sample is then moved to position B by the action of the mobile solvent. The three components are unresolved and the fiducial mark at 0 represents the movement of the solvent without retardation. It is the solvent front. At position C, the sample is further retarded and the components 1, 2 and 3 are evident. Finally, at position D, the initial solvent front is about to leave the column and the three components are resolved from each other. The area under the curve represents the mass of each component and the position of each peak is a qualitative function of each component. While the components are yet in the column, they are referred to as solutes. Note that solutes 1 and 2 have a symetrical distribution of the ideal Gaussian form, while solute 3 is asymetrically distributed and shows a tailing common in chromatography. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		7. Revival of interest in liquid chromatography
(Transition A.-B.) From a general and historical description to the basis of chromatography.	(0:10) 0:15	B. Basis for chromatographic separation
Visual 12-1		1. Example of separation in a column

2. If the solvent movement is stopped at position D, the three solutes may be fixed and observed directly on the column as in the case of paper and thin layer chromatography. Further movement of the solvent will cause the three components to be removed sequentially from the column with measurement by the detector. The components are then said to be eluted from the column and they are referred to as eluates. (2)

3. This elution chromatography is typical for a partition process where there is an exchange between the mobile and stationary phases all down the length of the column. The solvent, in effect, has pulled the solutes through the column, with some reluctance. If, on the other hand, the three solutes are quite dissimilar in their attraction to the stationary phase, as per ion exchange chromatography, solute 1 may pass entirely through the column, while solutes 2 and 3 remain near the column inlet. A change in solvent character is then necessary to force solute 2 down the column, and a further solvent change will finally elute solute 3. In aqueous ion exchange chromatography, these solvent changes may be changes in the acid or salt concentration of the solvent. This is displacement chromatography where, effectively, additional components of the solvent have pushed the solutes through the column by preferred substitution at the active adsorption sites. (2)

4. Chromatographic methods may be classified by the types of thin stationary phases in combination with the bulk mobile phases as shown in Visual 12-2. In types 1 and 2 the distribution between phases is controlled by solution and vaporization effects; this is partition chromatography. In types 3 and 4 the distribution is largely controlled by the nature of the solid and the manner in which the solute from the mobile phase is attracted to active sites in the solid phase. This is adsorption chromatography. (2)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		2. Eluted from column
		3. Displacement chromatography
Visual 12-2		4. Types of chromatographic methods

5. Considering these four types, the chromatographic fields may be classified as follows:

- Paper chromatography is usually a liquid-liquid type 2 partition process in which the solutes are developed or resolved behind the solvent front. Visualization or measurement of the solute is most often made directly on the paper.

- Thin layer chromatography is usually a liquid-solid type 4 adsorption process where the adsorbent is prepared from a slurry as a thin layer on a glass or plastic surface with or without a binder.

- Ion exchange chromatography is a liquid-solid type 4 adsorption process wherein organics or more often organic resins of controlled particle size and structure are used in a column to separate both inorganic and organic ions.

- Column chromatography is a liquid-solid type 4 or a liquid-liquid type 2 process where usually an organic liquid sample is placed at the head of a glass column and solvent is added to elute the components sequentially. The solid stationary phase is typically silica gel or alumina and the liquid stationary phase is typically water adsorbed on a support of silica gel, diatomaceous earth or cellulose. This might be called gravity column or classical chromatography.

- High speed liquid chromatography is a type 4 or type 2 process in which the solvent is forced through a longer and narrower column than in classical gravity column chromatography. It forms the basis of a complete analytical instrument in that a dynamic detector is placed at the column outlet to measure the eluates. Ion exchange and gel permeation chromatography are also included in the high speed liquid chromatography category. Gel permeation is based on a molecular sizing exclusion principle. The column is loaded with a semi-solid gel of a discrete porosity. Molecules greater than a critical size related to the porosity are not retarded by the column and molecules less than the critical size are eluted according to their size.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		5. Classification of chromatography: . Paper--liquid-liquid . Thin layer--liquid-solid . Ion exchange--liquid-solid . Column--liquid-solid or liquid-liquid . High speed liquid--liquid-liquid or liquid-solid

- Gas chromatography is a gas-liquid type 1 or a gas-solid type 3 process where the gas sample or vaporized sample is placed at the head of a long narrow column and a carrier gas elutes the sample components through a dynamic detector similar to high speed liquid chromatography. Gas chromatographic columns may be packed with a solid phase or with a liquid supported phase, as in liquid chromatography. For greater power of separation, the column may be an open tubular capillary column with the liquid or solid phase deposited on the inner wall of the tubing. (2) (3)

6. The separation of component 1 from component 2 is due to the concentration of each component in the stationary and mobile phases. This is the partition coefficient, K, expressed as:

$$K = \frac{C_S}{C_M}$$

when C_S and C_M are concentrations in the stationary and mobile phases, respectively. Through all the stages of a separation process, the partition coefficient may be nearly constant under controlled conditions, especially if the concentrations of the component are low, as is the case in gas chromatography. Rarely will a single separation stage suffice. The separation stages may be made stepwise, as in solvent extraction with separatory funnels, or they may be continuous as in elution chromatography. Multiple stepwise separations are in theory the same as a truly continuous method. Conversely, it is often mathematically convenient to treat a continuous method as though it were stepwise, using the theoretical plate concept, originally developed in fractional distillation. (4)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		. Gas--gas-liquid or gas-solid
Write on blackboard. It is not important for the students to memorize formulae. It is important for them to understand the concepts.		6. $K = \frac{C_S}{C_M}$

7. The results of a separation process in a given system yields a distribution or partition ratio known as the capacity factor, k:

$$k = \frac{C_S V_S}{C_M V_M} = K \frac{V_S}{V_M}$$

where K = partition coefficient

C_S and C_M are concentrations in stationary and mobile phases

V_S and V_M are the volumes of stationary and mobile phases

In the separation of components 1 and 2 it is desirable that their partition ratios k_1 and k_2 be widely different, so a minimum number of separation stages are needed. The separation factor, α is defined as:

$$\alpha = \frac{k_2}{k_1}$$

This ratio should be much larger or smaller than unity for best separation. (4)

8. Separation processes are known as crosscurrent and countercurrent separations. Crosscurrent separation refers to a stepwise or continuous extraction process in which a component is moved from one phase to another by sequential or continuous introduction of new portions of the second phase. The removal is exponential and dependent upon the partition ratio, k. Examples of crosscurrent separation are the extractions from liquids and porous solids by gases and liquids. Countercurrent separations refer to stepwise or continuous processes where both phases are renewed and move in opposite directions. It is generally not convenient to have both phases mobile.

NOTES	Times (elapsed) projected	LESSON OUTLINE
Write on blackboard.		7. $k = K \frac{V_S}{V_M}$; $\alpha = \frac{k_2}{k_1}$
		8. Crosscurrent and countercurrent separation

Thus, in several varieties of chromatography just discussed, one of the phases is a stationary solid or liquid retained on a solid and the other phase is a mobile gas of liquid. In this general discussion of interphase separations, the mechanism of transfer of components between phases is not considered. These may include ion exchange, surface adsorption solubility, volatility or other mechanisms. (4)

C. Partition Chromatography Theory

1. The volume of carrier gas or the mobile phase required to transport a solute band the length of a column is the most fundamental parameter in chromatography. This is the retention volume, V_R, and the length of time that each component spends in the column is the retention time, t_R. For a given column at temperature T_o, with a carrier gas flow rate, F_o, the retention volume and time are a constant. The total time spent in the column from the start when the sample is injected is the uncorrected retention time and the corresponding uncorrected retention volume is the product of the time and the flow rate:

$$V_R = t_R F_o$$

The gas flow rate must be corrected to column temperatures and outlet pressure. (5)

2. Visual 12-3 shows the elution of two components. The air peak represents a substance not retarded by the column. This is eluted by a a volume, V_M, which is the interstitial volume of the column, plus that in the inlet section and the detector. The retention volume from the air peak to the peaks of components 1 and 2 is the adjusted retention volume V_R':

$$V_R' = t_R F_o - t_{Air} F_o$$

$$V_R' = V_R - V_M$$

NOTES	Times (elapsed) projected	LESSON OUTLINE
	(0:25)	C. Partition Chromatography Theory
(Transition B.-C.) From a general discussion to specifics of partition theory.	0:25	1. $V_R = t_R F_o$
Since gas chromatography is the most used instrumental analytical separation method, this discussion is centered on gas chromatography. The same principles hold for other forms of chromatography.		
Visual 12-3		2. $V'_R = V_R - V_M$

3. This adjusted retention volume, V_R', changes slightly with the amount of the component, and the true value is obtained when the mass of the component is extrapolated to zero. In gas chromatography, the mobile phase is compressible. The pressure is higher at the inlet of the column than at the outlet, and the flow rate is higher at the outlet than the inlet. Therefore, a pressure-gradient correction or compressibility factor, V', must be applied to the adjusted retention volume to obtain the net retention volume, V_N:

$$V_N = j V_R'$$

This compressibility factor, j, is given by:

$$j = \frac{3 \left[(P_i/P_o)^2 - 1 \right]}{2 \left[(P_i/P_o)^3 - 1 \right]}$$

where P_i and P_o are the carrier gas pressures at the inlet and outlet of the column respectively. (6) (7)

4. As indicated in Visual 12-3, one half of the component has eluted in the retention volume, V_R. The other half remains in the volume of the gas phase, V_M, and in the volume of the stationary liquid phase, V_S, at the temperature of the column. The two halves are equal thus:

$$V_R C_M = V_M C_M + V_S C_S$$

C_M and C_S are the concentrations in the mobile and stationary phases. By rearrangement:

$$(V_R - V_M) C_M = V_S C_S$$

$$V_R' = V_R - V_M = V_S \frac{C_S}{C_M}$$

NOTES	Times (elapsed) projected	LESSON OUTLINE
		3. Compressibility factor: $V_N = j V'_R$ 4. $V_N = K V_S$

C_S / C_M is equal to the partition coefficient and therefore providing the factor j is applied:

$$V_N = K V_S \qquad (5)$$

5. When the volume of the stationary liquid phase is increased either by using a thicker layer on the support material or by using a longer column, the retention volumes are increased. Therefore, the specific retention volume, V_g, is defined as:

$$V_g = \left(\frac{273}{T_o}\right) \left(\frac{V_N}{w_S}\right)$$

where w_S is the weight of the stationary phase and T_o is the column temperature. (6)

6. Under linear elution conditions, each eluate will produce a Gaussian distribution as shown in Visual 12-3. The width of the elution peak, W, is measured when the tangents to the Gaussian curve cross the baseline. W is equivalent to four standard deviations. The column efficiency is empirically defined as the theoretical plate number, N, where:

$$N = 16 \left(\frac{V'_R}{W}\right)^2$$

Thus the theoretical plate number, N, is inversely proportional to the square of the peak width. Alternately, N is given as:

$$N = 5.54 \left(\frac{V'_R}{W_{1/2}}\right)^2$$

where $W_{1/2}$ is the width of the peak at one half the height of the peak. In gas chromotagraphy values of N range from 1500 to 3000 plates per meter for packed columns and 3000 or more plates per meter for narrow bore open tubular columns when the stationary phase is on the inner walls of the column. (5)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		5. $V_g = \left(\frac{273}{T_o}\right) \left(\frac{V_N}{w_s}\right)$
Visual 12-3		6. Plate number: $N = 16 \left(\frac{V'_R}{W}\right)^2$ or $N = 5.54 \left(\frac{V'_R}{W_{1/2}}\right)^2$

7. The theoretical plate height, H, also noted as HETP, is given by:

$$H = \frac{L}{N}$$

where L is the column length. Typical plate heights are 0.1 to 2 mm. The broadening of a peak is the summation of effects from several interacting sources. (5)

8. From a theoretical approach at a constant temperature, the Van Deemtes equation relates the plate height, H to the average carrier gas velocity, v as:

$$H = A + \frac{B}{v} + Cv$$

where A,B and C are constants. The A term relates eddy diffusion to solid support particle size and packing. The A term is zero for an open tubular column. The B term is due to molecular diffusion in both column directions in one or both phases. The C term generated the resistance to mass transfer between phases, which means that the system departs from equilibrium with excess velocity v. Visual 12-4 is a typical plot of this function. (5)

9. Peak broadening may be due to:

- Excessively large sample causing large original bandwidth
- Slow introduction of sample, again causing large original bandwidth
- Excessive inlet dead volume between the injection port and the column causing prediffusion
- Excessive dead volume between the column and the detector, causing post-diffusion
- Excessive volume in the detector, causing diffusion and loss of resolution. (5)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		7. $H = \frac{L}{N}$
Visual 12-4		8. $H = A + \frac{B}{v} + Cv$
		9. Additional peak broadening: . Large sample . Slow introduction . Excessive inlet dead volume . Excessive dead volume--between column and detector . Excessive volume in detector

10. The separation factor, α is defined as the ratio of the capacity factor of components 1 and 2

$$\alpha = \frac{k_2}{k_1}$$

This is also known as the relative retention, α and can be derived from experimentally measured values, i.e.,

$$\alpha = \frac{k_2}{k_1} = \frac{V'_{R,2}}{V'_{R,1}} \qquad (5)$$

11. This separation factor and the average peak width of the two components influence the resolution, R_1 thus:

$$R = \frac{V_{R,2} - V_{R,1}}{0.5\ (W_2 + W_1)}$$

A value of R = 1.5 yields complete resolution to the baseline, whereas a value of R = 1.0 means a 3% overlap of peak areas. The resolution is about 94% complete at R = 1. If equal amounts of the two components are present, the resolution can also be expressed in terms of α, N and k thus:

$$R = \frac{1}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_2}{1 + k_2} \right) (N)^{\frac{1}{2}}$$

Then two solutes moving through a column are separated according to their relative retention, the capacity factor, and the number of theoretical plates. By rearrangement the number of theoretical plates required can be expressed as:

$$N_{req.} = 16\, R^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{k_2 + 1}{k_2} \right)^2 \qquad (5)$$

NOTES	Times (elapsed) projected	LESSON OUTLINE

10. Separation factor or relative retention:

$$\alpha = \frac{V'_{R,2}}{V'_{R,1}}$$

11. $$R = \frac{V_{R,2} - V_{R,1}}{0.5\ (W_2 + W_1)}$$

12. As previously stated, large relative retentions are desirable. The relative retention is dependent upon the nature of the stationary phase, the column temperature and the type of phase (the most important parameter). In practice, a packed column cannot be used when the relative retention, α, is less than 1.05 or even 1.10. For instance, for $\alpha = 1.10$, the required number of plates is 9,800. With a plate height of 0.6 mm. the required column length is 5.8 meters. (5)

13. Partition chromatography generally yields a higher resolution than absorption, which is a more complex process, primarily because the distribution coefficients between fluids and solids are not nearly constant. Therefore, in partition chromatography the members of a homologous series of compounds may be resolved, and the retention volume or the relative retention may be related to properties of the compounds as shown in Visual 12-5. In this case the relative retention is related to the number of carbon atoms in a regular series. A semi-log plot yields straight lines for several series. The log of the relative retention plotted against the boiling point of each member of a series will also yield smooth curves. (4)

14. The retention volumes or times from two different types of columns may be compared for a series. A log-log plot of the retention times will yield almost parallel lines for each series with the molecular weight of each member spaced linearly along each series line, as in Visual 12-6. This is a useful comparison of two liquid stationary phases. Evidently, column B retards the alcohols more than the alkanes. (4)

15. Further correlation among different compounds on the stationary phase is provided by the Kovats retention index, R. I. This index indicates when a compound will elute relative to the normal paraffins. By definition the index is 500 for pentane, 600 for hexane, 700 for heptane, etc. After the Kovats retention index has been determined for a specific compound it is only necessary to run a standard hydrocarbon on a new column to predict the retention time of the compound. (4)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		12. Relative retention--stationary phase, column temperature, type of phase
Visual 12-5		13. Relative retention related to compound properties
Visual 12-6		14. Retention times of different columns compared
		15. Kovats retention index

16. In gas-liquid and liquid-liquid chromatography, the polarity of the solute and of the stationary phase determines the relative retention or separation factor. Polarity refers to the positive and negative centers which exist within a molecule as evidenced by the measurement of dielectric constants. The use of the term polarity is broadened in chromatography so that it is a relative term applied to solutes, solvents and adsorbents. The following solutes and solvents are listed in a decreasing order of polarity: water, methanol, acetone, ethyl ether, chloroform, benzene, toluene, carbon tetrachloride, cyclohexane and n. hexane. The polar compounds used for the liquid phase in the separation of polar solutes are polyethylene glycol, polyesters, ethers and derivatives of ethylenediamines. The nonpolar liquid phases, typically used to separate nonpolar solutes such as hydrocarbons, esters, aldehydes and polycyclic aromatics, are squalene, silicone oil, dibasic acids and high molecular weight alcohols. Thus, in the example in Visual 12-6, column B has a polar liquid phase, best for the separation of alcohols, and column A has a nonpolar phase for the separation of saturated hydrocarbons, the alkanes. (8)

17. The Rohrschneider system is a method of classification whereby separations can be predicted in any column. It compares the Kovats retention indices for at least five separate columns with that of a squalene coated column. Although highly empirical, the system does determine the polarity of a liquid phase used in chromatographic separations. (6) (9)

D. Column Parameters in Gas Chromatography

1. The relative retention or separation factor, α, in gas chromatography depends upon the temperature as well as the type of stationary support. The relative retention is related to the column temperature by:

$$\log \alpha = \frac{(\Delta H_2 - \Delta H_1)}{RT}$$

where ΔH_1 and ΔH_2 are the enthalpy changes of vaporization for components 1 and 2, R is the gas constant and T is the temperature. Thus, an increase in temperature decreases the separation factor. In general, increasing the temperature lowers the retention time about five percent per degree increase. A decrease of 20°C will approximately double the retention time. Temperature control is critical in gas chromatography. (5)

NOTES	Times (elapsed) projected	LESSON OUTLINE
		16. Polarity--definition in chromatography
		17. Rohrschneider system
	(0:50)	D. Column Parameters in Gas Chromatography
(Transition C.-D.) From the theory behind partition to the factors of gas chromatography columns.	0:10	1. $\log \alpha = \frac{(\Delta H_2 - \Delta H_1)}{RT}$

2. When a sample contains solutes of a wide boiling range, the separation of the solutes and the speed of analysis can be improved by raising the temperature of the entire column at a constant rate during the analysis. Such temperature programming on a given system has a greater effect upon the separation process than any other variable . The effect of temperature programming is illustrated in Visual 12-7. In this hypothetical example, eight components are separated in A. at constant temperature with low resolution between eluates 1 and 2 and with spreading and tailing on eluates 6, 7, and 8. In B. with programmed temperature, the initial temperature starts lower than in A. and the resolution of eluates 1 and 2 is improved. Eluates 6, 7 and 8 appear in less total time with improved peak to base line ratios. Generally, the product of the heating rate in degrees per minute, r, and the air peak retention time, t_m, should be equal to or less than 12°C with column temperature ranges of 30° to 200° C. (5) (8)

3. Basically two types of gas chromatographic columns are used--packed and open tubular columns. Packed columns usually have an inner diameter of 1 to 4 mm. and are used in lengths of 0.6 to 2 meters. The support material porosity is controlled to yield a high surface area balanced against the total pressure drop of the column. Gas samples of 0.1 to 5 milliliters are injected with carrier gas flow rates typically 20 to 80 milliliters per minute. With a volatile sample dissolved in a solvent, typically 1 to 20 microliters of the solution are injected. Excessive sample size is a common cause of peak broadening. In partition gas chromatography, the support is usually loaded with 5 to 20 percent by weight of the liquid phase. Porous polymer beads are used for adsorption chromatography. (5)

4. Open tubular columns have inner diameters of 0.1 to 0.5 mm. and may range from 30 to 300 meters in length. The separation factors per meter do not differ from packed columns greatly. However, with pressure drops of one-tenth or more less than the packed column, very long columns with 6000 to 15,000 plates are used to separate components that have small differences in their physical characteristics. Sample sizes of 0.5 microliters or less are used with sensitive detectors of low dead volume. Originally the liquid phase was coated directly on the wall of the capillary column. (WCOT). In order to increase the amount of liquid phase with the same thin film layer, the wall is coated

NOTES	Times (elapsed) projected	LESSON OUTLINE
Visual 12-7		2. Temperature programming--effect upon separation 3. Two types of columns--packed and open tubular 4. Open tubular columns--WCOT, SCOT, PLOT

with a finely divided support. This is the support coated open tubular (SCOT) column. Recently impregnated graphitized carbon and metallo-organic compounds have been used to form a porous layer open tube (PLOT) column. This is a gas-solid adsorption chromatography versus the gas-liquid partition chromatography of the SCOT column. The sample loading is so small in open tube columns that frequently the sample is split; only 1 to 5 percent of the injected sample goes to the column. (3) (10)

E. Column Parameters in Liquid Chromatography

1. High pressure liquid chromatography is analogous to gas chromatography in both partition and adsorption modes. Column diameters are usually 2 to 3 mm. with column lengths of 0.5 to 1 meter or more. Sample size is similar also, in the range of 5 to 50 microliters. Columns are packed with porous surface supports, porous layer beads and surface active ion exchangers as well as the more commonly used diatomacious earths, silica gel and alumina. Columns are operated in the pressure range of 200 to 5000 psi. (11)

2. Temperature control is important and columns are usually held at a constant temperature $\pm$ 0.2°C. Programmed temperature control is not common as in gas chromatography. An effect similar to programmed temperatures is produced by gradient elution. In this mode the composition of the carrier solvent is changed during progression from a weaker solvent action to a stronger solvent action. This may be a change in pH or the progression from a nonpolar to a polar character. Similar effects to those shown in Visual 12-7 are obtained. The efficiency of high pressure liquid chromatography approaches that of packed gas chromatographic columns, while the nonpressured classical gravity flow liquid column is 1/100 as efficient. Therefore, hours are required for a separation with the classical packed column as against minutes with the high pressure column. (11)

NOTES	Times (elapsed) projected	LESSON OUTLINE
(Transition D.-E.) From column parameters for gas to those for liquid chromatography.	(1:00) 0:05	E. Column Parameters in Liquid Chromatography 1. High pressure analogous to gas 2. Temperature control and gradient elution

F. Sample Preparation in Chromatography

1. The operation of the chromatographic column for efficient separations imposes restrictions on the sample that is introduced. First, in order to maintain the capacity factors, the sample size must be small (relative to normal analytical chemistry). That is, chromatographic analytical methods are micro or semimicro techniques. However, this makes these methods most useful for trace analysis in the industrial hygiene field. In gas chromatography volatile liquid samples of 0.1 to 50 microliters are used and, as noted previously, in the case of SCOT and PLOT columns, the injected sample of 1 microliter may be split in the inlet system so that only 0.05 microliters enters the column. For gas samples, volumes of 0.05 to 50 milliliters are injected directly by hand or by means of a calibrated mechanical bypass system. Similarly, the planar paper and thin layer techniques employ samples of 1 to 50 microliters or total solutes of 0.1 to 10 milligrams. Even in open column ion exhange chromatography the sample size seldom exceeds 50 milligrams. (5)

2. A second constraint which effects sample preparation is that, ideally, all components of the sample must pass through the column before the introduction of the next sample. A latent or residual component, eluted broadly over a long period of time is seen as a drift in the baseline or background of the detector. Also the sample must not contain inert material which remains in the column and coats or otherwise occludes the active sites on the column. Therefore, a filtration of suspended material may be required, or the sample may have to be distilled. In gas chromatography, particulate matters will plug micro-syringe needles and contaminate the heated vaporization inlet chamber. (4)

3. A third constraint on the sample and its preparation is that the small subsample must be representative of the bulk sample and that it must be free of contaminations. Many of the precautions taken in the preparation of samples for molecular absorption, fluorescence, atomic absorption and optical emission apply here. Since the same equipment is often used for the sequential injection of samples, cross contamination between samples is a common occurence unless the equipment is adequately cleaned and equilibrated. (4)

4. A fourth constraint, which is particularly applicable to liquid chromatography and ion exchange in that the inorganic or organic ionic species must be in solution form and at the required pH level for the intended separation. (4)

NOTES	Times (elapsed) projected	LESSON OUTLINE
(Transition E.-F.) From column parameter to sampling considerations.	(1:05) 0:05	F. Sample Preparation in Chromatography 1. Small sample size 2. All components of sample pass through column before introduction of new sample. 3. Representative and not contaminated. 4. In solution and proper pH.

5. In gas chromatography, many samples can be directly injected into the chromatographic system. However, when several analytes are similar or have low vapor pressures, derivations are made to shift chromatographic peaks for greater distinction and, in some cases, to create lower boiling compounds. Trimethylsilyl ether derivatives of hydroxy-compounds can be used as a prime example of chemical modification in sample preparation. In other instances, the pre-treatment may consist of the removel of one or more class of components before analysis. This is termed selective abstration. (6)

G. Self Test

1. Test instructions and review of questions are presented.

NOTES	Times (elapsed) projected	LESSON OUTLINE
		5. Derivations and selective abstraction
Self Test	(1:10) 0:10	G. Self Test
	1:20	1. Instruction and review

LESSON TITLE	LESSON NUMBER
Introduction to Chromatography	12

1. What is the difference between high pressure and high speed liquid chromatography (briefly)?

There is no difference.

2. What are the basic differences between partition and adsorption chromatography (briefly)?

See : B. 4

3. Fill in the blanks:

Crosscurrent separation refers to a stepwise or continuous extraction process in which a component is moved from one phase to another by sequential or continuous introduction of new partitions of the second phase.

Countercurrent separation refers to a stepwise or continuous extraction process where both phases are reversed and move in opposite directions.

LESSON TITLE	LESSON NUMBER
Introduction to Chromatography	12

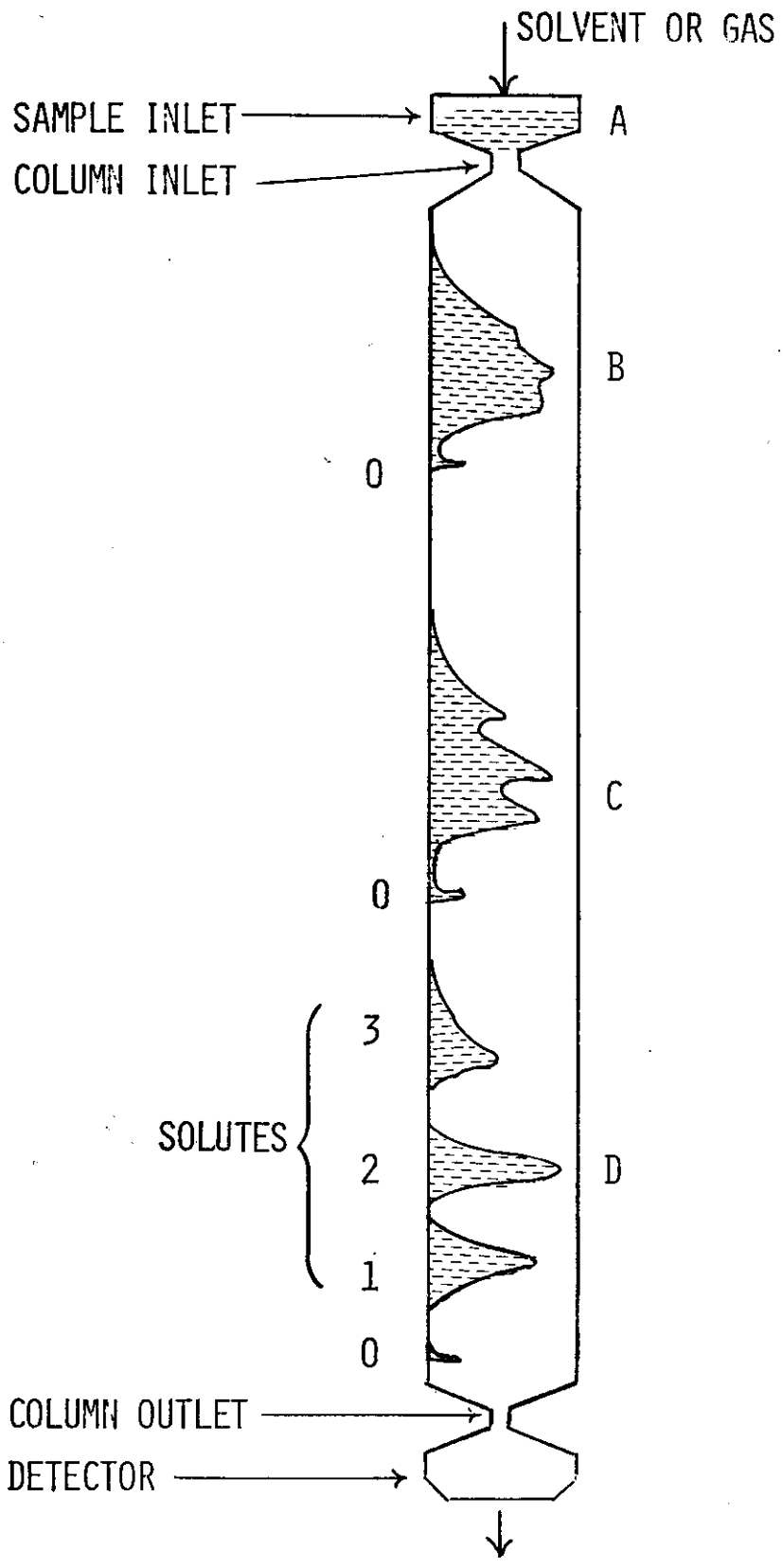
4. Which of the following are correct statements? (Circle True or False)

(T) F a. In partitition chromatography, the members of a homolgous series of compounds may be resolved, and the retention volumes or the relative retention may be related to properties of the compounds.

(T) F b. Temperature programming in gas chromatography has a greater effect upon the separation process than any other variable.

T (F) c. An effect similar to programmed temperatures is produced by gradient elution in gas chromatography.

VISUALS, TABLES, FIGURES AND EXHIBITS

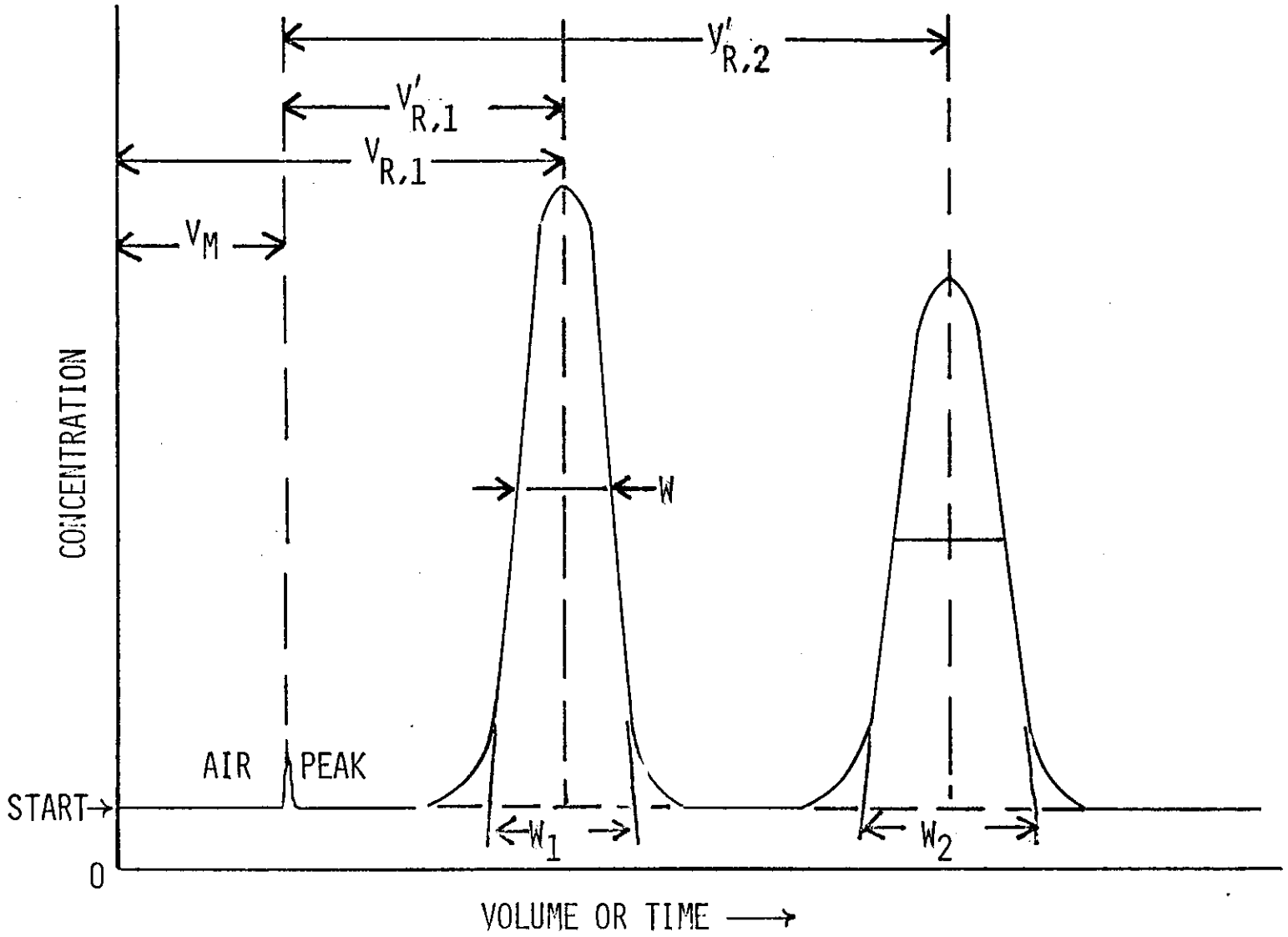


SCHEMATIC COLUMN CHROMATOGRAPHIC SEPARATION

TYPES OF CHROMATOGRAPHIC METHODS

TYPE	MOBILE PHASE	STATIONARY PHASE
1	GAS	LIQUID
2	LIQUID	LIQUID
3	GAS	SOLID
4	LIQUID	SOLID

Visual 12 - 2



IDEAL CHROMATOGRAM

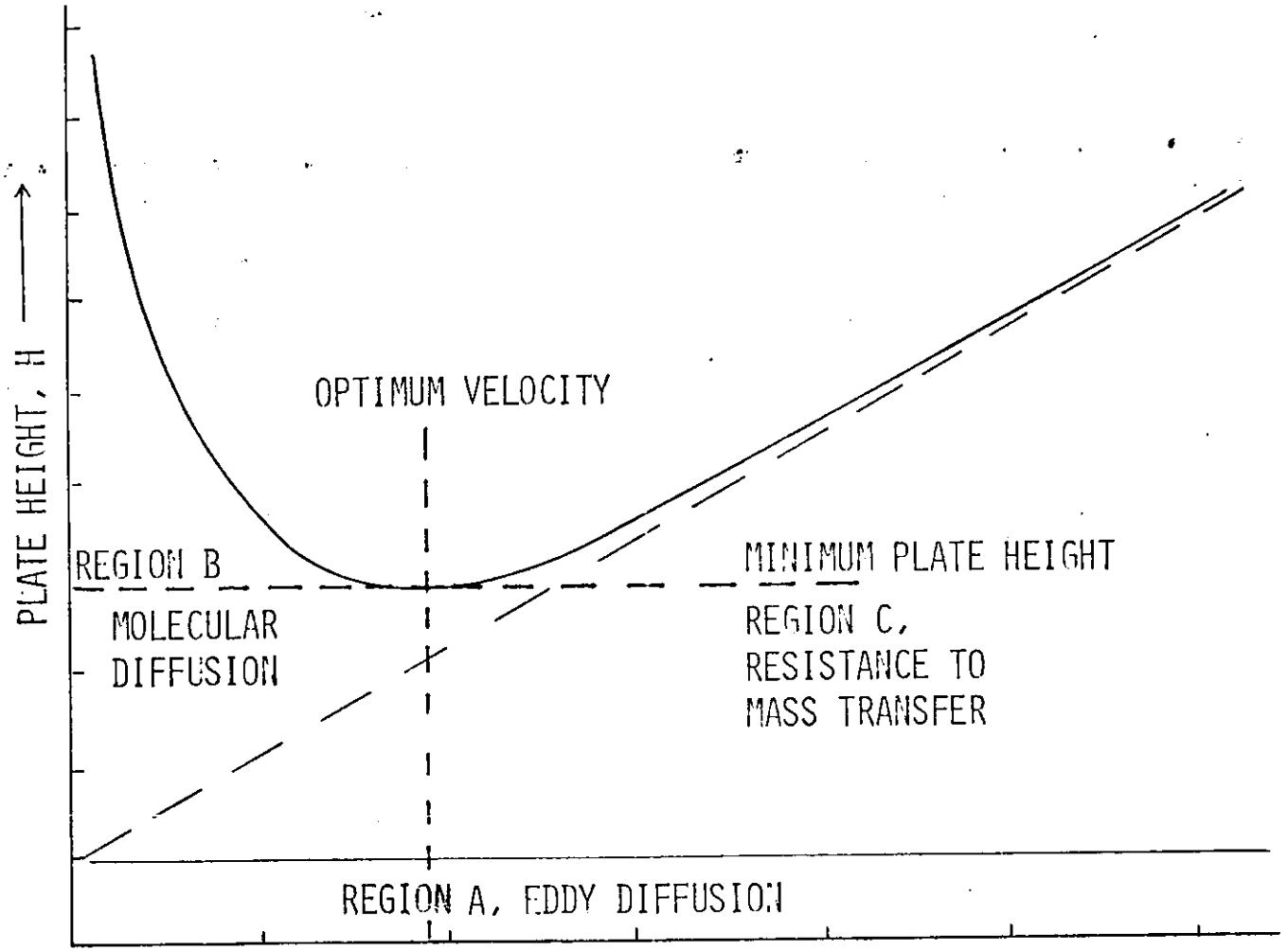
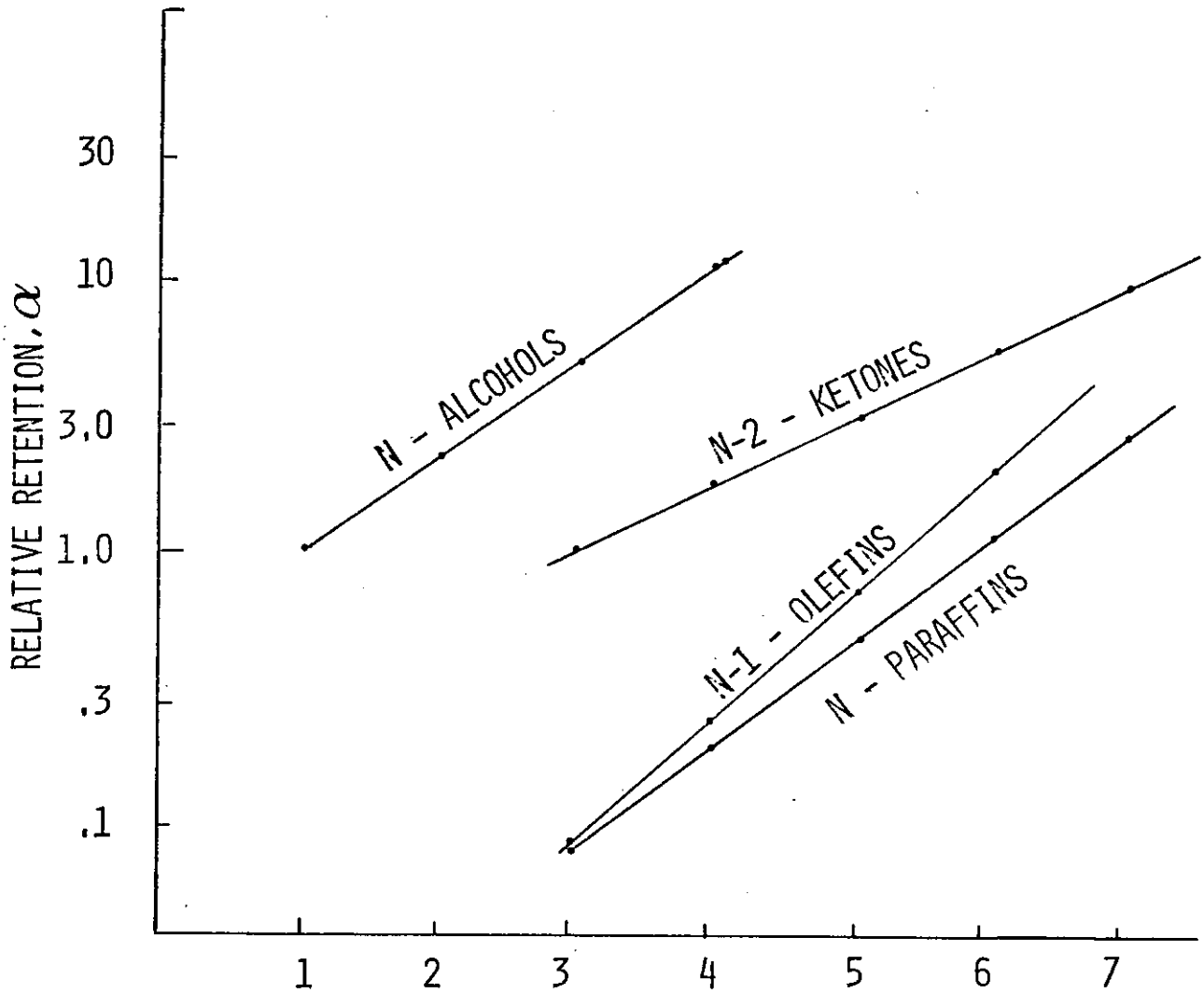


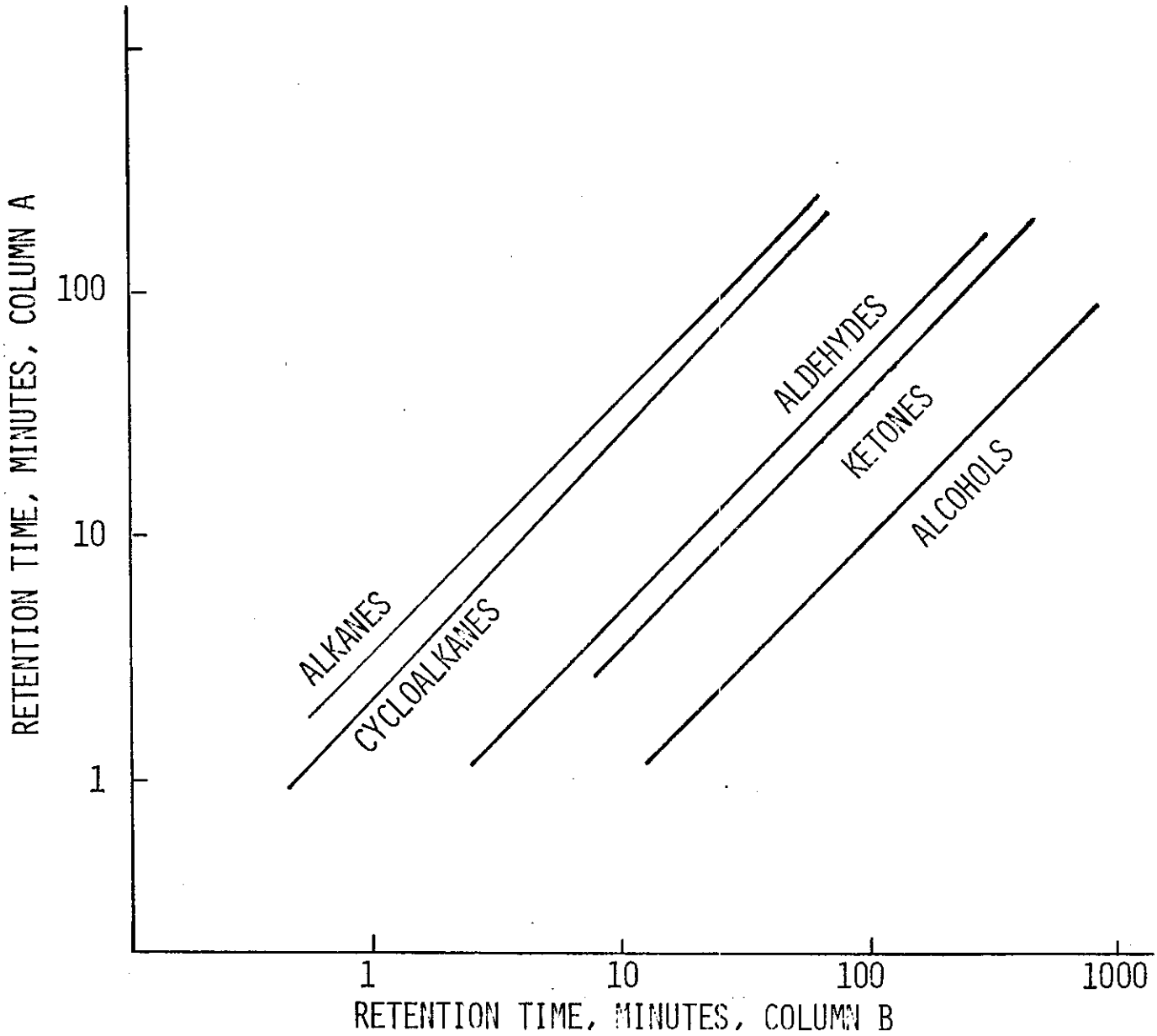
PLATE HEIGHT VS. MOBILE PHASE VELOCITY



RELATIVE RETENTION VS. CARBON ATOMS IN

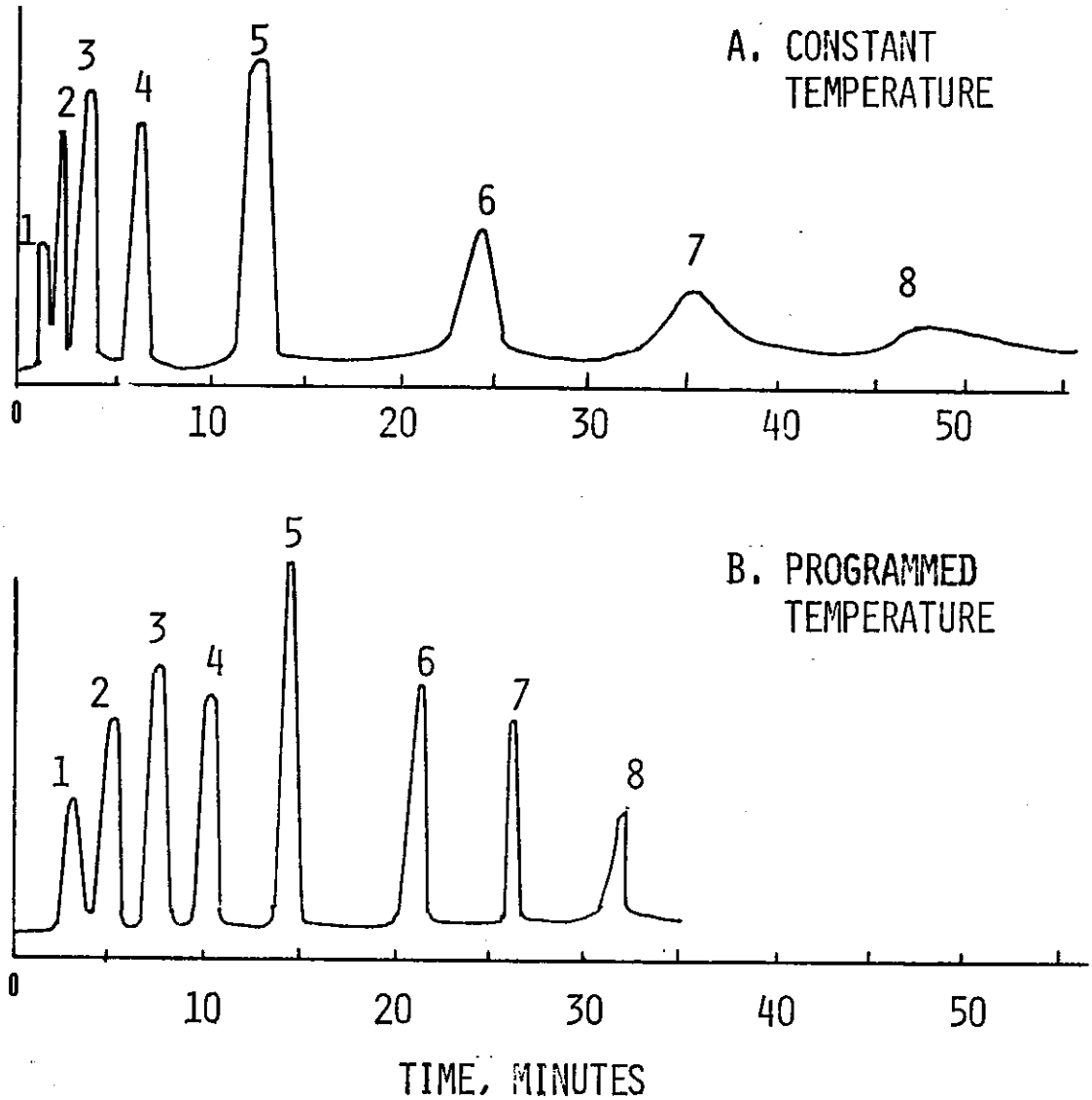
IN A HOMOLOGOUS SERIES

Visual 12 - 5



TWO COLUMN LOGARITHMIC PLOT

Visual 12 - 6



CONSTANT VS. PROGRAMMED TEMPERATURE CONTROL

Visual 12 - 7