CHROMATOGRAPHY IN LABORATORY MEDICINE

ESSAY

SUBMITTED IN PARTIAL FULFILMENT OF THE
MASTER DEGREE (M.S.)
IN CLINICAL AND CHEMICAL PATHOLOGY

BY

SUZAN ABOU EL-FOUTOUH YONICE
M.B. B. CH.

UNDER THE SUPERVISION OF

PROF. DR. *SAWSAN HOSNY HAMZA*
PROFESSOR OF CLINICAL PATHOLOGY
AIN SHAMS UNIVERSITY

DR. *HANZADA IBRAHIM ABD EL-FATTAH*
LECTURER OF CLINICAL PATHOLOGY
AIN SHAMS UNIVERSITY

CLINICAL PATHOLOGY DEPARTMENT
FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY

1990

Central Library - Ain Shams University

CHROMATOGRAPHY IN LABORATORY MEDICINE

ESSAY

SUBMITTED IN PARTIAL FULFILMENT OF THE
MASTER DEGREE (M.S.)
IN CLINICAL AND CHEMICAL PATHOLOGY

BY

SUZAN ABOU EL-FOUTOUH YONICE
M.B. B. CH.

UNDER THE SUPERVISION OF

PROF. DR. *SAWSAN HOSNY HAMZA*
PROFESSOR OF CLINICAL PATHOLOGY
AIN SHAMS UNIVERSITY

DR. *HANZADA IBRAHIM ABD EL-FATTAH*
LECTURER OF CLINICAL PATHOLOGY
AIN SHAMS UNIVERSITY

CLINICAL PATHOLOGY DEPARTMENT
FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY

1990

Central Library - Ain Shams University

بسم الله الرحمن الرحيم

Central Library - Ain Shams University

بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا إلا ما علمتنا

إنك أنت العليم الحكيم

صدق الله العظيم

سورة البقرة الآية ٣٢

Central Library - Ain Shams University

Table of Contents

	Page
* Acknowledgement	
* Abbreviations	
* Introduction and Aim of the Work	1
* Review of Literature	2
I. Introduction to Chromatography	2
A. History of chromatography	2
B. Definition of some terms used in chromatography.	4
C. Classification of chromatography:	8
(1) According to Separation Procedure	8
(2) According to Development Procedure	11
D. Uses of chromatography	14
II. Separation Mechanisms	15
A. Adsorption chromatography	15
B. Partition chromatography	19
C. Gel filtration chromatography	21
D. Ion Exchange Chromatography	25
E. Affinity Chromatography	29
III. Chromatographic Parameters	35
IV. Different Chromatographic Techniques	42
A. Paper chromatography	42
B. Thin layer chromatography	57
C. Gas chromatography	73

Central Library - Ain Shams University

D. High performance liquid chromatography (H.P.L.C) 81

1) Introduction 81

2) Modes of separation in H.P.L.C. 82

3) Instrumentation of H.P.L.C. 85

a) Pumps 86

b) Detectors 93

c) Column and injection system. 102

4) The practice of H.P.L.C. 108

5) Developing a chromatographic method 116

6) Applications of H.P.L.C. 122

* Summary 125

* References 128

* Arabic Summary

Central Library - Ain Shams University

ACKNOWLEDGEMENT

I would like to express my deep thanks and gratitude to Prof. Sawsan Hosny Hamza, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her kind and indispensible guidance, her patience with me in writing and rewriting, together with her continuous encouragement throughout the work. It is really wonderful to work under her supervision.

I would like to express my deep thanks and gratitude to Dr.Hanzada Ibrahim Abd El-Fattah, Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University for her interest, encouragement and helpful discussions. Her kind advice and guidance were of paramount importance in bringing this work to light.

Finally, I am really greatful to every one who helped me in this piece of work. I wish that this work may be of value in the clinical laboratory.

Central Library - Ain Shams University

ABBREVIATIONS

GC	:	Gas chromatography
GLC	:	Gas Liquid Chromatography
GPC	:	Gel Permeation Chromatography
GSC	:	Gas Solid Chromatography
HPLC	:	High Performance Liquid Chromatography
IEC	:	Ion-Exchange Chromatography
LLC	:	Liquid-Liquid Chromatography
LSC	:	Liquid Solid Chromatography
MP	:	Mobile Phase
PC	:	Paper Chromatography
SP	:	Stationary Phase
TLC	:	Thin-Layer chromatography

Central Library - Ain Shams University

INTRODUCTION AND AIM OF THE WORK

Central Library - Ain Shams University

Introduction

Chromatography is the name given to a particular family of separation techniques of great effectiveness. It was introduced into the laboratory as early as 1930. Since then, it has emerged in its various manifestations, as perhaps the most powerful single analytical and preparative method available in the clinical laboratory (Ettre, 1971). The wide variety of chromatographic techniques available, makes it possible to separate molecular species of all sizes and forms. These include gases, ions, vitamins, drugs, steroids, proteins, nucleic acids, viruses and bacteria. Also, it can establish the identity of one or more substances in a mixture, aids in structure elucidation and concentrates the solute molecules.

Chromatography has different techniques and methods. Most important of these is the high performance liquid chromatography (H.P.L.C.). Recently HPLC has been introduced into the Ain Shams Specialized University Hospital Laboratory, representing a new technique.

Aim of the Work

This study aims to give a detailed review of chromatography, its different techniques, methods of separation, as well as its various applications. A special emphasis will be directed to HPLC with its methods, uses, equipment and applications in clinical laboratory.

Central Library - Ain Shams University

REVIEW OF LITERATURE

Central Library - Ain Shams University

I. INTRODUCTION TO CHROMATOGRAPHY

A. History of Chromatography:

The name "chromatography" originated from the Greek words Chroma, meaning colour and Graphein, meaning write. The first person to use the term chromatography was the Russian Chemist, Tswett (1906). He was interested in the separation of plant pigments which presented peculiar analytic difficulties. Tswett was able to separate plant pigments dissolved in petroleum ether by pouring the extract over finely divided calcium carbonate contained in a vertical glass tube. He observed that the pigments were adsorbed near the top of the column. With the addition of more solution, the region of pigmentation became broader, and there was a separation of colours, becoming more marked when the column was washed with pure petroleum ether. The different pigments were being separated by adsorption at different levels on the column. By prolonged washing, the various coloured bands became separated from each other by colourless regions.

Tswett named the coloured column a chromatogram and the process, chromatography. This was perhaps, a poor choice since the method is far more universally applicable to colourless than to coloured substances Tswett's discovery lay neglected for about 25 years.

In 1930, chromatography was in the form of thin layer and ion-exchange techniques and became a regularly

Central Library - Ain Shams University

used analytical procedure. The 1940s saw the development of partition and paper chromatography, with gas chromatography following in 1950.

In the 1960s a rapid rise was observed in the routine use of chromatography as a universal technique particularly in chemistry, biology and medicine. Although it is now used as a production process, yet it is sensitive enough for trace analytical techniques (Ettre, 1971).

Recent developments and technologies, such as microelectronics and microcomputers, have enabled manufacturers to produce highly sophisticated instruments. These are reliable, with parameters that can be precisely set and measured, and can give reproducible chromatograms. The modern liquid column chromatography which is now called high performance liquid chromatography (H.P.L.C.) is a flexible and powerful analytical procedure. It essentially remains a separation technique. However, the ideal instruments for complete analysis of complex mixtures are becoming more feasible, with the coupling of rapid scanning spectroscopic instruments to chromatographs providing spectro-analytical information for each separated component (Julin et al., 1986). It is doubtful that the rapid progress in the fields of vitamins, hormones and numerous other natural products would have been so rapid and so successful, had it not been for this technique.

Central Library - Ain Shams University

B. **Definitions of Some Terms Used in Chromatography**:

In the process of describing types of chromatography, we have already used some terms commonly used in this field of analysis, but there are a few more that need to be defined. The solid material that serves as the stationary phase in adsorption chromatography is called the adsorbent. The solid base which supports the liquid film in partition chromatography is called the support. When the mobile phase is caused to flow over the adsorbent or support the process is termed development. After the substances on the chromatogram have been separated by development, they are detected or visualized. If the substances on the chromatogram are actually washed off the adsorbent, they have been eluted. The substances being separated are usually called solutes or as a group, the **sample**.

adsorption: The process whereby one substance adheres to another because of attractive forces between surface atoms of the two substances.

analyte: The substance or component in a sample for which the analysis is being conducted or that is being measured.

bands: A chromatographic zone, that is, a region where the separated substance is concentrated.

Central Library - Ain Shams University

chromatogram: A series of separated bands or zones detected either visually, as in some paper chromatographic or thin-layer chromatographic separations, or indirectly by the detection system. In the latter case, the detection system usually outputs an electrical signal, which is graphically plotted through time, to display the series of separated bands or zones.

chromatography: A method of analysis, in which the flow of a mobile phase (gas or liquid) containing the sample promotes the separation of sample components by a differential distribution between this phase and a stationary phase. The stationary phase may be a solid, or a liquid coated or bonded on a solid.

detector: A device that responds to the presence of a compound, usually in a proportional or linear manner.

efficiency: A measure of chromatographic performance usually related to the sharpness of the peaks in the chromatogram and quantitated by the number, N, of theoretical plates of a column.

electrostatic attraction: The attractive force of compounds having formal positive or negative charges.

Central Library - Ain Shams University