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microcolumn separations

columns, instrumentation and ancillary techniques

edited by
Milos V. Novotny and Daido Ishii

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PREFACE

The analytical importance of modern liquid chromatography (LC) has now been widely recognized. Indeed, high-performance liquid chromatography (HPLC) is frequently referred to as the single most successful method in modern analytical chemistry. Its numerous applications span many diverse branches of science and technology, and its potential for even greater improvements in technique provides an unusually strong incentive for further development and instrumentation marketing. Of the many recent trends in the field, one which is assuming an increasing importance is that associated with the miniaturization of modern LC.

Miniaturization, in general, has numerous merits, and has long been a well-recognized direction in science and technology, as its broad range of applications so clearly demonstrates. Component miniaturization is frequently sought to improve the performance and versatility of various analyzers throughout industry. The remarkable success of both modern electronics and computer technology has been primarily based on miniaturization. Clearly, without significant component miniaturization efforts, the space flight technology and the related scientific measurements in the upper atmosphere, or even on the surface of other planets, would not have been feasible. In modern biology and medicine, increasing demands will soon be placed on the scientist's ability to handle individual cells and subcellular structures and to reliably analyze their content for a variety of molecules. This trend is already seen in modern physiology, however, certain manipulation techniques will first need to be developed for the accurate sampling of various biological fluids in volumes far below the microliter range. This capability, when combined with ultrasensitive measurement principles, may provide qualitatively new directions toward a better understanding of life processes.

Elements of miniaturization in the field of separation science have been evident for some time. Indeed, the introduction of open tubular (capillary) columns for gas chromatography by M.J.E. Golay in the late 1950's can be viewed as a miniaturization step. In the second opening lecture to the 4th International Symposium on Gas Chromatography (Hamburg, West Germany, 1962), A.J.P. Martin emphasized the future importance of micro-manipulations not only to chromatography, but to science, in general, as he observed that "the appetite of the chemist to work on a small scale will grow as it becomes more possible. He will be able to analyze and experiment on single cells." Undoubtedly, many other scientists have also been aware of the various positive aspects to be derived from miniaturization in chromatography.

Decreasing the particle size in modern LC has obviously been pursued since the very beginning of HPLC. However, consistent efforts toward decreasing

the column diameter can only be traced to the mid-1970's. Our laboratories in Japan and the United States were among the first to realize the various advantages of miniaturization in LC. As a result, an informal exchange of ideas, publication manuscripts, and visiting scholars between the laboratories began in 1976 and led eventually to the idea of a small seminar on the subject of microcolumn separation techniques. Since the following years not only reinforced our research programs in the area but also saw an increase in the number of other laboratories (both academic and industrial) participating in investigations on microcolumn LC, we felt increasingly that such a meeting would be highly beneficial. Recognizing the fact that most developments in the area had originated in U.S. and Japanese laboratories, the U.S. National Science Foundation together with the Japan Society for Promotion of Sciences kindly agreed to sponsor a seminar, restricted to less than forty participants, to be held in 1982 in Honolulu, Hawaii. Several instrument manufacturers, who had interests in the area and agreed to send their observers, provided further financial support toward the travel expenses of additional overseas participants. On August 25 - 28, 1982, thirty-two experts from Japan and the United States, joined by four participants from other countries, met in the East-West Center, situated on the campus of the University of Hawaii at Manoa, to discuss advances in the field of microcolumn separation methods and compatible ancillary techniques, and to outline future directions for this newly emerging field. In addition to a selected number of researchers from U.S. and Japanese academic and industrial laboratories, additional participants arrived from Australia, Israel, Sweden, and Switzerland. While the central theme of this seminar was the miniaturization of chromatographic techniques, some participants were also chosen to provide perspectives in the interfacing areas of small-volume technology, electrochemistry and spectroscopy.

As the successful meeting approached its conclusion, several participants urged us, the seminar coordinators, to document this information in the open literature for the benefit of those who could not participate. It was after considerable thought that we decided to proceed with the present volume. These days, the proceedings of many symposia appear in book form, but their impact on the scientific community is frequently less than was originally intended. They are seldom more than random collections of the presented talks, lacking organization and exhibiting a considerable overlap in the subjects presented. What we decided for this volume, instead, was to provide a balanced treatment of the most representative directions in the field. We have, consequently, asked less than half of the seminar participants to provide a survey-type contribution in their area of expertise. Since more than a year has passed since the Honolulu seminar, the individual chapters have been

updated with the most current information.

The contributions to this volume have been divided into four sections: (a.) column studies; (b.) miniaturized instrumentation and new techniques; (c.) spectroscopic detection; and, (d.) electrochemical detection. The seminar itself was structured similarly.

While there were somewhat different reasons for the initial work in microcolumn LC by the pioneering laboratories, it has been increasingly evident that microcolumn techniques offer the following advantages over the commonly used HPLC: (a.) increased separation efficiencies; (b.) the ability to use "exotic" or expensive mobile phases because of extremely low volumetric flow-rates; (c.) increased mass sensitivities with the concentration-sensitive detectors; and, (d.) chances for novel detection modes. All of these aspects are discussed in the following chapters together with the instrumental demands and technologies which are unique to this area. The chapters on capillary supercritical fluid chromatography and high-voltage capillary electrophoresis were added, since these techniques share a variety of common goals and instrumental features with the LC microcolumn methods. As microcolumn techniques provide unique opportunities for the use of lasers, microelectrodes, microflames, and mass spectrometers in solute detection and identification schemes, we felt a need to provide adequate treatment of these subjects, as well, in this volume.

This book attempts to summarize all of the important aspects of miniaturized separation techniques. Since this direction is relatively new, it will be undoubtedly subject to various interpretations, and perhaps even to criticism. We welcome all constructive critical remarks by the readership.

The timely completion of this book would not have been possible without the cooperation of the individual contributors. Our special thanks are due to Ms. Jennifer Gluckman and Dr. Susan Olesik for their help with scientific corrections. The assistance of Mrs. Cathy Keith is gratefully acknowledged for typing and retyping many of the chapters, correcting the references, and preparing the figures for reproduction. Finally, we are grateful to our families who provided an atmosphere conducive for us to prepare this book.

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CONTENTS

COLUMN STUDIES

OPEN-TUBULAR MICRO-HPLC

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ABSTRACT

Various types of open-tubular capillary columns are prepared and employed as separation tools in liquid chromatography(LC). Preparation procedures and the apparatus for open-tubular capillary LC are described and the performance of capillary columns is discussed.

INTRODUCTION

Open-tubular capillary columns can produce larger theoretical plate numbers per unit time and unit pressure drop than packed columns in gas chromatography(GC); thus, they have been widely applied to the separation of complex mixtures. Open-tubular capillary GC provides a powerful tool for the identification of components in complex mixtures along with mass spectrometry(MS).

In this decade, there has been a trend towards the use of micro columns in LC. One of the various advantages arising from miniaturization of LC is the possibility of attaining higher efficiencies, in terms of theoretical plate numbers, compared with conventional high-performance LC(HPLC). At present, three types of columns are available : (1)packed micro or microbore ; (2)packed microcapillary and (3)open-tubular capillary, which are classified according to their state of packing.

Open-tubular capillary columns in LC are not as successful as those in GC, which is mainly due to the large differences in diffusion speed between the liquid and the gaseous states. H(or HETP: the height equivalent to a theoretical plate) is a function of the linear velocity of the mobile phase(u), as shown by the following Golay equations[1-3]:

$$H = \frac{2D_m}{u} + \frac{2k'd^2u}{3(1 + k')^2 D_s} + \frac{(11k'^2 + 6k' + 1)r_c^2 u}{24(1 + k')^2 D_m} \qquad (1)$$

$$\equiv \frac{B}{u} + C_s u + C_m u \qquad (2)$$

where D_m is the diffusion coefficient of a solute in the mobile phase, k' is the capacity factor, d is the thickness (or depth) of the stationary phase, D_s is the solute diffusion coefficient in the stationary phase and r_c is the radius of an open tube. The third term, the contribution of the resistance to mass transfer in the mobile phase, is too large to operate capillary columns with the same dimensions as used in GC. Thus, in order to decrease the third-term contribution, the column bore should be reduced and a low-viscosity mobile phase should be used.

The performance and the limitation of open tubular capillary columns in LC are discussed and compared theoretically with those of packed columns[4-6]. The injection and the detection volume should be reduced to around 1 nl(10^{-9} l) when open-tubular capillary columns are operated under optimum conditions. Under these conditions, larger theoretical plate numbers are produced with open-tubular capillary columns than with packed columns. Injection and detection of such a small volume can be carried out by split injection and on-column detection, respectively. However, it requires expertise.

We have studied and developed instruments for open-tubular capillary LC. The standard deviation of the unretained solute eluting from capillary tubing was examined, and it was found that instruments and techniques which have previously been developed in micro-HPLC[7] could be applied to open-tubular capillary LC after some improvements. The instruments developed for open-tubular capillary LC are suitable for capillary columns which have a volume of more than 10 µl. Therefore, columns of 30 to 60 µm i.d. are practical, taking into account the limitations of the instruments, although narrow-bore columns are required in order to obtain higher theoretical plate numbers per unit time.

To date, we have prepared physically-coated[8,9], support-deposited[10], chemically bonded[11,12], polystyrene[13] and ion-exchange open-tubular capillary columns[14] for LC. When open-tubular capillary columns are employed as the separation columns, the extra-column effect becomes serious. Injection, connection and detection systems which do not cause an unfavorable effect should be developed. Observed band broadening($V_{w,obs.}$) is described

as the summation of the contribution of the band broadening in the injection, the connection, the column and the detection parts:

$$(V_{w,obs.})^2 = (V_{w,inj.})^2 + (V_{w,con.})^2 + (V_{w,col.})^2 + (V_{w,det.})^2 \quad (3)$$

where $V_{w,inj.}$ is the band broadening in the injector, $V_{w,con.}$ is that in the connecting tubing, $V_{w,col.}$ is that in the column and $V_{w,det.}$ is that in the detector. The dimensions of the connecting tubing which cause 5 % additional band broadening, are shown in Table 1 for capillaries of 10 µl volume and an unretained solute [15].

TABLE 1
Dimensions of connecting tubing which cause a 5 % additional band broadening.

Column dimensions	Length of connecting tubing (mm)				
volume=10 µl	0.25 mm i.d.	0.15 mm i.d.	0.13 mm i.d.	0.10 mm i.d.	0.07 mm
3.54m x 60µm i.d.	1.2	9.2	16.3	46.6	194
5.10m x 50µm i.d.	0.8	6.4	11.3	32.3	134
7.96m x 40µm i.d.	0.5	4.1	7.3	20.8	86.5
14.15m x 30µm i.d.	0.3	2.3	4.1	11.6	48.4

APPARATUS

Pumping system

A Microfeeder(Azumadenkikogyo, Tokyo, Japan) equipped with a gas-tight syringe and LC-5A(Shimadzu, Kyoto, Japan) were generally employed as a pump in the constant-flow mode and the constant-pressure mode, respectively. With the former pump, the mobile phase could be supplied to the column at a low flow-rate(0.14 to 16.7 µl/min). Cross-section areas of these syringes are so small that relatively high pressure can be obtained with a low mechanical force, e.g., 50 kg/cm^2 can be obtained by a weight of 0.83 kg for the 100-µl gas-tight syringe, which has a cross-section area of 0.017 cm^2. These gas-tight syringe pumps withstood 70 kg/cm^2. LC-5A withstood 500 kg/cm^2, but the maximum operation pressure was limited by the durability of the injector or the connection parts.

Gradient elution was carried out by using home-made simple gradient equipment comprising a small-volume mixing vessel and a magnetic stirrer[16,17]. The gradient profile was exponential and determined by the ratio of the mobile phase to the volume of the

mixing vessel.

Injection system

The injection volume should be made as small as possible, in case the composition of the sample solution differs considerably from that of the mobile phase. Otherwise, deterioration of column efficiencies cannot be avoided and sometimes peaks are skewed. The volume of around 1 cm in length of the column is permissible injection volume.

Stop-flow on-column injection and micro-valve injection methods were adopted. In the former method, a sample solution was sucked into a stainless-steel tube(0.13 mm i.d. and 0.31 mm o.d.) by oprating a Microfeeder manually. The stainless-steel tube was inserted into the PTFE tubing(0.1 to 0.25 mm i.d.) which was attached to one end of the capillary column. A micro valve injector (JASCO:Japan Spectroscopic, Tokyo, Japan) with an injection volume of 0.02 µl was employed in the latter method.

Detection system

An ultraviolet spectrophotometer and a spectrofluorometer were employed as detectors for open-tubular capillary LC. The flow cell for these detectors had to be designed for open-tubular capillary LC. Quartz tubing(0.07 to 0.2 mm i.d.) was employed as the flow cell and connected to the column with a narrow-bore tubing. Dimensions of the flow cell and the connecting tubing were dependent on those of the separation column.

The concentration of solutes eluting from the column is so high that solutes can be detected sensitively in spite of a small cell volume(less than 40 nl). Figure 1 shows a chromatogram of polynuclear aromatic hydrocarbons by fluorimetric detection. Samples at the level of 10^{-11} g are easily detected by using a small-bore flow cell(6 X 0.07 mm i.d.). The structure and the dimension of the flow cell should be carefully designed so that the bore of the open-tubular micro capillary column is less than 30 µm. In such a case, on-column detection is usually adopted[18].

Low flow-rate of the mobile phase in open-tubular LC enabled the direct coupling to a mass spectrometer[12,19]. The column works as a kind of enricher in open-tubular capillary LC, owing to the low dispersion of solutes and a low mobile phase flow-rate.

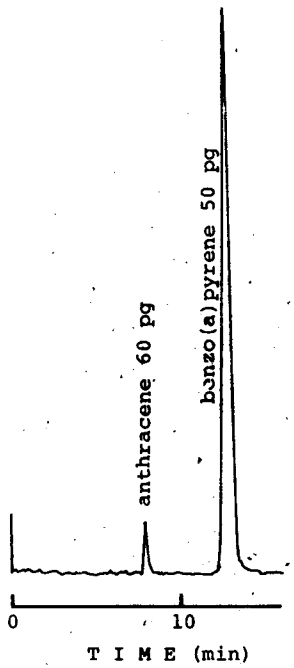

Fig.1 Fluorimetric detection of polynuclear aromatic hydrocarbons. Column:ODS, 5.3 m X 40 μm i.d. Mobile phase:acetonitrile-water= 6:4. Flow-rate:1.0 μl/min. Detector:FP-110C, Ex. 365 nm; Em. 430 nm.

PREPARATION PROCEDURES AND PERFORMANCE OF OPEN-TUBULAR CAPILLARY COLUMNS

Pretreatment

The bare inner surface of open-tubular glass capillary tubing is so small that it should be roughened in order to increase the surface area. The treatment of a soda-lime glass capillary with an aqueous sodium hydroxide solution was effective in roughening the surface[10]. The treated surface was polar, leading to good dispersion of polar liquid stationary phase[9]. Deposited solids worked as adsorbents in the normal-phase mode similarly to silica gel in packed LC. This suggested that stationary phases could effectively be chemically bonded onto the treated glass surface, as