Recent Developments in Analytical Chromatography

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Capillary gas chromatography (GC) is rapidly reaching a state of maturity. Recent advances in GC include new highly selective and stable open-tubular columns, capillary sampling techniques, and element-sensitive (plasma) detectors. An impressive growth of modern liquid chromatography continues with an emphasis on the separation of biopolymers, new silica-based and stable polymeric packings, microcolumn techniques, electroosmotically driven separations, and ultrasensitive detection. Supercritical fluid chromatography has gained considerable attention primarily because of its distinctive detection capabilities. Novel combinations of chromatographic techniques with each other, or with spectroscopic methods, continue to be explored.

CHROMATOGRAPHY CONTINUES TO PLAY A SIGNIFICANT role in various branches of science and technology as both an effective analytical tool and a powerful approach to large-scale separations. Recently, both directions have been stimulated by the needs and opportunities of modern biochemistry and materials science. While, on one hand, there is a distinct trend to scale-up highly efficient chromatographic processes (for instance, in purification of recombinant DNA products), there is also a continued quest for more reliable determinations of ever smaller concentrations that are characteristic of the needs of biological sciences and environmental monitoring.

The purpose of this article is not to provide an exhaustive review of the many advances in chromatographic separations that have occurred during the last several years, but to focus on analytically oriented developments with an emphasis on instrumental aspects. Whereas an attempt is made to preserve the historical links between new directions in chromatography and some earlier developments, the period primarily described in this article is approximately 5 to 6 years since the trends in analytical-scale separations were reviewed by Jorgenson (*1*). Gas chromatography (GC), liquid chromatography (LC), and supercritical fluid chromatography (SFC) are the main topics; a section on "combined techniques" has also been added to emphasize an increasing trend to couple different separation methods into one integrated analytical system.

Gas Chromatography

Gas chromatography is the oldest and the most mature of all instrumental chromatographic methods. The use of packed columns in GC was once widespread, and only a minority of laboratories dealt with capillary (open tubular) columns; the situation is reversed today. Analysts have found that in addition to superior resolution, capillary columns offer additional advantages: shortened time of analysis, better trace analysis capabilities, and superior detector performance due to less contamination originating from the separation column. The main strength of this method still resides in its excellent capability to deal with complex mixtures of volatile organic compounds. In particular, scientists in the fields of environmental chemistry, biomedical sciences, and natural products chemistry are more frequently dealing with such complex mixtures.

The recent popularity of capillary GC stems from several developments of the late 1970s; additional, minor advances are still being made. The use of flexible fused-silica columns and the development of reliable commercial instrumentation have been the key to progress in the area. Most analysts find such columns more inert toward labile samples, more adaptable to different combined detection techniques, and relatively easier to use than any other previously developed column type.

The most significant developments in GC column technology of recent years include (i) improvement of surface deactivation; (ii) immobilization of various stationary phases on the column wall; (iii) ability to vary the thickness of the stationary phase film to suit different analytical applications; and (iv) design of highly selective phases. The surface deactivation procedures now make use of predominantly silicon-containing reagents (various halogenated silanes, disilazanes, or hydrosilanes) to react with the surface silanol groups and thus form an "umbrella" over the surface that prevents irreversible adsorption of sensitive compounds. This treatment must furthermore be compatible with the layer of stationary phase coated over the modified surface. In fact, this treatment now often serves as a link between the surface and the immobilized stationary film.

Film immobilization is desirable for improved stability of capillary columns at high temperatures and resistance toward the degrading effects of sample injections. Many modern sampling methods that deposit the sample directly inside the capillary ("on-column injection") cannot be effectively used with mechanically deposited stationary phases. The literature of the last several years reveals numerous procedures for immobilization of silicone polymers to the surface. The adjacent polymer chains in a mechanically coated layer are usually cross-linked through a free-radical–induced reaction

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(traces of peroxides, aliphatic azo-compounds, or γ-radiation can be used). Although such procedures primarily refer to various silicone polymeric phases, additional efforts are being made with polyglycol phases. The 1984 book on capillary GC by Lee, Yang, and Bartle (*2*) describes various aspects of column technology.

Previously, there were only limited options to vary the stationary film thickness. Today, one can choose from highly efficient, thin-film columns (typically, 0.1 to 0.2 μm), or less efficient, but more adaptable thick-film columns (typically, 1 to 8 μm), to suit a given analytical problem. In fact, 0.5-mm [inside diameter (ID)] capillary columns ("wide-bore capillaries") coated with thicker films are finding considerable appreciation as a highly effective substitute for the packed columns.

Thermal stability of contemporary GC columns has improved dramatically. From several outstanding examples published to date, a high-temperature separation of vanadyl porphyrin complexes (*3*) extracted from Serpiano oil shale has been chosen (Fig. 1). Here, Blum *et al.* (*3*) have been able to observe the natural petroporphyrins by capillary GC combined with a mass spectrometer (a GC/MS) at column temperatures as high as 420°C. Other impressive high-temperature separations reported in the literature include various triglycerides (*4*) and hydrocarbon mixtures up to C_{120} (*5*). Since such excessively high temperatures render the polymeric overcoat of the fused-silica capillaries brittle, aluminum-clad capillaries have been developed (*6*).

Although there is a tendency to consolidate the overall number of stationary phases in use for the sake of method standardization, fascination with highly selective phases continues. During previous years, the stationary phases used were mostly the purified versions of synthetic polymers produced by the chemical industry for purposes other than chromatography. More recently, a rational design of chromatographic substrates has been seen. The use of liquid crystals as stationary phases has become of greater interest because of their ability to discriminate various molecules according to their size and shape. Although liquid crystalline compounds were investigated previously, only recently has it been feasible to synthesize them in polymeric forms (*7, 8*) that provide the necessary stability and performance. The degree of retention on such selective phases is determined by a structural match between the solute molecular geometry and the oriented mesomorphic side chains of the polymeric phase. A retention model for this behavior has been proposed (*9*).

Chiral separations continue to interest biochemists, natural products chemists, and pharmaceutical scientists. In capillary GC, thermal stability and enantioselectivity of chiral stationary phases have been a focus of numerous studies. Silicone stationary phases with various chiral selectors have been synthesized and evaluated for the GC separation of amino acid enantiomers and other racemic mixtures (*10–12*).

Advances in complexation GC made during the last several years may be used in the future by researchers who attempt to clarify various stereochemical problems, such as those encountered with insect and mammalian pheromones and biosynthetic pathways. Schurig and Weber (*13*) have used several types of metal chelates to resolve enantiomers of various esters, aliphatic alcohols, ketones, and various terpenes. More recently, various cyclodextrin derivatives have been explored as a new class of selective stationary phases. These distinctive carbohydrate macrocyclic structures can be prepared with different sizes of inner cavities (α-, β-, or γ-cyclodextrins), whereas suitable chemical modifications of their polar groups can further aid in selective interactions. Formation of various inclusion complexes with different solutes is primarily thought to account for highly selective separations on the cyclodextrin stationary phases. An example of specific interaction between a derivatized α-cyclodextrin and enantiomeric γ-lactones is shown in Fig. 2 (*14*).

Gas chromatography instrumentation has also advanced during the last several years. In contrast to the rapid standardization of certain basic functions of the gas chromatograph (such as oven temperature control, use of conventional detectors, and signal recording and amplification), the questions of optimum sampling procedures have not been entirely settled. This is particularly evident in the use of capillary columns for quantitative analysis. Nevertheless, considerable progress is being made through more frequent uses of direct injection methods, which do not discriminate against the high-boiling sample components as much as the sample-splitting techniques do. Various "dirty" (routine) samples are now effectively handled by the so-called programmed-temperature vaporizing (PTV) injector (*15*). Automation of most sampling techniques, leading to superior quantitative results, is now becoming widespread.

Perhaps the most interesting and analytically useful advances in GC instrumentation have been in detection technology. With increasing demands on sensitivity and selectivity of measurements in biochemical and environmental studies, this is not surprising. Recent advances in atomic emission spectroscopy, such as the development of various effective atom reservoirs and new optical-recording devices, have considerably revived interest in GC element-selective detection. In particular, electrical discharges (plasmas) have been explored as a way to cause molecular fragmentation of the components separated by GC, after which the emitted spectrum (characteristic of a solute) is focused on the entrance slit of a high-resolution monochromator. The monochromator settings at different wavelengths then allow selective detection of various elements in the picogram-per-second range. Alternatively, simultaneous multi-element detection is feasible with a photodiode array or a rapid-scanning spectrometer, making the overall system as instrumentally complex as, for example, a GC/MS.

Various types of plasma detectors have been investigated. Helium or argon plasmas can be maintained within a microwave cavity, whereas the type of cavity can be crucial to a particular detection problem. Systems working at atmospheric pressure are generally

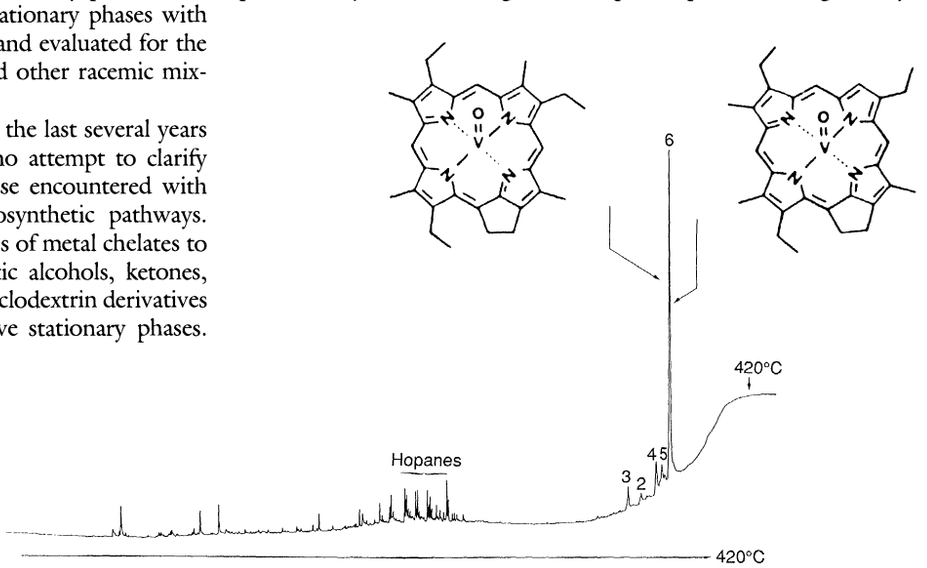

Fig. 1. High-temperature GC separation of porphyrin vanadyl complexes extracted from Serpiano oil shale. Conditions: 20 m by 0.3 mm ID capillary coated with 0.2-μm film of PS-089 silicone; hydrogen carrier gas; and heating rate 4°C per minute. [Adapted from (*3*) with permission of Dr. Alfred Huethig Verlag]

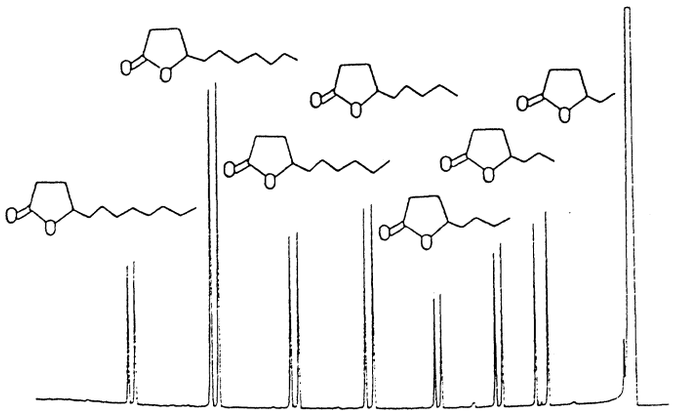

Fig. 2. Separation of a racemic mixture on a glass capillary column coated with hexakis (3-*O*-acetyl-2, 6-di-*O*-pentyl)-α-cyclodextrin. [Reproduced from (*14*) with permission of Dr. Alfred Huethig Verlag]

preferred in chromatographic applications. However, the low flow rates of capillary GC also make it feasible to combine this separation technique with microwave cavities where plasmas can only be sustained at reduced pressures. Traditionally, the ultraviolet (UV)-visible range has been used to monitor emission from a variety of elements. More recently, near-infrared (IR) atomic emission has also been explored (*16, 17*). Various plasma dopant gases further extend the scope of detection.

Although volatile metal chelates and mercury- and lead-containing organic compounds are readily detectable by the microwave plasma devices, measurement of nonmetal elements is of primary interest in GC applications. Detection of selenium and arsenic compounds has been reported (*18*) with an atmospheric-pressure system, whereas halogenated and sulfur-containing polycyclics showed relatively good sensitivity with a new radio frequency plasma detector (*19*). Selective detection of oxygenated substances with a low-pressure system (*20*) has also been reported. Element-selective plasma detectors seem to be entering an era of extensive commercial exploration. A novel detector type, based on redox chemiluminescence (*21*), further extends possibilities of element-specific GC measurements.

The present-day applications of capillary GC are both impressive and numerous. Reliable state-of-the-art columns and instrumentation have made it feasible to separate extremely complex organic mixtures. The method is now well established in the analysis of fossil fuels, fatty acids, essential oils, aroma constituents, and various environmental pollutants. Although some biologically oriented investigators use capillary GC routinely, various advances in modern LC make GC less attractive as the primary analytical tool for biological substances, because most biochemically important molecules (amino acids, peptides, carbohydrates, prostaglandins, and so on) can only be made sufficiently volatile for GC after their polar functional groups have been blocked through a suitable derivatization.

Liquid Chromatography

In modern liquid chromatography, high-pressure systems are used to enable the flow of liquid through columns that are packed tightly with small particles. This optimized form of chromatography has now revolutionized many analytical methods due to its inherent efficiency, speed, and quantitative capabilities. Its growth continues with emphasis on new columns, instrumentation, and an enormous scope of applications.

In his 1984 review (*1*), Jorgenson noted that no column dimension could be considered typical for the field of LC. Today, while 25 cm by 4.6 mm ID columns packed with 5-μm particles are most popular among analysts, many uses of shorter columns packed with 5-μm particles are evident from the literature. Although there are theoretical reasons for exploring even smaller particles, the general trend toward particle miniaturization has slowed compared to previous years. Interesting developments in particle technology include highly regular monodisperse beads (*22*) and rejuvenation of the pellicular packing approach by Horvath and co-workers (*23*).

Interest in microcolumn LC continues to be strong, now with a particular emphasis on various sample-size–limited applications and the separation of biopolymers. From different microcolumn types (*24*), fused-silica capillaries (typically 200-μm ID) packed with 3- or 3-μm particles appear most versatile, as they are compatible with a number of miniaturized detectors and do not demand the most stringent instrumentation. For fast separations featuring up to about 30,000 plates, short microcolumns are used. However, in resolution of very complex mixtures (requiring up to several hours of analysis time), as many as 250,000 plates can be achieved with long columns. Yet such separations do not require excessive inlet pressures. Recently, further gains in efficiency were realized through the decrease of the column ID below 50 μm (*25, 26*). For example, a 44-μm ID column of less than 2 m in length that was packed with 5-μm C_{18}-bonded particles produced 226,000 plates in 33 min (*25*). The interest in open tubular LC is now somewhat less evident, presumably because of the extreme instrumental demands for work with microcolumns with IDs less than 10 μm. Nevertheless, some very interesting work in this direction has been reported by the groups of Jorgenson (*27*) and Simon (*28*), who used extremely sensitive miniaturized electrochemical detectors. Tijssen *et al.* (*29*) have also explored LC open tubular columns for the separation of small particles by means of hydrodynamic chromatography. Overall, the area of microcolumn separation techniques will be significantly aided by commercial developments, so that less laborious and more reliable instrumentation, including quantitative sampling and gradient elution devices, will become available.

The use of microcolumns together with miniaturized instrumentation may have important applications in the fields of modern biochemistry and medical science. This is due to their capability of handling extremely small samples, separating their constituents, and detecting them at extremely high sensitivity. This trend is perhaps best exemplified by a recent report on the analysis of single neurons of *Helix aspersa* (*30*).

In the area of biomacromolecular separations, microcolumn techniques provide the benefit of isolation of extremely small amounts. It is a combination of the enhanced mass sensitivity of miniaturized concentration-sensitive detectors (for example, a UV detector) and the drastically reduced amount of the sorptive material that allows recovery of proteins at subnanogram levels (*31*). (With conventional LC columns, difficulties with labile biomacromolecules are experienced even at microgram levels.) Microcolumn techniques are likely to play a major role in characterization of the biopolymers isolated in such minute quantities. Such tasks include reduced-scale methods for composition, sequencing, and "mapping" of biomacromolecules. Microcolumn LC is viewed here as a tool complementary to capillary electrophoresis (another microcolumn method that differs in its separation principle but shares common instrumental characteristics).

The advent of miniaturized separations has been a catalyst to the development of electroosmotically driven capillary chromatographic methods, such as capillary electrochromatography (*32*), micellar electrokinetic capillary chromatography (*33*), and chromatographic separations where the mobile phase is transported by an electroos-

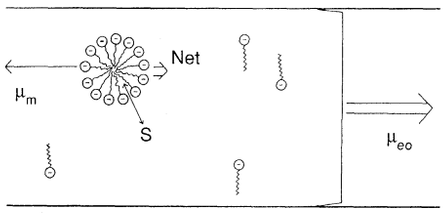

Fig. 3. The separation principle of the micellar electrokinetic capillary chromatography. The electroosmotic flow effect causes the liquid to flow at velocity μ_{eo} in one direction, while the charged micelles tend to migrate (μ_m) in the opposite direction. The solutes (S) partition between the micelles and their buffer environment. [Reproduced from (*35*) with permission of Marcel Dekker, Inc.]

motic flow generated by high-voltage gradients (*34*). Such techniques are related both in principle and instrumental aspects to chromatography as well as capillary electrophoresis. In all instances, the small column diameter is essential to conduct away the Joule heat generated at high voltages. Excellent results have been obtained in systems proposed by Terabe and co-workers (*33, 35*). The principle of micellar electrokinetic capillary chromatography is illustrated in Fig. 3. In such a system, the separated solutes are partitioned between the electroosmotically driven mobile phase and the micellar aggregates that tend to migrate to the respective electrode (*35*). A degree of chemical selectivity can further be enhanced by addition of chelating agents.

There have been numerous advances in LC packing technology. Siliceous materials still dominate the field, but numerous efforts to design rigid organic materials are also evident. Silica-based materials suffer from instability in high *p*H media and tend to present difficulties in the chromatography of basic compounds. Additional adverse effects of siliceous surfaces are often realized with particularly sensitive compounds, such as proteins. One approach to solve such problems is to mask effectively the surface structures through a controlled deposition of polymers. Another approach that is perhaps potentially more effective in the design of polymeric materials that are stable over an extensive *p*H range. Notable examples of these technologies in the protein separations area are rigid polystyrene-divinylbenzene (*36*) and fluorocarbon resins (*37*), and polymethacrylate-based materials (*38*). New latex-based ion-exchangers were recently introduced for chromatography of small ions (*39*).

While reversed-phase chromatography continues to be the most popular LC operational mode, selective materials are needed for separation of more "sophisticated" molecules, such as proteins and nucleotides. Hydrophobic interaction and ion-exchange chromatography are increasingly recognized as versatile techniques, and a number of new stationary phases, both silica-based and organic, have been explored. Multifunctional phases show some merit for the separation of biological molecules (*40*).

The extremes of separation selectivity are sometimes needed in purification and determination of biological macromolecules. While bioaffinity separations represent the ultimate in this direction, the rational design of a column can be a very tedious task. Trends in affinity chromatography have been recently reviewed by Chaiken (*41*). Intermediate on the scale of selectivity are metal complexation phenomena, which can often be responsible for useful separations of protein mixtures (*42*).

Owing to its importance in biological processes and drug design, chiral recognition continues to be a subject of intense interest at chromatographic conferences. Various approaches to chiral LC separations have been shown in the recent literature. The general understanding of chiral interactions has been further advanced (*43*), while cyclodextrin-based phases (*44*) and human α_1-acid glycoprotein (*45*) were described as new chiral selectors.

The mobile phase interactions in LC are becoming fairly well understood. Modern liquid chromatographs are provided with various options of manipulating the eluting strength of solvents in a variety of gradient modes. Secondary chemical equilibria are now widely used in selective separations. Micellar systems have also been explored (*46*) as an interesting adjunct to the mobile phase manipulations.

The search for new LC detection techniques continues to be an active area. A fairly comprehensive treatise of LC detectors has recently appeared (*47*), and therefore only the main directions and selected recent developments will be highlighted here. A majority of typical LC analytical problems in industry are being satisfied by the common detectors in use (UV-visible, refractive index, conductivity, fluorescence, and so forth), which have been gradually improved by instrument manufacturers in terms of their operational range and sensitivity. The wide use of spectroscopic photodiode array devices has further increased the analytical scope. For numerous compound classes, the use of precolumn and postcolumn derivatization has at least partially overcome the need for a universal detector.

The challenges posed by research areas such as life sciences and analytical biotechnology will undoubtedly provide further impetus for improvements in high-sensitivity determinations, as has been clearly demonstrated with the recent uses of electrochemical detection in neurochemistry and increasing interest in laser-based and mass spectroscopic detectors in protein biochemistry. In most of these areas, microcolumn techniques seem to provide the best hope for dramatic improvements. Particular chemical approaches must often be combined with such detection methods. As an example, amperometric detection of the amino acids in the attomole (10^{-18} mole) range has been shown (*48*), based on precolumn derivatization into electrochemically active isoindoles. A further example with respect to this standard class of compounds is shown in Fig. 4, where the standard amino acids are displayed together with a hydrolysate from 171 fmol (1 fmol = 10^{-15} mole) of myoglobin (*49*). In this instance, highly fluorescent isoindoles were formed through the reaction with 3-benzoyl-2-quinolinecarboxaldehyde, which had been synthesized so that the resulting isoindoles match the spectral characteristics of a helium-cadmium laser. The laser-based detectors and microcolumns hold the promise of the ultimate in LC detection sensitivity; a recently estimated detection limit of 6000 molecules (*50*) in a flow system supports this notion.

Commercial LC instrumentation has been dramatically improved in recent years and has been augmented by additional optional devices, such as autosamplers, robotics systems, postcolumn reactors, and so on, to enhance its versatility and routine use. For LC of labile biopolymers, "biocompatible" instruments made from special materials are being designed (*51*).

Supercritical Fluid Chromatography

Because SFC was in its initial stages of development, it received only a cursory acknowledgment in Jorgenson's review (*1*). During the last several years, numerous developments have taken place that justify specialized symposia and attract commercial interest. Although SFC was first reported and investigated already during the late 1960s, the method has undergone its "renaissance" only since the early 1980s. Certain technologies, borrowed from the areas of capillary GC and modern LC, have become available to overcome the widely perceived technical problems of SFC. An additional important impetus was the development of open tubular SFC (*52*), which provided the potential of higher separation efficiency together with an enhanced compatibility of the method with certain chromatographic detectors.

The main rationale for using SFC has been its favorable mobile-

phase properties that are pertinent to effectiveness of a separation process. Although supercritical fluids (dense-gas media) possess some of the solvating properties of true liquids, they are much less viscous. Less dense supercritical fluids permit faster solute diffusion than liquids and, consequently, enhanced radial mass transfer. This translates into efficient separations with open tubular (50-μm ID or narrower) columns, or equally efficient but faster separations with conventional LC columns when 3- to 10-μm particles are used. Other analytical advantages of SFC reside in easy desolvation of the separated materials during micropreparative runs. The relative insensitivity of some detectors to certain compressed mobile phases is another advantage. In its relation to GC and LC, SFC is both complementary and competitive. Its main areas of application are in improved separations of substances that are insufficiently volatile or stable for high-temperature capillary GC, on one hand, and for those compounds that are difficult to detect by the LC systems, on the other.

With SFC, several laboratories have at present reached the elution of compounds in excess of 10,000 daltons, clearly a range inaccessible by capillary GC. At the same time, such materials can be detected and quantified by means of the universal flame ionization detector, a technique which is not feasible in conventional LC.

Carbon dioxide and nitrous oxide are the most appealing supercritical mobile phases from the practical point of view (relatively low critical pressures and temperatures). The use of small-size columns (either open tubular or packed microcolumns) has advantages in selecting either an expensive, flammable, or otherwise hazardous mobile phase if it provides some analytical advantage. Attempts have been made to rate supercritical media in terms of their solubilization properties and potential capability to chromatograph polar molecules at routinely obtainable pressures. Being an intermediate between GC and LC, SFC provides some distinctive opportunities to study mobile phase–solute and mobile phase–stationary phase interactions.

It is unfortunate that the more polar supercritical media, such as ammonia or sulfur dioxide, are not compatible with the present-day chromatographic columns and hardware. To overcome the limited solubilization of polar molecules in carbon dioxide or nitrous oxide, numerous investigations have used a polar mobile phase modifier. Methanol, acetonitrile, ether, propylene carbonate, formic acid, or water are typically added to the bulk mobile phase in amounts ranging from 0.1 to 20% in order to shorten the retention times of polar compounds. These modifiers appear to function quite differently for packed and open tubular columns. Some interesting phenomena, such as ion-pairing (*53*) and formation of reverse micelles (*54*) in supercritical media, were discovered during such studies.

Rapid development of SFC instrumentation has taken place during the last several years. Since solvation processes generally improve at elevated pressures (greater densities), commercial instruments are equipped with high-precision pumps that are computer controlled to produce linear density ramps. In some instances, simultaneous density and temperature programming is used for optimized component resolution. Programming the mobile phase composition in SFC (unlike in LC) is not common.

Being intermediate between GC and LC, SFC can draw on detection methods from both areas. Detection in SFC can be accomplished either in the compressed state (in a high-pressure cell) or after efficient expansion through a suitable pneumatic restrictor. The design of such a restrictor appears crucially important to the success of detection in the flame and plasma-based detectors, or combined SFC/MS.

The flame ionization detector (FID) is perhaps the most important SFC detector today, since certain supercritical fluids generate only a minimum background signal, permitting a universal detection of organic compounds. Other flame-based devices, such as the flame photometric and thermionic detectors, have also been adopted from the GC field and optimized for the element-selective (nitrogen, phosphorus, and sulfur) analyses at levels comparable to or lower than that for the FID. The use of plasma detectors also appears feasible. Spectrofluorimetric and UV-visible detection in SFC are useful and straightforward. Technically they differ little from the optimized detection techniques developed for microcolumn LC.

The major application areas for SFC have been the analysis of fossil fuels (heavy constituents), synthetic oligomeric mixtures, polymer additives, food products, and agricultural chemicals. Most of these instruments use FID or a mass spectrometer as the detectors. An example of such an analysis is shown in Fig. 5, where alcohol-ethoxylate oligomers have been resolved on a 5-m biphenylsiloxane capillary column (*55*). Although the high resolution of capillary SFC was mandatory to observe additional minor peaks in the sample, the universal FID can measure all sample components. Clearly, it is not feasible to obtain such a chromatogram with modern LC because of the lack of a suitable detector as well as its less adequate separation efficiency in the size-exclusion mode. A large number of separations made only by SFC can be found in recent compilations of various applications (*55*), which confirms that SFC has found its "niche" in the arsenal of powerful chromatographic methods.

At present, SFC works considerably better for nonpolar rather than polar substances, which is undoubtedly the result of the limited choice of suitable mobile phases. Although the use of more polar or modified mobile phases for biochemical analyses may be inherently limited, it appears feasible to derivatize polar molecules for better

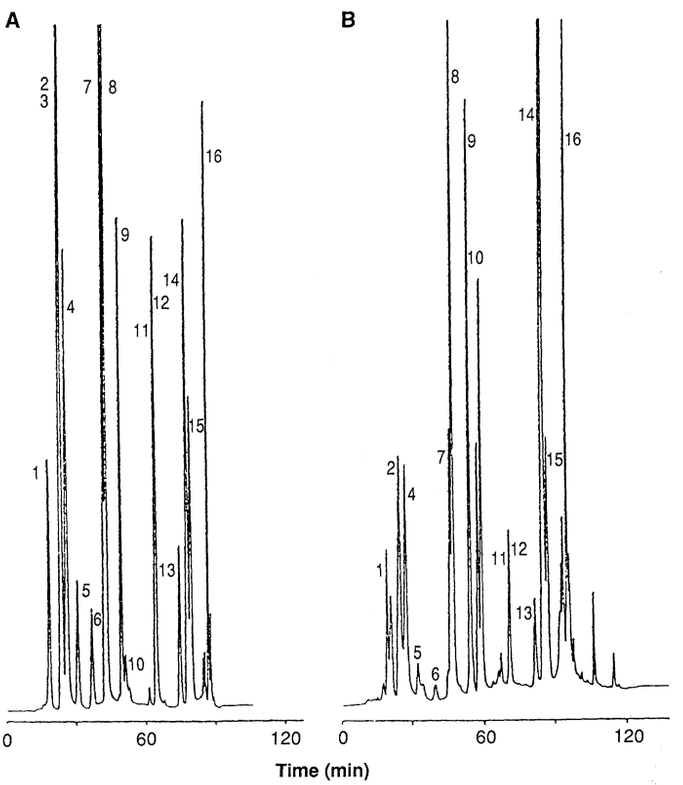

Fig. 4. Chromatogram of (**A**) a standard amino acid mixture and (**B**) hydrolysis products from 171 fmol of myoglobin. Peaks: (1) His; (2) Glu; (3) Gln; (4) Thr; (5) Asp; (6) Ser; (7) Tyr; (8) Gly; (9) Ala; (10) Arg; (11) Met; (12) Val; (13) Ile; (14) Leu; (15) Phe; and (16) Lys. [Reproduced from (*49*) with permission of Pergamon Press]

solubility in "comfortable" mobile phases, such as CO_2 or N_2O. This approach has been powerfully illustrated by applications to oligosaccharides (*56*) and ganglioside mixtures (*57*). Yet another advantage of sample derivatization lies in the possibility to incorporate easily detectable heteroatoms into the molecules of interest (*58*).

Combined Techniques

Combined ("hyphenated") techniques represent a rapidly growing area of analytical chemistry. Historically, the key developments here were associated with coupling GC to MS and to IR spectroscopy to aid identification of the separated components. Although this trend has now been extended into coupling LC and SFC with a growing number of spectroscopic methods, there has also been an extensive search for new ways to combine different separation techniques with each other (GC/GC, LC/GC, SFC/SFC, SFC/GC, and so on). This latter direction is primarily stimulated by the need to analyze very complex mixtures as well as trends toward special-purpose analytical instruments that integrate the tasks of preconcentration, separation, and the final measurement. Through various advances in pneumatic and instrument computer control, these valuable concepts of "multidimensional chromatography" are becoming increasingly attractive for commercialization. The rationale, instrumentation, and selected applications of various combined separation techniques have been reviewed by Bartle and co-workers (*59*).

Ancillary methods of capillary GC (GC/MS and GC/IR) are rapidly reaching full maturity. The merits of IR spectroscopy as a structural tool, complementary to MS, have recently resulted in sensitivity improvements through the use of Fourier-transform (FT) techniques and matrix isolation. Although MS is inherently more sensitive than FTIR spectroscopy, the gap between these methods has been gradually narrowed, and it has become practical to combine GC, FTIR spectroscopy, and MS into a single analytical unit capable of nanogram sensitivities. Combining SFC and LC to MS and FTIR spectroscopy has been more technically involved than the respective GC combinations. Easy desolvation at the column exit is one of the favorable points for SFC/MS, where the column effluent is either deposited onto a moving belt prior to MS investigations for packed columns, or directly to the ion source of a mass spectrometer for capillary columns. Depending on the type of ionization technique, sensitivities in the nanogram-to-picogram range appear feasible in capillary SFC/MS (*60*). FTIR measurements are feasible in combination with SFC, since unlike in LC/FTIR, a nonabsorbing mobile phase can be chosen, or, alternatively, any supercritical medium can be easily removed during the solute deposition on a suitable matrix (*61*). Supercritical xenon has also been explored in SFC/FTIR (*62, 63*) due to its total transparency in the IR spectral region.

Although the LC/MS combination has been successfully commercialized during the last several years with the use of thermospray, "dynamic FAB" (fast atom bombardment), and monodisperse aerosol generator interfacing techniques, a search for better coupling methods and ionization phenomena continues. While reviewing various approaches to these technical problems would be beyond the limits of this article, it should be emphasized that the use of chromatographic microcolumns is among the most promising routes toward improvements in sensitivity and spectroscopic information (*64–66*) because of the tolerance of MS instruments to low flow rates.

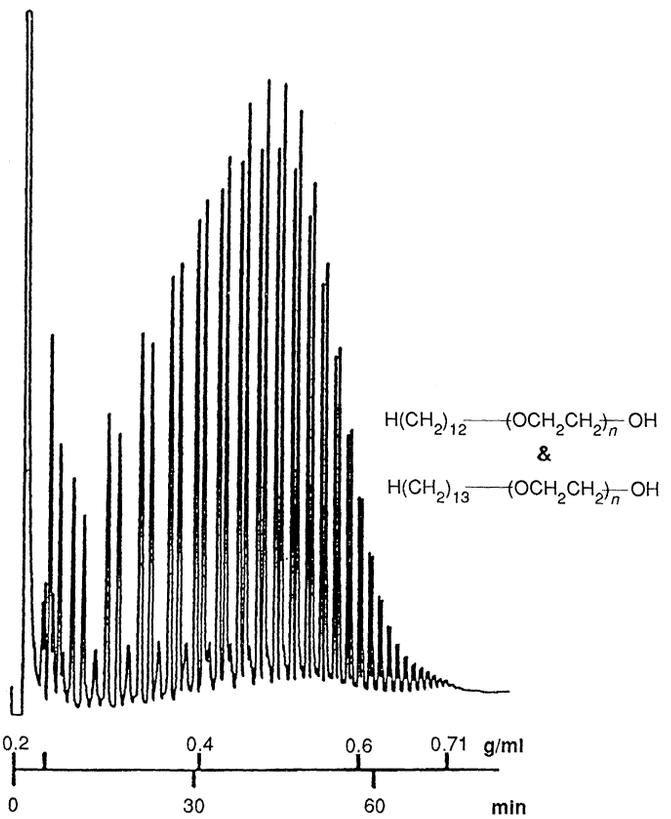

Fig. 5. Chromatogram of a sample of alcohol-ethoxylates obtained with a supercritical carbon dioxide column (density program used) and flame ionization detection. Conditions: 5 m by 25 μm, ID capillary column coated with 25% biphenyl silicone phase; and column temperature, 120°C. [Reproduced from (*55*) [ed. 1, p. 296] with permission of Brigham Young University Press]

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Microcolumn Separations and the Analysis of Single Cells

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Capillary zone electrophoresis and open tubular liquid chromatography are two examples of an emerging area of analytical instrumentation known as microcolumn separations. The high resolution and small sample requirements of these methods make them suitable for the quantitative, multicomponent chemical analysis of single cells. Appropriate instrumentation for the analysis of nanoliter and subnanoliter samples is discussed. Data from the analysis of individual neurons are presented, including amino acid and neurotransmitter content.

IN THE DEVELOPMENT OF ANALYTICAL INSTRUMENTATION, miniaturization is often a fruitful endeavor. During the past 20 years, numerous scientific advances arising from the development of microelectrodes, microsensors, and ion, electron, and light microprobes have been made. Two distinct benefits are associated with the miniaturization of analytical methods. The first is a change in the properties of the analytical tool, which often can be turned to advantage. The second is that the miniaturized instrument allows analysis of smaller samples and with higher spatial resolution.

Capillary electrophoresis (CE) (*1–3*) and open tubular liquid chromatography (OTLC) (*4*) are two examples of miniaturized, or microcolumn, separation methods that have analytical advantages over their conventional counterparts. In CE the separation of compounds is based on the different mobilities of molecules in an electric field. The separation takes place inside a capillary tube, typically with an inner diameter (ID) of 5 to 100 μm and a length of 10 to 100 cm. The capillary tube may be filled with only a buffer, as in capillary zone electrophoresis (CZE) (*2*), or with a gel, as in capillary gel electrophoresis (*5*). In both types of CE, the small dimensions of the capillary allow the rapid dissipation of Joule heat, which in turn allows potentials as high as 30 kV to be applied across the capillary. The strong electric field makes possible rapid, high-resolution separations. More than 1 million theoretical plates have been achieved in the separation of proteins by CZE (*6*).

In OTLC the separation takes place inside a capillary tube with an ID of 1 to 50 μm and a typical length of 1 m or more. The stationary phase is attached to the inner wall of the capillary instead of to particles packed into the column as in conventional high-performance liquid chromatography (HPLC). Open tubular columns, if their ID is small enough, have considerably greater resolving power than packed-bed columns (*7, 8*). The theory for OTLC predicts that, given certain time and pressure constraints, the optimum ID is approximately 2 μm. Such an OTLC column would generate over 1 million theoretical plates for a well-retained compound with an analysis time of less than 1 hour (*9*). Microcolumns have also been of interest because the inherently low volumetric flow rates facilitate the coupling of the separation to other analytical techniques such as mass spectroscopy.

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