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Column Liquid Chromatography: Equipment and Instrumentation

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Review Contents

This review covers fundamental developments in column liquid chromatography (LC) equipment and instrumentation for the period of January 2000 through December 2001. As with past issues, separate reviews on theory, size exclusion chromatography (SEC), supercritical fluid chromatography (SFC), and capillary electrophoresis (CE) are not included in this review unless the reference has technology pertinent to LC. With the growing trend in miniaturization, this reviewer has chosen to include significant articles in the area of capillary liquid chromatography (CLC), capillary electrochromatography (CEC), and microchip technologies that have a focus on LC. High-performance liquid chromatography (HPLC) and LC are considered synonymous. Although this review is not intended to cover applications, applications that contain developments and improvements to LC equipment and instrumentation or were used to demonstrate equipment performance were also included.

The databases for the majority of this review were CAPLUS, CHEMCATS, and CHEMLIST. This paper was not intended to be a comprehensive review of all published papers during this time period, but those papers that were considered to be significant developments or improvements. This review has also been largely restricted to the English language literature. Although the vast majority of articles were from peer-reviewed publications, included are relevant articles from widely read periodicals such as LC-GC and American Laboratory.

INSTRUMENTATION

Fundamental developments in column LC equipment and instrumentation for the period of October 1997 through October 1999 were reviewed by LaCourse (*A1*). This paper is a continuation of that effort. Presently, major trends are toward miniaturization, speed, and throughput, important qualities when handling complex, analysis-intensive samples such as those derived from the needs of research in proteomics, metabolomics, and combi-

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natorial chemistry. There has been literally an explosion of articles on microchromatographic techniques (e.g., CLC, CEC, and microchip techniques), which also drives new detector design and developments. Ultrafast separations, on-line analyte identification, high-throughput technology, and an orientation to biological applications are all a reflection of the advances in genomics and biotechnology.

Hyphenated Instrumentation. This section is not inclusive of all hyphenated techniques, in that numerous papers in the following sections, especially detectors, also highlight hyphenated techniques. One area that underscores the goals of hyphenated instrumentation is in the full identification of eluting species in LC. To accomplish analyte identification, the approach is often to combine mass spectrometry (MS) and nuclear magnetic resonance (NMR) on-line. Bailey et al. (*A2*) used an HPLC NMR-MS system for identification of three metabolites (i.e., *N*-glucoside, *N*-malonylglucoside, and *O*-malonylglucoside) of 5-nitropyridone (2-hydroxy-5-nitropyridine), in maize plants grown hydroponically. Another approach used by Schrader et al. (*A3*) was to couple HPLC-infrared (IR) spectroscopy-NMR-MS to identify reaction products of pinene ozonolysis, which relates to the "blue haze" phenomenon above forests. Huber and Holzl (*A4*) reviewed in detail the coupling of CEC with MS. The article also covers instrumental aspects, separation systems, and applications.

Multidimensional multimodal instrumentation greatly improves peak capacity in complex mixtures. An on-line system for coupling a normal-phase LC column to a reversed-phase LC column is described by Moret and co-workers (*A5*). The interface consisted of an on-line solvent evaporator working on the principles of concurrent eluent evaporation and vapor overflow and two additional 10-port valves. Optimization of the on-line procedure and recoveries, repeatability, and linearity characteristics were tested in a simplified system simulating detection of heavy polycyclic aromatic hydrocarbons (PAHs) in edible oils. Isaaq et al. (*A6*) presented a simple approach to the application of HPLC/CE for the separation of complex mixtures. A two-dimensional HPLC/CE instrumental setup was assembled from commercially available equipment. Fractions of the effluent from the HPLC system were collected into microtiter plates with the aid of a microfraction collector, which allows the collection of samples by time, drops, or external signal (peaks). The fractions are then dried under vacuum at room temperature in a special unit, reconstituted, and analyzed by CE. Any size or type of HPLC or CE column can be used with no limitation on the amount of sample injected into the HPLC, and any CE detection procedure, such as laser-induced fluorescence (LIF), MS, or UV, or other, can be used. Preliminary results show the utility of this system for the analysis of a mixture of two protein digests, cytochrome *c* and myoglobin.

High-Throughput Advances. Morand et al. (*A7*) discussed generally recent advances in high-throughput MS. Hiller et al. (*A8*) describes the application of a prototype dual sprayer electrospray ionization (ESI) source for the quantitative analysis of biological samples. Quantitative performance for 180 compounds in a microsomal stability assay was adequate when compared with a conventional single sprayer measurement. Sage and Giles (*A9*) reported on the design and implementation of a multiplexed (MUX) electrospray interface capable of sampling four individual liquid streams in rapid succession. Data from single compounds and mixed analytes under fast-gradient LC conditions using the four-channel MUX technology source were also described. Using fast-gradient LC on small-bore LC columns, samples can be analyzed with inject-to-inject cycle times of 3 min. Another approach is to process several inlet streams at one time to decrease cycle time, such as a new parallel LC/tandem mass spectrometry (LC/MS/MS) system, in which the mass detector was shared between two staggered parallel chromatographic runs (*A10*). Shen et al. (*A11*) reported on the design and application of a high-efficiency multiple-capillary LC system for high-throughput proteome analysis. The multiple-capillary LC system using commercial LC pumps was operated at a pressure of 10 000 psi to deliver mobile phases through a novel passive feedback valve arrangement that permitted mobile-phase flow path switching and efficient sample introduction. A high magnetic field (11.4 T) Fourier transform ion cyclotron resonance (FTICR)-MS was coupled on-line with this high-efficiency multiple-capillary LC system using an ESI interface. For yeast cytosolic tryptic digests, >100 000 polypeptides were detected and ~1000 proteins could be characterized from a single capillary LC-FTICR analysis using the high mass measurement accuracy (~1 ppm) of FTICR and likely more if LC retention time information was also exploited for peptide identification. Bayliss et al. (*A12*) present parallel ultrahigh flow rate LC using four columns in parallel and a four-way multiple sprayer interface to the mass spectrometer. This technique enables the quantification of drugs from four plasma samples simultaneously, at nanogram per milliliter concentrations, from small aliquots of plasma without sample preparation and with throughputs of >120 samples/h. High-throughput analysis in MS can also be accomplished via the use of an automatic function switching with direct infusion microspray quadrupole time-of-flight (TOF)-MS for peptide and protein analysis (*A13*).

The Karger group (*A14*) described an approach, based on a subatmospheric ESI interface, which allows sample introduction from a commercially available microtiter plate without the need for a separation fluid delivery system. The microtiter plate was placed vertically on a three-dimensional translation stage in front of the sampling ESI interface. A flow-through wash device was positioned between the microtiter plate and the ESI interface. This design allowed alternate filling of the capillary with (a) sample from the wells and (b) wash solution from the wash device. Sample turnaround times of 10 s/sample, with a 120-nL sample consumption/analysis, and a duty cycle (percentage of total analysis time spent acquiring data) of 40% were achieved. Hoke et al. (*A15*) increased throughput using packed-column supercritical fluid (pcSFC) coupled to MS/MS. In contrast to traditional reversed-phase LC, the addition of a volatile component to the mobile phase, such as CO_2, produces a lower mobile-phase viscosity. This allows the use of higher flow rates, which can translate into faster analysis times. In addition, the resulting mobile phase is considerably more volatile than the aqueous-based mobile phases that are typically used with LC/MS, allowing the entire effluent to be directed into the MS interface. Using pcSFC and MS/MS, dextromethorphan was quantified in 96-well plates at a rate of ~10 min/plate with an average intraday accuracy of 9% or better. Daily relative standard deviations (RSDs) were less than 10% for the 2.21 and 14.8 ng/mL quality control (QC) samples, while the RSDs were less than 15% at the 0.554 ng/mL QC level. Several configurations

using 6- and 10-port switching valves were studied for high-flow, on-line extraction of rat plasma coupled to an ESI-MS by Mallet and co-workers (*A16*). After sample loading and sample cleanup, the analytes were eluted from the extraction column with a 1.0-min gradient at 0.4 mL/min. The samples were analyzed either directly after elution from the extraction column or after additional separation using a short HPLC column, which resulted in a total time per sample of 3 min.

Capillary Chromatography/Electrochromatography. One of the reasons for the immense interest in capillary electrochromatography is its promise to combine chromatographic selectivity with the high efficiency and the miniaturization potential of CE. This interest is reflected in the numerous review articles of this technique. Instrumentation for CEC was reviewed by Steiner and Scherer (*A17*) and Rozing et al. (*A18*). General requirements, solvent delivery, and detection are covered in some detail. Other reviews have focused on modes of separation (*A19*), stationary phases (*A20*), and migration of charged species and electroosmotic flow in CEC (*A21*). Open-tubular CEC (*A22*) and its coupling with on-line MS (*A23*) have also been reviewed. Both CEC and capillary LC are discussed in Tsuda's (*A24*) review. Powell and Tempst (*A25*) described a microflow-based instrument, consisting of multiple rotary valves, capillary tubing, and miniaturized reaction vessels, for the purpose of performing automated chemical and biochemical reactions on a very small scale (i.e., submicroliter volumes). The novelty is that close to 100% of the reaction end products are available in a minimal volume (<5 μL) inside a pressurized microvial for subsequent analysis. This makes the system compatible with capillary LC and, in principle, with continuous-flow nanoelectrospray MS. Instrument performance was convincingly demonstrated by partially sequencing 100 fmol of an intact protein using classical Edman chemistry in combination with CLC.

Ye et al. (*A26*) reported on a novel mode of affinity chromatography (AC) based on an open-tubular (OT) capillary column. The OTAC column is prepared by immobilizing Cibacron blue F3GA onto the inner surface of a 50-μm-i.d. capillary column. The AC experiment is performed on a CE instrument by using its pressure system as the driving force. Bovine serum albumin and lysozyme (Lys) are successfully separated with stepwise gradient elution. The RSD for the elution time of the retained Lys is 0.08%, and good repeatability of its peak area and peak height with an RSD value lower than 2.12% for 10 consecutive runs was observed. The loading capacity and detection limit for the retained Lys are about 36 and 8.6 ng, respectively.

Injectors/Autosamplers. A series of articles by Dolan (*A27*−*A29*) focused on autosamplers in LC systems. The areas covered are the common designs, advantages and disadvantages, problems and solutions, and sample carryover. Kehl and Meyer (*A30*) described an approach to the determination of LC injection reproducibility based upon argentometric titration. The authors found the following repeatabilities (RSDs): titration, 0.07%; 10-μL pull loop injection (partial filling of the loop), 0.6%; and 20-μL full loop injection, 0.1%. Heron et al. (*A31*) looked at the influence of the injection volume and the sample solvent on column efficiency in packed nano LC using columns of 150-μm i.d.. When compounds are dissolved in a weak solvent (such as acetonitrile/H_2O, 30:70) and whatever the injection volume (i.e., 60 or 200 nL), a gain in efficiency can be observed due to the known on-column focusing phenomenon. Layne et al. (*A32*) studied the effects of sample solvent composition and injection volumes on the chromatographic performance of ODS-bonded silica columns under fast-gradient running conditions. Chromatographic performance was compromised as a function of both sample injection volume and sample solvent strength, with earlier eluting analytes affected much more than later eluting ones. In general, when injecting samples dissolved in a strong solvent, performance was improved by diluting the strong injection solvent and injecting a proportionally larger volume. Volume loading capacity can be increased by using a longer column or by using a column of equivalent length, but with a larger inner diameter. It was conjectured that sample solvent strength, not viscosity, is responsible for the noted effects.

Temperature Effects In LC. The present state of temperature programming in LC, including CEC, is reviewed by Greibrokk and Andersen (*A33*). As a part of the ongoing trend of miniaturization in chromatography, the active use of temperature as a variable in LC is expected to increase significantly. This paper includes an overview of the effects of temperature on column efficiency, retention, and selectivity, particularly in relation to narrow-bore columns, instrumental features, and some selected applications. The Greibrokk group (*A34*) also investigated temperature programming for gradient elution in packed capillary LC coupled to electrochemical detection (EC).

Xiao and Oefner (*A35*) reviewed denaturing HPLC (dHPLC), which compares two or more chromosomes as a mixture of denatured and reannealed PCR amplicons, revealing the presence of a mutation by the differential retention of homo- and heteroduplexes. This technique relies on temperature programming, and the authors discuss temperature-related resolution sensitivity and its optimum prediction by computational means. This work is extended further in an article by Premstaller et al. (*A36*) dealing with temperature-modulated arrays in HPLC for mutation detection. Analyses times are on the order of a few minutes and turnaround time is extremely short as there is no need for the replenishment of the separation matrix between runs. Issaq and co-workers (*A37*) investigated the effect of experimental temperature on the separation of DNA fragments (i.e., 21−587 bp) by both HPLC and CE. The optimum temperature was found to be between 40 and 50 °C for HPLC, while 25 °C was the optimum temperature for the CE separation.

The advantages of packed capillary columns in LC as well as in supercritical fluids were reviewed by Greibrokk et al. (*A38*). In liquids, the active use of temperature as a variable resulted in reduced analyses time, increased column efficiency, increased signal-to-noise ratio, and allowed temperature programming for controlling retention and replacing solvent gradients in HPLC. The advantages of high-temperature separations lend themselves to ultrafast LC, and the Carr group (*A39*) described a novel LC system, which enabled high-temperature ultrafast liquid chromatography (HTUFLC). Careful consideration was given to heat transfer, band broadening, and pressure drop. Using pure water as the mobile phase, five phenols were separated in <30 s.

Thompson et al. (*A40*) compared a narrow-bore column (2.1-mm i.d.) to a conventional-bore column (4.6-mm i.d.) at elevated temperatures under conditions where thermal mismatch broadening is serious and showed that narrow-bore columns offer signifi-

cant advantages in terms of efficiency and peak shape at higher linear velocities. The so-called thermal mismatch broadening effect is largely due to a radial retention factor gradient and not a radial viscosity gradient. The lower volumetric flow rates inherent using narrower columns lead to lower linear velocity in the heater tubing and longer eluent residence times in the heater. Thus, with the same heater tubing at the same column linear velocity, narrow-bore columns give better thermal equilibration between the eluent and the column compared to wider bore columns. This means that HTUFLC no longer requires excessively long preheater tubing to thermally equilibrate the eluent to the column temperature. Consequently, the use of narrow-bore columns at high-temperature improves analysis speed and efficiency over wider bore columns.

Sample Preparation/Derivatization. The use of preconcentration steps based on phase separation by the cloud point technique offers a convenient alternative to more conventional extraction systems. It was used successfully for the preconcentration of species of widely differing character and nature, such as metal ions, proteins and other biomaterials, or organic compounds of strongly differing polarity. The most recent analytical applications of this methodology as an isolation and trace enrichment step prior to LC, GC, or CE are reviewed by Carabias-Martinez et al. (*A41*). Klink and Majors (*A42*) summarized current sample preparation procedures for LC/MS as applied to samples from biological matrixes.

Zhu (*A43*) described a simple liquid−liquid−liquid microextraction device using a 2 cm × 0.6 mm i.d. hollow fiber membrane to preconcentrate nitrophenols from a water sample prior to CLC analysis. The extraction procedure was induced by the pH difference inside and outside the hollow fiber. The donor phase outside the hollow fiber was adjusted to pH ∼1 with HCl; the acceptor phase was NaOH solution used at various concentrations. Organic solvent was immobilized into the pores of the hollow fiber. With stirring, the neutral nitrophenols outside the fiber were extracted into the organic solvent and then back-extracted into 2 μL of alkaline acceptor solution inside the fiber. The acceptor phase was then withdrawn into a microsyringe and injected into the CLC system directly. Up to 380-fold enrichment of analytes could be achieved with a RSD ($n = 6$) of <6.2%. Using a similar approach, a simple, inexpensive, and disposable device for liquid-phase microextraction (LPME) was presented by Rasmussen et al. (*A44*) for use in combination with capillary GC, CE, and HPLC.

Ramsey and co-workers (*A45*) described the direct coupling of supercritical fluid extraction (SFE) with HPLC. The technique used a system of coupled octadecyl silane (ODS)-aminopropyl HPLC columns connected to the outlet of the SFE vessel. Moderately polar analytes that could be extracted were trapped onto the nonpolar ODS column during SFE. After SFE, these compounds were eluted from the ODS column, with band focusing, onto the polar aminopropyl column using a nonpolar organic solvent. Finally, HPLC analysis was performed using a gradient program that slowly introduced a polar solvent into the mobile phase. An on-line coupling of solid-phase extraction (SPE) and micro-LC was developed requiring minimal sample preparation and providing maximal sensitivity by Schoenzetter et al. (*A46*). Selectivity was greatly enhanced using an immunoaffinity extraction sorbent obtained by bonding antichlortoluron antibodies. Antibody cross-reactivity allowed extraction of a mixture of seven phenylurea herbicides with recovery of >75% for sample volumes of <10 mL. Quantification limits were as low as 10 ng/L in drinking water and seawater from 5-mL samples.

Milofsky (*A47*) investigated the parameters effecting the sensitivity and selectivity of a photochemical reaction detection scheme based on the reaction of 3-substituted pyrroles with singlet molecular oxygen ($^1O_2$) in HPLC. Polychlorinated biphenyls (PCBs) were chosen as model compounds for the detection scheme. Following separation by reversed-phase LC, PCBs are excited by a Hg pen-ray lamp in a crocheted PTFE photochemical reactor. Detection was based on the loss of pyrrole. The reaction was catalytic in nature since one analyte molecule may absorb light many times, producing large amounts of $^1O_2$. Detection limits for 4,4′-dichlorobiphenyl and Aroclors 1242, 1248, and 1254 were improved by 1−2 orders of magnitude over optimized UV absorbance detection.

Microchip Technology in LC. After a brief introduction to the area of miniaturized analytical separation systems, McEnery et al. (*A48*) detailed the fabrication of components for a miniaturized LC system. The microchannel walls were chemically modified with *n*-octyltriethoxysilane, and the reversed-phase retention of the drug caffeine was observed using off-chip injection (20 nL) and UV detection (3-nL flow cell). On-chip platinum electrodes were used for the amperometric detection of phenol. The latest results obtained with on-chip pressure-driven injection allowed for injections in the low-nanoliter to -picoliter range (*A49*).

Ujiie et al. (*A50*) detailed methodology to fabricate quartz microCE chips using plasma etching. Deep quartz etching technology was demonstrated by fabricating vertical trench features with 50 μm depth and 20 μm width, i.e., the aspect ratio of 2.5, which cannot be attained by the wet chemical etching technology conventionally used. No significant difference was found in the separation performances between dry-etched and wet-etched chips. Also, the advantage of pattern transfer with high resolution and high fidelity was demonstrated by fabricating functional microstructures such as a slit or a filter within a capillary. Samsonov (*A51*) proposed a novel way of fabricating miniaturized multicapillary columns for gas and liquid chromatographs or electrophoresis devices containing many thousands of identical channels with a width (or depth) of ∼1−30 μm by industrial technology for the production of optical plane reflecting diffraction gratings.

A microfabricated fluidic device that combines micellar electrokinetic chromatography and high-speed open-channel electrophoresis on a single structure for the rapid, automated two-dimensional analysis of peptides has been devised and demonstrated by the Ramsey group (*A52*). The microchip operated by rapidly sampling and analyzing effluent in the second dimension from the first dimension. Second-dimension analyses were performed and completed every few seconds, with total analysis times of less than 10 min for tryptic peptides. The peak capacity of the two-dimensional separations was estimated to be in the 500−1000 range. The two-dimensional separation strategy was found to greatly increase the resolving power over that obtained for either dimension alone. Ericson et al. (*A53*) showed that the application range of microchips can be extended to any mode of chromatography by filling the narrow channels with continuous polymer

beds, exemplified by electrochromatography and ion-exchange chromatography. Wall effects were eliminated by anchoring the bed to the wall of the channel, an arrangement that had the additional advantage that no frits to support the bed were required. A sample of uracil, phenol, and benzyl alcohol were separated by CEC in <20 s.

Automation. He et al. (*A54*) investigated the feasibility of a Culex automated blood-sampling system in conjunction with LC/MS/MS analysis for the quantification of ketoconazole in rat, after an oral dose of 10 mg/kg of the test article. Powell and Tempst (*A55*) described a microflow-based instrument, consisting of multiple rotary valves, capillary tubing, and miniaturized reaction vessels, for the purpose of performing automated chemical and biochemical reactions on a very small scale (i.e. submicroliter volumes). The novelty is that close to 100% of the reaction end products were available in a minimal volume (<5 μL) inside a pressurized microvial for subsequent analysis. This makes the system compatible with CLC and, in principle, with continuous-flow nanoESI-MS. Instrument performance was convincingly demonstrated by partially sequencing 100 fmol of an intact protein using classical Edman chemistry in combination with CLC.

Other Articles of Interest. Siouffi (*A56*) briefly reviewed all aspects of HPLC in relation to food analysis. Lee and Bowerbank (*A57*) reviewed fast separations in all forms of chromatography. In truly high-speed chromatography, temperature and composition gradient programming are not practical, primarily because of the relatively long times required for reequilibration. These and other requirements of fast separations lead to the strongest case for universal chromatography, where "universal" implies that the mobile phase can be in any form (i.e., gaseous, supercritical fluid, or liquid) at any point in the chromatography column and the instrumentation, including the column, can be used for all forms of column chromatography. Coupled, unified, and universal approaches to chromatography are reviewed, and universal instrumentation and column technology are described, particularly applicable to fast chromatography. A general overview illustrated with selected examples is given. Bartle et al. (*A58*) reviewed packed capillary LC as a unified chromatography with gas, supercritical, and liquid mobile phases. Also, a comparison of CEC and HPLC was made.

COLUMNS

Reviews. Majors (*B1*) examined the trends in column introductions at Pittcon 2000. He described HPLC columns and packings for reversed-, normal- and bonded-phase; ion-exchange; ion; size exclusion; and large- and preparative-scale chromatography. He also looked at specialty HPLC columns. Current trends in HPLC column development are toward robust columns that extend the usable pH range (*B2*). The life science arena with small sample amounts demands smaller inner diameter columns. Capillary LC instruments are now commercially available, and the presentation will shed some light on various capillary column applications. Comprehensive reviews of ion-exchange chromatography (IEC) and reversed-phase chromatography, including many aspects of instrumentation, were done by Roos (*B3*) and Schluter (*B4*), respectively. Zirconia composite materials have been shown to surpass standard silicon and carbon for stability and robustness in complicated reversed-phase column applications (*B5*).

Columns Materials, Effects, and Packing. Using optical on-column visualization for the study of the migration of sample bands, the radial variations of the local migration rate were studied in the region near the column wall (*B6*). Photographs of small sample bands migrating along the column at various radial locations were obtained. On-column chromatograms extracted from these photographs showed evidence of two wall effects including the classical wall effect that chromatographers have long discussed. Collins et al. (*B7*) investigated the effects of inner surface roughness of microbore column blanks on column performance. These results suggested that two categories of packed column structure relate to the surface features and yield high (75 000 plates/m)- and low (20 000 plates/m)-efficiency columns with 5-μm particles. Bed−wall friction in axially compressed packed chromatographic columns was studied by Cherrak and Guiochon (*B8*). The extent of the consolidation of the column beds, their permeabilities, and the friction shear stress of these beds against the column wall were determined, as well as the column efficiencies (for an unretained tracer). The bed−wall friction shear stress increases rapidly with increasing bed length and varies widely with the nature of the solvent used.

An improved and easy electrokinetic packing procedure was presented by Stol et al. (*B9*) for the production of stable capillary columns suitable for CEC. In pseudoelectrokinetic packing, a high electric field is used in conjunction with a hydrodynamic flow. The packing of silica-based reversed-phase columns can be achieved with basic, commercially available CE equipment in <15 min. The procedure is robust, and a high success rate is achieved. Columns are stable for at least 100 runs and were tested using mixtures of polycyclic aromatic hydrocarbons and positively charged drugs. Separations were performed in a relatively high conducting NH_4Ac buffer, with efficiencies of >283 000 plates/m. Chirica and Remcho (*B10*) developed a new design of immobilized particle separation media for CLC and CEC. A mixture of porogenic solvents and methacrylate-based monomers is pumped through a packed column to provide, following a polymerization step, an organic matrix capable of holding the sorbent particles in place, thus rendering the end frits unnecessary. The new columns demonstrated excellent chromatographic performance in both CEC (reduced plate height (h) = 1.1−1.5) and micro LC modes (h = 2.2−2.5), while minimizing secondary interactions encountered when silica-based entrapment matrixes are employed.

Supports. The effect of acid treatment on the trace metal content of chromatographic silica, as determined by bulk analysis, surface analysis, and chromatographic performance of bonded phases, was investigated by Barrett et al. (*B11*). Surface-specific analysis suggested that trace metals were removed more rapidly from the surface of the silica compared to the bulk matrix and that the acid treatments resulted in halide contamination of the silica surface. Evidence is presented to suggest that the bulk metal content of the silica is not representative of the concentration of metals at the chromatographic surface. The chromatographic studies showed that HF-treated silica gave substantially better performance toward weak bases than the HCl-treated silicas. Kirkland et al. (*B12*) discussed how very fast reversed-phase separations of biomacromolecules are performed using columns made with superficially porous silica microsphere column packings

(Poroshell). These column packings consist of ultrapure "bio-friendly" silica microspheres composed of solid cores and thin outer shells with uniform pores. The excellent kinetic properties of these new column packings allowed stable, high-resolution gradient chromatography of polypeptides, proteins, nucleic acids, DNA fragments, etc., in a fraction of the time required for conventional separations.

Hybrid organic−inorganic particle technology is breaking through traditional barriers of HPLC separations (*B13*). Hybrid organic−inorganic particles combine the best properties of silica—high efficiency and excellent mechanical strength—with the best properties of organic polymers—a wide pH stability range and reduced silanol effects. The authors describe reversed-phase HPLC packings based on hybrid particles and demonstrate the packing's retention and selectivity characteristics. They also showed that these media have long lifetimes at elevated temperature under conditions at which conventional bonded silica fails rapidly.

Stationary Phases. New developments in LC stationary phases were reviewed by Hanai (*B14*). The topics include electron microscopic analysis of the silica surface, STM analysis of the inside of silica particles, impurities of silica gels, measurement of surface activity of chemically modified silica gels, stability of chemically modified silica gels, development of polar bonded phases and of silica-based packing materials, spectroscopic analysis of the surface of bonded phases, and computational chemical analysis of bonded phases. Majors (*B15*) focused on new developments in stationary-phase LC techniques. Ultrafast reversed-phase LC separations are often needed for analyses related to combinatorial chemistry, studies in LC/MS, and other applications in which very rapid sample turnaround is paramount. Unfortunately, no consensus exists regarding the best column technology for optimally performing the desired rapid separations. Kirkland (*B16*) compares the advantages and limitations for columns of ultramicroporous, ultramicrononporous, and superficially porous particles and monolith structures for the very fast separation of solutes by reversed-phase HPLC. Data from literature and the author's laboratory were used to illustrate the strengths and limitations of the various approaches that can be used for ultrafast separations.

To reduce the influence of surface silanol groups on the retention of basic solutes, reversed phases (RP) with polar groups embedded in the anchored alkyl chain were introduced. Engelhardt and co-workers (*B17*) showed that this polar selectivity improved the separation of the dinitrophenylhydrazones of C3 aldehydes and ketones and resulted in better selectivity in the detection of flavonoids in wine. Different selectivities for classical RP columns and shielded phases with methanol and acetonitrile as organic modifiers are demonstrated. Chowdhury et al. (*B18*) showed that silica-supported comb-shaped polymer (Sil-ODA18) provided a specific selectivity toward aromatic and nonaromatic solutes. The retention versatility of this phase arises from the orientation change on transition from the ordered crystalline to the less ordered noncrystalline state. Selectivity in the crystalline and noncrystalline regions of Sil-ODA18 was similar to that of polymeric and monomeric ODS phases, respectively. The Sil-ODA18 column also exhibited the characteristic temperature dependency of liquid crystal phases, i.e., an increase in retention with increasing temperature on transition from crystalline to isotropic state.

Reversed-phase packing materials were prepared by Melo et al. (*B19*) from HPLC silica and from zirconized HPLC silica support particles having sorbed poly(methyloctylsiloxane) (PMOS) as the stationary phase. Portions of zirconized material were subjected to ionizing radiation. Columns prepared from these packing materials were subjected to 5000 column volumes each of neutral and alkaline (pH 10) mobile phases, with periodic tests to evaluate chromatographic performance. The PMOS stationary phase sorbed onto zirconized silica required an immobilization treatment (e.g., γ-irradiation) for long-term stability while prior surface zirconization of the silica support surface greatly improved the chromatographic stability of the stationary phase when using alkaline mobile phases were used. Porous ceria−zirconia spherules were surface modified by Hu et al. with alkylphosphonic acid to create reversed-phase material (*B20*). The chromatographic properties of the ceria−zirconia-based stationary phase were evaluated with neutral, basic, and acidic compounds as probes, over a wide range of mobile-phase composition and pH. Similarly, the effects in LC of a high pH in the mobile phase on poly(methyloctylsiloxane) immobilized by γ-radiation on titanium-grafted silica were noted (*B21*).

Okusa et al. (*B22*) developed a unique cyano-bonded column for HPLC by chemically bonding cyanopropyl groups to the silica gel support. Eight cyanopropyl-bonded silica gels were prepared under different conditions. These packing materials were packed into a stainless steel column, the chromatographic properties and durability of which were studied in both normal- and reversed-phase partition modes. The separating selectivity and the durability of cyanopropyl-bonded silica (CN) columns were dependent on the preparation conditions.

Porous graphitic carbon provides unique properties as a stationary-phase HPLC. Majors (*B23*) briefly reviewed the history of porous graphitic carbon development, the retention mechanism responsible for analyzing compounds of increasing polarity, and application areas in which the phase's unique properties have been used. A denatured avidin-bonded column was found to be suitable for use in the reversed-phase HPLC by Tanaka (*B24*). The retention property of the denatured avidin-bonded column was very nearly similar to a nondenatured avidin-bonded column already reported and, however, showed very high stability to organic solvent.

Kiseleva and Nesterenko (*B25*) investigated the chromatographic properties of a new stationary phase, phenylaminopropyl silica (PhA-silica), containing phenylaminopropyl residues covalently bonded to the silica surface. The effects of mobile-phase pH, ionic strength, and the nature and concentration of organic modifier on the retention of phenols on PhA-silica were studied under conditions of reversed-phase HPLC. The isocratic separation of phenol, and its nine methyl-, chloro-, and nitro-substituted derivatives, was achieved on a 150 × 4.6 mm i.d. chromatographic column packed with 7-μm particles of PhA-silica. Ryoo et al. (*B26*) prepared a bonded silica stationary phase (SP1) by connecting *N*-(3,5-dinitrobenzoyl) aminoundecylsilane to silica gel. The stationary phase was applied in resolving a liquid crystal mixture with a reversed-phase HPLC mode, and the chromatographic resolution results were compared with those on an ODS column.

The authors found the new stationary phase was better than the ODS column in resolving liquid crystal mixtures and the elution orders of some liquid crystals were changed.

Ion-Exchange Phases. The inability to separate fluoride, phosphate, and sulfate by electrostatic ion chromatography (EIC) was overcome by using an ODS SiO_2 column coated with mixed zwitterionic−cationic surfactants as the stationary phase (*B27*). The best results were obtained using the zwitterionic surfactant, 3-(*N*,*N*-dimethylmyristylammonium)propanesulfonate, and the cationic surfactant, myristyltrimethylammonium, in a 10:1 molar ratio in the column coating solution. With a dilute solution of sodium tetraborate as the eluent, the model analyte anions were completely separated in the following elution order: F^-, HPO_4^{2-}, SO_4^{2-}, Cl^-, NO_2^-, Br^-, NO_3^-. The very early elution of phosphate and sulfate is most unusual and is unique to this system. Detection limits better than 1.1×10^{-4}mM and linear calibration plots up to 7.0 mM were obtained with a suppressed conductivity system. Yu et al. (*B28*) used a column packed with titania stationary phase for the separation of ionic solutes. The retention behaviors of anions, cations, and benzoic carboxylic acids were investigated using various carboxylic acids as the eluents. The titania column exhibited significant ion-exchange and ligand-exchange behavior, which depended strongly on eluent pH and types of eluents. Separation of anions and cations, and simultaneous separation of anions and cations, could be obtained by careful selection of the conditions of the eluent.

Monolithic Columns. Until recently, HPLC columns have been made of particulate materials packed tightly into an HPLC steel column. This approach creates a significant obstacle to the flow of the solvent/sample mixture. To resolve these limitations, a sorbent material consisting of monolithic rods of highly porous metal-free silica has been developed. With no particles to become dislodged and moved around, less peak distortion and longer column life become a reality. Monolithic columns have been reviewed (*B29*), including the performances of Chromolith monolithic silica columns (Chromolith RP18e and Chromolith RP18e SpeedROD) (*B30*). Monolith columns are generally fabricated by polymerization of monomers within a column, but Regnier (*B31*) described an alternative strategy in which the bed is microfabricated in an inorganic material by ablation. Using this approach, chromatographic beds were constructed in which cubic support structures were created and arranged in rows to mimic particles in a conventional column. Podgornik et al. (*B32*) researched the development of large-volume (e.g., 80 mL) monolith columns.

Guiochon and Kele (*B33*) studied the column-to-column and batch-to-batch reproducibility of several commercial brands of silica-based C18 stationary phases for RPLC. The data characterizing the retention, the hydrophobic and steric selectivity, the column efficiency, the peak symmetry, and the hydrogen-bonding capacity were reported for a group of 30 neutral, acidic, and basic compounds selected as probes. The reproducibility of series of conventional packed columns (Symmetry from Waters, Kromasil from Eka Chems., Vydac from The Separation Group, Luna from Phenomenex, and HyPURITY Elite from Hypersil) and of a monolithic column (Chromolith Performance RP18e silica rod, from Merck) was compared.

Tanaka et al. (*B34*) evaluated two types of monolithic silica columns derivatized to form an ODS phase, one prepared in a fused-silica capillary (SR-FS) and the other prepared in a mold and clad with an engineering plastic (poly-ether-ether-ketone) (SR-PEEK). The evaluation of SR-FS columns in a CEC mode showed much higher efficiency than in a pressure-driven mode. Jiang et al. (*B35*) prepared and characterized monolith polymer columns for CEC, and Ishizuka and co-workers (*B36*) studied the performance of a monolithic silica capillary column under pressure-driven and electrodriven conditions. The performance of the continuous silica capillary column in the electrodriven mode was much better than that in the pressure-driven mode.

Molecular Imprinted Polymer (MIP) Phases. Molecular imprinted polymers, which offer improved selectivity for a particular analyte or group of analogues, are becoming more popular as selective sorbents and stationary phases. Andersson (*B37*) reviewed developments and applications in the analytical chemistry arena. The improved selectivity of imprinted polymers compared with conventional sorbents may lead to cleaner chromatographic traces in the subsequent analytical separations. Furthermore, the solid-phase extraction application does not suffer from drawbacks generally associated with imprinted polymers in chromatography, such as peak broadening and tailing. The work done during the past decade in order to adapt MIPs to the capillary format and subsequently use these highly selective matrixes for CEC is reviewed by Schweitz et al. (*B38*). In a review by Wilson (*B39*), the focus is on the preparation of these polymers and their use for chiral separations and as novel sorbents for sample preparations.

MIPs for sulfonamides (*B40*), *p*-hydroxybenzoic acid and analogues (*B41*), the herbicide 2,4,5-trichlorophenoxyacetic acid (*B42*), and cortisol (*B43*) were all prepared. In each application, the mechanism of molecular imprinting was confirmed and a good selectivity for analyte(s) of interest was found.

Tunable Stationary Phases. The separation selectivity of temperature-responsive poly(*N*-isopropylacrylamide)-modified silica as a packing material for HPLC was studied with steroids, alkaloids, and substituted anilines as solutes by Song et al. (*B44*). The elution profiles of the solutes depended on the temperature of the column and the methanol content of the mobile phase, indicating that the separation selectivity could be controlled by the column temperature or the mobile-phase composition. As noted by the Porter group (*B45*), electrochemically modulated liquid chromatography (EMLC) provides a novel pathway to manipulate the efficiency of analytical separations. The strength of this technique is derived from electrochemically induced changes in the effective composition of a conductive stationary phase that acts as the working electrode in an electrochemical cell. Using an EMLC column, applications of applied potentials (E_{appl}) negative of the potential of zero charge (pzc) generate an excess negative surface charge on the electrode. In contrast, at values of E_{appl} positive of the pzc, the surface of the electrode will have an excess positive charge. In addition to general column design, mechanistic aspects of the separations are discussed. EMLC was coupled to ESI-MS (*B46*).This combination takes advantage of the ability of EMLC to manipulate retention and enhance separation efficiency solely through changes in the potential applied to a conductive stationary phase, thereby minimizing complications because of possible changes in analyte ionization efficiencies when gradient elution techniques were used.

Three demonstrative examples of the utility of EMLC-MS are presented. Porter (*B47*) has also used this approach to study the influence of applied potential on the adsorption of organic compounds on glassy carbon (GC) electrodes.

Chiral Phases. The history and discovery of enantioselective ligand-exchange chromatography (LEC) is briefly reviewed by Davankov (*B48*). Some of the most important results of studies into the structure of Cu(II) complexes with nitrogen-substituted α-amino acids are summarized, and the role of water molecules that are coordinated in two axial positions of these complexes in the mediation of between-ligand interactions is underlined. Perspectives on further development of enantioselective LEC are discussed. Svec et al. (*B49*) designed two combinatorial approaches and used them for the rapid development of selectors suitable for brush-type chiral stationary phases (CSPs) based on porous polymer platform. First, a mixed library of enantiopure chiral selectors was attached to polymer beads, packed into a column, and tested. Deconvolution of the best selector required the preparation of a small number of additional columns with rapidly decreasing number of attached selectors. Second, libraries of novel racemic selectors were prepared and evaluated using the reciprocal technique. Further enhancement of the recognition capability was achieved using aliphatic dendritic linkers. Both the convergent and divergent approach on beads as well as direct polymerization of chiral monomers led to CSP with a high selectivity.

Unique stationary phases of ODS coated with acylcarnitines were developed for LC columns. Kamimori and Konishi (*B50*) labeled these column as having "intelligent" ligands. The chiral ODS column coated with an enantiomer of stearoylcarnitine, L-stearoylcarnitine (l-CN-18 column), could achieve direct enantiomeric separation of DL-tryptophan, α-methyl-DL-tryptophan, and DL-3-indolelactic acid using 100% water as the mobile phase.

Characterization and Assessment. Shalliker et al. (*B51*) performed quantitative on-column visualization of solute migration in LC columns by a matched refraction index phase system in high-pressure glass columns. In this case, the mobile phase was CCl_4 and the stationary phase was a C18 SiO_2. Because the refractive indexes of the two phases were the same, the column bed, otherwise opaque, was transparent to the eye. Zones of colored solutes (e.g., I_2) were injected and their migrations studied along the column. As an example of applications for this technique, the authors show the sample entry through various inlet fittings. Importantly, the frit porosity should be matched to the particle size of the packing. The efficiency of a compressible, packed chromatographic bed was characterized experimentally and by computer simulations by Ostergren and Tragardh (*B52*). The experimental measurements were performed in situ by monitoring the propagation of a tracer using three sets of concentrically located electrodes. The results obtained were compared with results obtained for the same column packed with rigid, nonporous glass beads. The hydrodynamic dispersion in the compressed column was found to be approximately twice that expected for a column packed with rigid particles under the same conditions.

Claessens (*B53*) reviewed the state of the art and progress in the characterization of RP-LC columns. Existing column tests were compared with tests under development. Included in the review are the validity of several test parameters (e.g., hydrophobicity and silanol activity), solvent and eluent properties in respect to retention and selectivity, and developments to improve the chemical stability of RPLC phases, and thus column lifetime is reviewed. In a study by Felinger et al. (*B54*), principal component analysis was used to identify the parameters that influence the column-to-column and batch-to-batch reproducibility of retention times and retention factors measured on Symmetry C18, Kromasil C18, Luna C18 (2), and Vydac RP C18, all reversed-phase silica columns. Principal component analysis of the retention times confirmed that the column-to-column variations of the column volume and the total porosity of the bed are the factors that influence the reproducibility of the retention times, the column volume being the major factor. All the C18 columns investigated proved to behave in a very similar fashion. The influence of the chemical structure of porous polymers on the chromatographic properties of HPLC columns was studied by Gawdzik (*B55*). Using the alkyl aryl ketone scale, the retention indexes of five homologous series (alkylbenzenes, alkyl aryl ketones, *N*-alkylanilines, alkyl aryl ethers, alkylbenzoates) and column test compounds (toluene, nitrobenzene, *p*-cresol, 2-phenylethanol, *N*-methylaniline) were calculated. Their values were used for comparison of the selectivities of the studied polymeric packings.

O'Gara et al. (*B56*) investigated the dependence of cyano-bonded phase stability on ligand structure and solution pH. They determined that a trifunctional and a sterically protected CN phase was notably stable under acidic test conditions but had poor stability under basic conditions. Conversely, chain extension afforded poor stability under acidic conditions but did afford improved stability at higher pH. In total, the data indicate that good CN column stability can be achieved by using a trifunctional or a sterically protected phase in acidic mobile phases. However, as mobile phases of intermediate or higher pH are employed, shorter column lifetimes can be expected due to an accelerated dissolution of the underlying silica substrate.

Other Articles of Interest. Majors (*B57*) offered up an extensive glossary of definitions and terms used in the liquid-phase separation techniques of HPLC, CE, and CEC. The glossary should be useful to those just starting to use these separation techniques and can serve as a refresher for longtime users. To help with the incredible challenge facing chromatographers, Brady et al. (*B58*) discussed the daunting task of finding the column that will solve their most difficult analytical challenges. Discussion topics include the following: finding the right chromatography column, the universal column, comparison of chromatography columns, the starting point for chromatographic research, and one source for all chromatography needs are discussed. Along the same lines, Dolan (*B59*) discussed approaches on selecting column conditions to increase resolution or to improve sample throughput.

An in-line stripper column packed with iminodiacetate chelation resin was placed between the pump and injection valve and shown to remove metallic impurities from an HPLC system (*B60*). Although metal-free pumping systems contribute significantly lower metallic impurities than stainless steel systems, metal is nevertheless present in the mobile phase and the chelating stripper columns were found useful in protecting the analytical columns from contamination. The stainless steel frits were not found to be significant contributors to the contamination.

ELEMENTAL DETECTORS

The majority of LC techniques have been coupled to an elemental detection system, and elemental speciation of environmental and biological samples using LC continues to increase. There were very few novel developments in instrumentation, with the only significant trend of note being that toward miniaturization. Hywel et al. (*C1*) presented an extensive review with over 700 references of atomic spectrometry, which focused on advances in atomic emission, absorption, and fluorescence spectrometry and related techniques. They note a marked decrease in the number of papers describing chemometric procedures, speciation using GC, and solid sampling using arcs and sparks. Publications describing applications involving some form of sample pretreatment, either on-line or batch, such as matrix removal, preconcentration, or vapor generation, was similar to previous years. Sanz-Medel (*C2*) summarized analytical atomic spectroscopy as having a bright future due to more ambitious goals, more powerful elemental detectors (based on both photon and ion measurements), more and more flexible hybrid techniques, and more active cross-fertilization with other fields of science.

Atomic Absorption/Emission. Krachler and Emons (*C3*) studied the potential of HPLC coupled to a hydride generation system connected to an atomic absorption spectrometer (HPLC−HG-AAS) as an element-specific detector for the speciation of Sb(III), Sb(V), and trimethylantimony dichloride ($TMSbCl_2$). Among the five anion-exchange columns tested, the Dionex AS14 provided the best results for the separation of Sb(V) and Sb(III) with 1.25 mM EDTA at pH 4.7. Calibration curves were linear between 2 and at least 100 mg/L, and detection limits of 0.4, 0.7, 1.0 mg/L for $TMSbCl_2$, Sb(III), and Sb(V), respectively, were obtained. An evaluation of the particle beam-hollow cathode glow discharge atomic emission spectroscopy (PB-HC-AES) system as an element-specific detector for LC was described for aromatic amino acids, organomercury, and organolead compounds by Dempster and Marcus (*C4*). A high-efficiency thermoconcentric nebulizer was used to introduce analyte particles into a heated hollow cathode glow discharge source for subsequent vaporization, atomization, and excitation. Emission responses for hydrogen and nitrogen of amino acids in flow injection mode using 200-μL injection volumes indicate detection limits of 0.13 and 3.6 ppm for elemental H and N, respectively, with RSDs of <10% for triplicate injections over a concentration range of 10^{-3}−10^{-2} M. Arsenic speciation was performed by using coupled techniques with anion- and cation-exchange columns and atomic fluorescence detection, with UV irradiation and hydride generation as derivatization steps (*C5*). The coupling design is described in detail.

Inductively Coupled Plasma (ICP)-Mass Spectrometry. Inductively coupled plasma-MS has made significant progress since its genesis in the late 1990s. Xu and Wang (*C6*) give an extensive overview on all aspects of this technique and its application in the life sciences. The authors emphasize the use of ICPMS in combination with laser ablation (LA), CE, and HPLC. In addition to a comprehensive review of fruitful applications, this paper gives a systematic overview on the use of this technique in new devices and technologies related to plasma source, sample-introducing devices, and detecting spectrometers. Szpunar et al. (*C7*) discussed recent advances in the coupling of GC and HPLC with ICPMS and their role in trace element speciation analysis of environmental materials. Particular attention is paid to the problem of signal identification in ICPMS chromatograms and the potential of ESI-MS/MS for this purpose is highlighted.

HPLC-isotope dilution (ID)-ICPMS provides the coupling of two established instrumental techniques to achieve accurate and precise measurements. The approach, which offers considerable potential for speciation studies, was reviewed by Hill et al. (*C8*). ID on its own is regarded as being a definitive technique that overcomes many problems associated with instrumental drift and incomplete extraction of analytes from samples, and these advantages are also inherent in HPLC−ID-ICPMS. Importantly, the technique requires isotopically pure standards and cannot overcome problems associated with speciation changes and contamination.

Chang and Jiang (*C9*) described an ICPMS equipped with a dynamic reaction cell (DRC) that was used as an LC detector for the determination of Cr species. The repeatability of the peak area was <2% at m/z 52. The limits of detection for Cr(III) and Cr(VI) were 0.063 and 0.061 ng/L Cr at m/z 52, respectively. The use of HPLC coupled to ICPMS and orthogonal acceleration time-of-flight (oa-TOF) for the profiling, identification, and quantification of metabolites in rat urine following the administration of 2-bromo-4-trifluoromethylacetanilide was described by Nicholson et al. (*C10*). Dundar et al. (*C11*) described a study into the presence of artifacts associated with the column properties on metal speciation in chromatography systems and identified possible limitations of columns used. The study illustrated that samples may interact with the residual silanol groups and as a result produce artifacts of the chromatographic retention mechanism, peak tailing and loss of chromatographic resolution.

A simple, relatively low-cost interface for HPLC−ICPMS detection was studied by Acon et al. (*C12*). The interface consisted of a one-piece micronebulizer (direct injection high-efficiency nebulizer, DIHEN), positioned in the ICP torch for the direct nebulization of solution into the base of the argon plasma. Absolute detection limits were in the low-picogram to subpicogram range. Importantly, no plasma instability or carbon deposition on the nebulizer tip was observed using organic modifiers in the mobile phase of up to 20%. Ackley and co-workers (*C13*) compared a glass concentric nebulizer, a microconcentric nebulizer (MCN), and a Micro Mist nebulizer for the introduction of mobile phases containing varying concentrations of methanol into microbore LC−ICPMS. At a flow rate of 0.2 mL/min, the absolute detection limits for the MCN were slightly higher than those obtained with the Micro Mist nebulizer or the glass concentric nebulizer. Precision and linearity of the calibration curves were comparable for all three nebulizers.

Microwave-Induced Plasma. Microwave plasma torch (MPT), as a relatively new source, is gaining in popularity in atomic analytical spectrometry. In a review by Yang et al. (*C14*), the fundamental features and characteristics of the MPT are summarized and compared with other kinds of analytical atomic sources (e.g., ICP, DCP, MIP, etc.). The advantages of MPT have accelerated its interfacing with GC, LC, and SFC. With the goal of atomic emission detection of LC analytes, a tubular poly(tetrafluoroethylene) membrane desolvator interface was used to enhance the performance of a 120-W helium microwave-induced plasma atomic emission detector. Membrane desolvator conditions

such as temperature, solvent composition, and countercurrent gas flow were studied and optimized by Das and Carnahan (*C15*). Chatrerjee et al. (*C16*) et al. successfully coupled a high-power N_2-MIP-MS with a hydride generation system that was attached to a PRP-X100 anion-exchange column for the determination of selenite and selenomethionine (Semet) in urine. The detection limits of selenous acid and Semet obtained with the optimized HPLC−HG-N_2-MIP-MS system were 0.73 and 8.7 mg/L, respectively, with a repeatability of 2−5% RSD.

As with ICP, MIP can be coupled with MS, and Kwon and Moini (*C17*) have used a dual oscillating capillary nebulizer (OCN) in conjunction with an atmospheric pressure microwave-induced plasma ionization (AP-MIPI) source for the analysis of underivatized amino acid mixtures. By using water/acetonitrile containing 0.1% nonafluoropentanoic acid as the HPLC mobile phase and a C18 column, all common amino acids were separated and detected.

OPTICAL DETECTORS

Tran (*D1*) reviewed the acoustooptic tunable filter (AOTF) as a compact, all-solid-state electronic monochromator. Compared to conventional grating monochromators, the AOTF has no moving parts, wider spectral tuning range (from UV through visible and near-IR to IR), higher throughput, higher resolution, faster scanning speed, and imaging capability. These features make it possible to use the filter to develop novel instruments that are not possible otherwise. The instrumentation development and unique advantages of such AOTF-based instruments including a multidimensional fluorometer, detectors for HPLC and flow injection analysis, and a multispectral imaging instrument are described.

UV/Visible. Wang et al. (*D2*) designed a remote flow cell based on a single strand of fused-silica fiber optic that was built for UV absorbance detection with a packed capillary HPLC system, using commercially available pumps, detection electronics, and fittings. This "off-column" flow cell design is applicable to both pressure and electroosmotically driven systems. The illuminated volume of ~3 nL was optimized for capillaries with inner diameters in the range of 50−100 μm and flow rates from 100 nL/min to 1 μL/min. A linear dynamic range of 10^3 (reserpine, $\lambda = 220$ nm) and a concentration detection limit of 5.1×10^{-8} mol/L were observed. Baseline noise was measured at 3.5×10^{-5} absorbance units (AU), with a standard deviation of 1.7×10^{-5} AU. Hibbert et al. (*D3*) investigated the propagation of uncertainty in peak area or peak height in HPLC with UV/visible detection, as derived in terms of injection volume, flow rate, injected concentration, retention volume, and temperature.

IR/Raman. The compatibility of ion-pair reversed-phase LC and surface-enhanced resonance Raman spectroscopy (SERRS) for the separation and identification of anionic dyes was investigated by Seifar and co-workers (*D4*). They emphasized the at-line coupling via a thin-layer chromatography (TLC) plate. At-line coupling of LC and SERRS was successfully achieved when silica, but not aluminum oxide, plates were used. The application of a gradient, a high water content, and the presence of ion-pair reagents needed for the separation did not adversely affect the deposition and the recording of SERR spectra. The identification limits were 10−20 ng of deposited material, depending on the dye selected. A distributed feedback quantum cascade laser was applied for the first time as a powerful light source for mid-IR (MIR) detection in LC by Edelmann et al. (*D5*). Fructose and glucose in red wine were separated with an isocratic HPLC system, which was connected to a custom-made flow cell. This flow cell was constructed of two diamond windows with adjustable spacing and two hollow waveguides for guiding the incoming and outgoing light. Since the emission of the laser at 1067 cm^{-1} matches the absorption maximum of fructose and glucose and the mobile phase does not, group-specific detection of carbohydrates was achieved.

Optical Activity. In a review by Edkins (*D6*), he notes that combinations of polarimetry, CD, and LC are replacing UV for effective detection and isolation of enantiomers. The use of hyphenated chromatographic instrumentation is expected to grow rapidly in the next few years. Driffield et al. (*D7*) extended the application of optical rotation detection in HPLC to areas that were previously difficult by use of an instrument developed to enable simultaneous monitoring of optical rotation (OR) and transmittance in the presence of high levels of absorbance, scattering, or other effects that change the intensity of the plane-polarized light at the photodiode detector. Examples include the analytical-scale separation of fructose and sucrose and the semipreparative separation of enantiomers of warfarin and Trogers base. A signal-to-noise improvement of up to 150% is found when signals with and without correction for transmittance changes are compared. A novel multibeam polarized photometric detector (PPD) for LC was described by Yamamoto et al. (*D8*). This detector was designed to measure the optical rotation of an analyte at its absorption band. Application of the proposed technique to an analyte carrying the Cotton absorption band provided good results. Hadley and Jonas (*D9*) evaluated the Jasco CD-995 in conjunction with achiral HPLC for the simultaneous determination of chemical and enantiomeric purity. This instrument is designed for the on-line monitoring of circular dichroism at a fixed wavelength as opposed to optical rotation.

Evaporative Light Scattering. The desire for sensitive detectors for nonchromophoric compounds is reflected in the growing number of articles citing evaporative light scattering detectors (ELSD) for LC. This review will focus on instrumentation and those applications that highlight novel aspects of ELSD. Kuch and Saari-Nordhaus (*D10*) describe a dual-mode ELSD that optimized performance for a wide range of applications. The impactor "off" mode provided the best sensitivity with nonvolatile analytes, organic mobile phases, or aqueous mobile phases at low flow rates. The impactor "on" mode provided optimum sensitivity and baseline stability for semivolatile compounds or with highly aqueous mobile phases at high flow rates, including steep or high-speed gradients. By adding triethylamine and an equimolar amount of formic acid, Deschamps and co-workers (*D11*) were able to enhance the response of ELSD for various classes of lipids. The generality of this phenomenon was demonstrated for different chromatographic techniques and various classes of lipids: for nonaqueous LC with a porous graphitic carbon packing for wax esters, fatty acid Me esters, and ceramides; for normal-phase LC with a PVA-Sil stationary phase for cerebrosides, digalactosyldiacylglycerols, and phospholipids; and for subcritical chromatography with an octadecyl grafted silica column for ceramides.

ELSD can be coupled with microcolumn chromatography to give better sensitivity (i.e., 0.5 ng of amino acid being detectable on-column with a 0.3-mm-i.d. column under gradient conditions) and improved linearity (i.e., 0.5−500 ng on-column; $R^2 = 0.98$ or better) compared with conventional chromatography (*D12*). Moucher et al. (*D13*) successfully used ESLD for the determination of inorganic cations (e.g., Na^+, K^+, Mg^{2+}, and Ca^{2+}) following ion-exchange chromatography, and Fang and co-workers (*D14*) used HPLC−UV-ELSD for combinatorial library quantitation.

Refractive Index. Westerbuhr and Rowlen (*D15*) described a universal detector for on-column analysis in flash chromatography. The detection scheme takes advantage of refractive index changes as analytes move through an illuminated region of the column. The column packing material is a diffuse scattering medium when the refractive index of the solvent is significantly different from that of the packing material. The magnitude of the "signal" depends on the degree to which the refractive index mismatch is changed.

LUMINESCENT DETECTORS

Fluorescent and luminescent techniques are especially attractive as the trend toward miniaturization continues. The literature is replete with articles describing preinjection or postcolumn derivatization reagents, procedures, and techniques in an effort to exploit the inherent advantages of high sensitivity and selectivity. Although these articles are not reviewed here, the following reviews are highly recommended. Oldham et al. (*E1*) reviewed in-depth molecular fluorescence, phosphorescence, and chemiluminescence spectrometry. The review contents are books, reviews, and chapters of general interest; general instrumentation; laser-based techniques; fiber-optic-based fluorescence; sensors; sample preparation, quenching, and related phenomena; data reduction; luminescence in organized media; low-temperature luminescence; total luminescence and synchronous excitation spectroscopies, and related techniques; solid surface luminescence; luminescence in chromatography, electrophoresis, and flow systems; dynamic measurements of luminescence; fluorescence polarization, molecular dynamics, and related phenomena; chemiluminescence; near-IR fluorescence; luminescence techniques in biological and clinical analysis; reagents and probes; and other techniques and applications. Roth and Uebelhart (*E2*) narrowed their review to LC with fluorescence detection in the analysis of biological fluids.

Fluorescence/Phosphorescence. Fluorescence line-narrowing detection in chromatography and electrophoresis was extensively reviewed by Jankowiak et al. (*E3*). They described the basic aspects fluorescence line-narrowing spectroscopy (FLNS) and its coupling to a variety of separation techniques for high-resolution, low-temperature spectroscopic identification. Interfacing with HPLC was only conjectured in the previously cited Janowiak article, until the same research group (*E4*) showed that HPLC can be interfaced with FLNS for the on-line identification and characterization of analytes. Interfacing centered primarily on the design and construction of a novel liquid helium cryostat that accommodates variable-sized quartz tubes/capillaries suitable for HPLC as well as CE and CEC. The proof of principle for the HPLC−FLNS system was first demonstrated with a mixture of four structurally similar PAHs and then applied to the analysis of DNA adducts from mouse skin exposed to the carcinogen dibenzo[*a*,*l*]pyrene. Femtomole detection limits were achieved.

Setford and Saini (*E5*, *E6*) described a three-dimensional spectrofluorometer incorporating charge-coupled-device (CCD) detection for chromatographic applications. This article described a detector for LC that provides three-dimensional, excitation−emission intensity data of fluorescent compounds. The extreme sensitivity of CCD detection coupled to a dual polychromator arrangement made possible the collection of fluorescence excitation−emission intensity data of polycyclic aromatic hydrocarbons in a flow stream in <0.05 s with nanogram per milliliter limits of detection.

Chemiluminescence/Bioluminescence. Chemiluminescence detection in the liquid phase was reviewed by Garcia-Campana et al. (*E7*). This article focused on several chemiluminescence-based reactions that are applicable to the determination of various biopharmaceutically important analytes, and they can be applied for monitoring chemiluminescence emission using flow injection, LC, and CE analysis, as well as for the development of chemiluminescence-based sensors or in immunoassays. Roda et al. (*E8*) extended their review to both bio- and chemiluminescence techniques in bioanalysis.

Petritis et al. (*E9*) demonstrated that ion-pair reversed-phase HPLC coupled with a chemiluminescent nitrogen detector (CLND) could be used to analyze underivatized amino acids. The linearity and equimolarity of this mass-dependent detector was confirmed. Amino acids can be detected in nutritional serum, tobacco extract and wine with a single calibration curve. Detection limits varied from 0.0025 to 0.0075 mM (0.33−0.86 mg/L) depending on the amino acid and its retention time.

ELECTROCHEMICAL DETECTORS

Reviews. Electrochemical detectors for LC were reviewed by Erickson (*F1*). She noted that bioanalytical applications were keeping a mature market strong. From an historical and methodological viewpoint, Budnikov and Kazakov (*F2*) reviewed electroanalytical instrumentation produced by Bioanalytical Systems Inc.. The most up-to-date instrumentation designed from technical innovations was developed for the use in medicine and environmental analysis. Attention was given to polarographs and peripheral devices. Yashin (*F3*) discussed novel applications of amperometric detection in LC, and LaCourse (*F4*) focused on the use of electrochemical detectors for functional group analysis. He also discussed the attributes of electrochemical detectors that make them suitable for consideration for inclusion in extraterrestrial exploration vehicles. In keeping with the trend of bioanalytical applications as a driving force behind electroanalytical research, Takenaka (*F5*) reviewed electrochemical approaches, including LC-EC, for gene analysis using redox-active probes.

Instrumentation. Chao and Huang (*F6*) developed a bipotentiostat based on a four-electrode detection system for HPLC analysis. An amperometric chromatogram and two three-dimensional chromatovoltammograms characterizing the electrochemical characteristics of analytes could be obtained in a single chromatographic run. The detection limits (based on $S/N = 3$) found were $\sim 1.0 \times 10^{-7}$ M for hydroquinone and catechol and 1.0×10^{-6} M for ascorbic acid. Cvacka and co-workers (*F7*) constructed an amperometric detector based on a platinum tubular

electrode that was made from a long narrow-bored platinum tube, which was directly connected to the column outlet. The tube is immersed in a vessel containing an electrolyte solution in which a common reference and counter electrodes were also immersed. Only the internal platinum wall is exposed to the electrolyte solution. The detector was tested in both conventional and micro-HPLC systems. The peak parameters obtained with the test detector are comparable with those obtained with a standard spectrophotometric detector for 1-aminonaphthalene.

Potentiometry. Poels and Nagels (*F8*) used a macrocycle-based liquid membrane, solid-sate electrode to potentiometrically detect amines following ion chromatography (IC). Detection limits of the order of 10^{-6} M (injected concentration) were measured for mono- and diamines for the macrocycle-based electrodes. Lee et al. (*F9*) used solid-state cation-selective electrodes (SSEs) for use as a detector in single-column IC. An SSE-based detector provided not only the overall chromatogram for the separated ion species (monensin methyl ester-nonactin-based membrane) but also the enhanced chromatogram for specified ions of interest (valinomycin for K^+ and nonactin for NH_4^+). This feature made it possible to perform highly quantitative analyses with low detection limits even if the separation efficiency of the ion exchange was not sufficient.

Novel Material/Modified Electrodes. Work continues on the development of chemically modified electrodes to be used in LC-EC. Pei and Li (*F10*) focused on xanthine oxidase immobilized on the surface of a $CuPtCl_6$/glassy carbon chemically modified electrode. The enzyme electrode displayed a quick and sensitive response to xanthine and hypoxanthine. The enzyme electrode was characterized as an amperometric sensor for xanthine (6×10^{-7}–2×10^{-4} M) and hypoxanthine (5×10^{-7}–2×10^{-4} M) with the detection limit of 1×10^{-7} M. Staes and Nagels (*F11*) were able to amperometrically detect electroinactive sulfonic acids, organic acids, and phosphate esters following their chromatographic separation. The working electrode consisted of a 3-mm-diameter glassy carbon electrode, coated with electrochemically deposited polypyrrole. Detection limits for sulfonic acids in LC with 4.6-mm-i.d. columns at a flow rate of 1 mL/min were 3 ng. The electrode had a linear response in the 1×10^{-6}–1×10^{-3} M concentration range.

Conductive B-doped diamond film electrodes were examined by Rao et al. (*F12*) for the electroanalysis of three sulfa drugs (i.e., sulfa drugs, sulfadiazine, sulfamerazine, and sulfamethazine). Diamond exhibited a highly reproducible amperometric response, with a peak variation of <5%, even at a concentration of 100 nM. A detection limit of 50 nM and a linear dynamic range of 3 orders of magnitude were obtained. No fouling of the electrode was observed within the experimental period of several hours. Sato and co-workers (*F13*) used a nickel–titanium alloy electrode as an electrochemical detector for the analysis of underivatized amino acids in flow systems. In strong alkaline solutions, an oxide film on the Ni–Ti alloy electrode surface exhibited a high catalytic activity toward the oxidation of amino acids. Consequently, the Ni–Ti alloy electrode exhibited an excellent stability for constant-potential amperometric detection of amino acids in flow systems. For example, the RSD for the repetitive 100 injections of 50 μM (1.2 nmol) glycine over 10 h was <1%. Detection limits were obtained in a range from 0.9 pmol for arginine to 90.2 pmol for leucine and isoleucine. Casella et al. (*F14*) performed amperometric detection of underivatized amino acids at a nickel-modified gold electrode following anion-exchange chromatography. The electrodeposition of nickel oxyhydroxide films was obtained by cycling a gold electrode between 0.0 and +1.0 V versus a SCE in a 80 μM Ni^{2+} solution buffered at pH 10 with $NaHCO_3/Na_2CO_3$. The resulting Au–Ni composite electrode exhibited good stability in alkaline medium and can be used as an amperometric sensor of underivatized amino acids at a fixed applied potential (+0.55 V vs Ag/AgCl). The detection limits (S/N = 3) for all studied compounds ranged between 5 and 30 pmol injected, while the linear ranges spanned over 2 or 3 orders of magnitude.

Array Electrodes. Interdigitated array (IDA) electrodes often exhibit higher sensitivities for analytes in LC-EC. Kurita et al. (*F15*) described the fabrication, evaluation, and application of an IDA electrode for a microfabricated wall-jet cell. The IDA electrode with eight pairs of microbands was fabricated by photolithography, sputter deposition, and the lift-off technique on a narrow glass plate. The active cell had a volume of 0.44 nL. The anodic current of the redox species reached its steady state value in 14 s because of the small cell volume even when the solution was injected into the flow cell at a flow rate of 20 nL/min. Their results clearly indicated that the IDA electrode is suitable as a detector for small-volume analyses such as microcolumn LC and CE. Bjorefors et al. (*F16*) exploited redox cycling using IDA microelectrodes to enhance sensitivity, and Senior et al. (*F17*) evaluated IDA microelectrodes for the detection of low levels of biogenic amines. They evaluated this technology as applied to HPLC with EC for the analysis of microdialyzates and tissue samples. Dopamine and serotonin were detected to a levels of 53×10^{-18} and 26×10^{-18} moles on column, which was ~10-fold better than existing technology.

Pulsed and Oscillometric Techniques. The Johnson group (*F18*) examined integrated square-wave electrochemical detection (ED) at Au electrodes for the determination of biogenic amines in soybeans seeds following their separation by LC. Estimated detection limits are significantly lower for cation-exchange LC-ED in comparison with LC with photometric detection. Detection of native amino acids and peptides was accomplished by Brazill et al. (*F19*) using sinusoidal voltammetry at a copper electrode in a flowing stream. The frequency spectrum due to the oxidation of each molecule has a unique "fingerprint" response resulting from the kinetics of oxidation at the electrode surface. Through examination of the frequency spectra, even structurally similar molecules can be easily distinguished from one another.

Indirect Electrochemical Detection Systems. Indirect amperometric detection of underivatized amino acids was developed by Sato et al. (*F20*) using a carbon film ring–disk electrode (CFBRDE) in microcolumn LC. Bromide present in the mobile phase could be efficiently oxidized to bromine at the upstream (disk) electrode and was subsequently detected at the downstream (ring) electrode. Most of the underivatized amino acids that are electroinactive under conventional amperometric conditions reacted rapidly with the electrogenerated bromine, and the concentration of amino acids can therefore be indirectly detected by continuously monitoring the reduction current of bromine. The detection limits for most of the amino acids were 0.2 pmol. Zhou et al. (*F21*) devised a similar system for peptides containing a

thiol or disulfide group. Osipova and co-workers (*F22*) demonstrated that Pb^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+}, Co^{2+}, and Cu^{2+} can be indirectly detected as their unithiol complexes by amperometric detection under static and HPLC conditions. The best sensitivity was attained for an amperometric detector with wall-jet flow cell and a graphite indicator electrode

Other Systems. The first hyphenation of HPLC, electrochemical on-line oxidation, and mass spectrometry was described by Diehl et al. (*F23*). Ferrocenecarboxylic acid esters of various alcohols and phenols were synthesized, separated by reversed-phase HPLC, and oxidized (ionized) coulometrically prior to single quadrupole MS analysis using ESI and atmospheric pressure chemical ionization (APCI) interfaces. Limits of detection for selected derivatives ranged from 4×10^{-9} to 4×10^{-7} mol/dm^3 depending on the individual compound and the selected interface.

MASS SPECTROMETRY DETECTORS

Reviews. Satisfactory performance of an LC/MS system in terms of resolution, precision, and reliability requires careful attention to several components of the HPLC system. Wher (*G1*) discusses how to configure HPLC systems for LC/MS in order to achieve maximal results. Slobodnik and Brinkman (*G2*) reviewed LC/MS interfacing systems (i.e., thermospray, particle beam, APCI, in-source collision-induced dissociation) in relation to environmental analysis. In a similar fashion, Van Bocxlaer et al. (*G3*) examined the area of LC/MS in regard to forensic toxicology. After a short introduction into LC/MS interfacing operational characteristics (or limitations), the review covered applications that range from illicit drugs to often abused prescription medicines and some natural poisons. The instrumentation involved in LC/MS/MS for the analysis of surface and wastewater was reviewed by Kienhuis and Geerdink (*G4*).

Electron capture (EC) is a sensitive and selective ionization technique for mass spectrometry, and Giese (*G5*) reviewed recent advances in this important area. Two applications were discussed in detail—bile acids and oxidized phenylalanine. EC-MS is well-established as a useful technique for trace analysis in special cases, and the scope of its usefulness is broadening to include qualitative analysis and detection of more polar and larger molecules, based on advances in both the chemical and instrumental aspects of this technique. Syage and Evans (*G6*) reviewed the advantages of photoionization mass spectrometry for high-throughput analysis of pharmaceutical samples, with emphasis on its capability to perform rapid screening of combinatorial chemistry libraries for drug discovery. The principal mechanism for photoionization (PI) of molecule M is photon absorption and electron ejection to form the molecular ion M^+. Compared with ESI and APCI, PI offers a broader range of ionizable compounds and is less susceptible to ion suppression effects. The PI-MS makes it possible to analyze mixtures without the use of LC to separate the components. Applications of PI-MS include chemical weapons detection and agrochemical analysis.

Time-of-Flight/MALDI. The attributes of TOFMS, such as unlimited mass range, high ion transmission efficiency, high duty cycle, and simplicity, fortunately complement the high-speed and sensitivity characteristics and qualify the TOFMS as one of the most powerful detectors for microcolumn separations. As a consequence, TOFMS is developing virtually exponentially compared to all other MS techniques. Jiang and Moini (*G7*) described the development of multi-ESI sprayer, multi-atmospheric-pressure-inlet MS and its application to accurate mass measurement using TOFMS. Using HPLC as a device for introduction of one liquid stream (sample) and a syringe pump as a device for introduction of the second liquid stream (i.e., reference standard), the accurate mass of a tryptic digest of cytochrome *c* was measured. The range of mass errors was from −6.1 to +3.6 ppm, a significant improvement over their previously reported mass accuracy for this digest using single-nozzle TOFMS. The results demonstrated that the dual-ESI sprayer, dual-inlet design provided reference peaks on every acquisition with minimum analyte−reference interaction and, therefore, higher consistent mass accuracy. Ekers et al. (*G8*) described the use of two separate electrosprays for introducing sample and reference for accurate mass LC/MS on an orthogonal acceleration TOFMS. This is carried out using an adaptation of the multiplexed electrospray ion source in which only two of the sprays were used

Chernushevich and co-workers (*G9*) reviewed quadrupole time-of-flight MS. The main features of reflecting TOF instruments with orthogonal injection of ions are discussed. Their operation and performance were compared with those of triple quadrupoles with ESI and matrix-assisted laser desorption/ionization (MALDI) TOF mass spectrometers. Basic algorithms for LC/MS/MS automation are discussed and illustrated by two applications. Miliotis et al. (*G10*) developed a piezoelectric flow-through microdispenser interfacing capillary LC with MALDI-TOFMS) for the identification of biomolecules. The MALDI target plate was placed on a computer-controlled high-resolution *x*−*y* stage, onto which the column effluent was deposited as discrete spots, which thereby facilitated tracing of the chromatographic separation. The entire target plate was sprayed with a homogeneous layer of α-cyano-4-cinnamic acid mixed with nitrocellulose by using an air brush. Minimizing dead volumes was crucial to maintain the chromatographic resolution. The volume of the ejected droplets was of the order of 60 pL. Successful separation of seven immunoregulating peptides was made. On-line sample dispensing on the target plate in combination with trace enrichment followed by automated MALDI-TOFMS identification was demonstrated, reaching a sensitivity of 100 amol.

An ultraHPLC (UHPLC) system was successfully coupled to TOFMS via a liquid−sheath electrospray interface by Wu et al. (*G11*). UHPLC has been shown to overcome the pressure limitations that small particles impose on conventional pumping systems, and high-speed separations produce peak widths that range between 100 and 1000 ms, many of which are too narrow to be monitored by scanning mass spectrometers. The only mass spectrometer that is fast enough for such separations is the time-of-flight mass spectrometer. Separations of selected combinatorial chemical samples, pharmaceutical compounds, and herbicides were completed in <100 s using UHPLC/TOFMS. Total column efficiencies ranged from 20 000 to 30 000 plates. Lazar et al. (*G12*) discussed ESI-TOFMS for fast liquid-phase separations. Fast LC TOFMS using sol−gel bonded continuous-bed capillary columns was described by Collins et al. (*G13*).

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS or FT-MS). Schmid et al. (*G14*) reviewed FTICR-MS as to its application for high resolution in combinatorial

chemistry. In combination with ESI, not only high-throughput measurements via flow injection analysis (FIA) but also coupling with separation techniques such as HPLC or CE is possible. Structural verification by way of decomposing ions using a variety of different dissociation techniques can be performed by FTICR-MS. This is the first review specifically covering applications of FTICR-MS in the field of combinatorial chemistry. Martin et al. (*G15*) achieved subfemtomole peptide sequence analysis using microcapillary HPLC columns, with integrated nanoelectrospray emitters, coupled directly to a FTICR-MS. A simple variable-flow HPLC application provides for on-line MS/MS analysis of tryptic peptides at the 400-amol level.

The combination of CLC with FTICR-MS and external ion accumulation has been shown to increase both sensitivity and analysis. A significant increase in the sensitivity, duty cycle, and dynamic range over that of the previously used accumulated trapping was achieved, exhibiting a detection limit of ~10 zmol (~6000 molecules) for smaller proteins such as cytochrome *c*. Capillary LC external accumulation interface with FTICR was successfully applied for the study of whole-proteome mouse tryptic digests by Belov et al. (*G16*, *G17*). External ion accumulation to improve instrument performance in capillary LC-ESI/FTICR was also done by Ostrander et al. (*G18*).

Electrospray/Thermospray. A simple and inexpensive approach to convert the electrospray nebulizer of a commercial LC/MS to accommodate lower flow rates has been proposed and evaluated by Carrier and Parent (*G19*). This modification consisted of simply replacing the nebulizer needle by a commercially available stainless steel needle with a smaller internal diameter. An inexpensive nanoflow gradient generator described by LeBihan et al. (*G20*) can be modulated to reproducibly deliver selected gradients. The performance of the μgrad-μLC/ESI-MS/MS system has been demonstrated for the identification of standard protein digests. Sheath liquid effects in capillary HPLC-ESI-MS for oligonucleotides were investigated by Huber and Krajete (*G21*). The incorporation of volatile acids or bases such as triethylamine or hexafluoro-2-propanol into the sheath liquid was found to influence the charge-state distribution of oligonucleotides longer than 20 nucleotide units whereas no significant effect was observed with shorter oligonucleotides. Organic acids and bases in the sheath liquid generally deteriorated the signal-to-noise ratios in the chromatograms and mass spectra mainly because of increased background noise.

Hiraoka et al. (*G22*) developed a negative-ion mode laser spray interface for use in LC/MS. The laser spray gave sensitivities that were orders of magnitude better than the negative-ion electrospray for all samples studied with the exception of sugars. Nonaqueous reversed-phase (NARP) HPLC coupled with a capacitive electrospray ion source was used for LC/MS analysis of complex mixtures of lipid A molecules, other membrane lipids, and membrane-bound proteins derived from Gram-negative pathogens (*G23*).

Atmospheric Pressure Ionization (API). Reemtsma (*G24*) summarized characteristic problems when using LC/API-MS for the qualitative and quantitative analysis of organic compounds in water. Qualitative analysis of completely unknown compounds suffers from the limited resolution of quadrupole and ion-trap MS and the limited fragmentation encountered in collision-induced dissociation. Quantitation of target analytes in complex samples may be impaired by matrix effects, especially when electrospray ionization is used.

Particle Beam. Gibeau and Marcus (*G25*) give a detailed evaluation of the analytical characteristics of an LC/particle beam-glow discharge mass spectrometry (LC/PB-GDMS) system for applications in the area of inorganic (i.e., free metals in solution) and organic compound analyses. A highly efficient particle beam interface was used to introduce analyte species into a glow discharge source for subsequent vaporization and ionization. The mass spectra obtained for inorganic species were composed exclusively of the respective elements' isotopic patterns, with no evidence of appreciable oxide species formation. Organic species introduced into the discharge through the particle beam interface yielded mass spectra that were virtually identical to those from standard electron impact (70 eV) ionization. As an example of the feasibility of the PB-GDMS system as a detector for LC, the separation and identification of the organic constituents in diet soda was performed.

Other Articles of Interest. The first hyphenation of HPLC, electrochemical on-line oxidation, and MS was described by Diehl et al. (*G26*). Ferrocenecarboxylic acid esters of various alcohols and phenols were synthesized, separated by reversed-phase HPLC, and oxidized (ionized) coulometrically prior to single quadrupole MS analysis using ESI and APCI interfaces. The dependence of the ionization on the electrochemical pretreatment is demonstrated. Limits of detection for selected derivatives range from 4×10^{-9} to 4×10^{-7} mol/dm^3 depending on the individual analyte.

OTHER DETECTION SYSTEMS

Detection techniques for ion analysis by ion-exchange chromatography and CZE were extensively reviewed by Buchberger (*H1*). Special attention was given to conductivity, UV/visible absorbance, amperometric and potentiometric detection, mass spectrometry (including inductively coupled plasma MS and atmospheric pressure ionization MS), and postseparation reaction detection.

Gas Chromatography Detectors for LC. Scott et al. (*H2*, *H3*) described a device that enabled the use of GC-type detectors in conjunction with HPLC, wherein the solvent is removed from the LC eluent prior to immobilizing the solute onto a moving, oxidized, titanium tape. The latter is wetted by all solvents, including water. The tape then moves the remaining solute into a pyrolysis zone, integral with a GC detector. For high-sensitivity universal operation, a modified argon ionization detector was constructed. Gaseous pyrolysis products were swept into the detector. An alternative design also was developed comprising a dual argon ionization/electron capture detector for high sensitivity in both universal and selective modes. A subcritical chromatography system using water as the mobile phase and FID system was employed by Lu et al. (*H4*) to separate several alcohols with varying polarity.

Nuclear Magnetic Resonance. The advent of sensitive and reliable HPLC NMR and HPLC−MS systems has revolutionized the on-line identification of compounds eluting from chromatography systems. Wilson (*H5*) has reviewed the construction and application of combined HPLC NMR-MS systems to the analysis

of mixtures of pharmaceuticals, drug metabolites in biological fluids, and natural products in plant extracts. In addition, preliminary work with alternative systems such as HPLC−UV−NMR−FTIR-MS is highlighted and the prospects for such complex systems are considered. As with almost all other analytical techniques, miniaturization is on the forefront of research in LC NMR. The Sweedler group (*H6*) described the first coupling of capillary HPLC with a diode array spectrophotometric detector and a custom-built NMR flow microprobe. The eluent from the capillary LC column was linked to a 500-MHz ^{1}H NMR microcoil probe with an observe volume of 1.1 nL. The complementary nature of diode array and NMR detection allowed stopped-flow data collection from analytes that would otherwise go unnoticed in continuous-flow NMR. The separation and structurally rich detection of a mixture of terpenoids under both isocratic and gradient solvent elution conditions was presented. Gavaghan et al. (*H7*) reported the first combined use of NMR-pattern recognition (PR) analysis and directly coupled HPLC NMR to identify subpopulations in normal laboratory animals and their discriminating endogenous urinary biomarkers.

Radioactivity Detectors. DeVol and co-workers (*H8*) described a new sensor material, which combined extraction chromatography and scintillation detection. The sensor can be used in either an off-line or on-line format. For on-line measurements, the sensor materials are used in conjunction with a flow cell scintillation detection system to assess the activity retained by the extractant in real time as the activity is eluted from the column. Characterization of the detection efficiency and regeneration capability of the extractive scintillator materials is evaluated.

Surface Plasmon Resonance. On-line detection and characterization of carbohydrates separated by LC was accomplished by an immunosensing system based on surface plasmon resonance (SPR). The sensitivity of the LC-SPR system developed by Jungar et al. (*H9*) was dependent on molecular weight of the carbohydrate, affinity of binding, and design of the sensor. By using weak and readily reversible monoclonal antibodies, the SPR system allowed specific on-line monitoring of the substances. To increase the specificity of the immunosensor, nonrelevant antibodies were used as reference in a serial flow cell.

Biosensors in LC. Tyrosinase, laccase, and coconut tissue were compared as active biocomponents in enzymatic detection of phenols (*H10*). The measurements were carried out in flow injection systems with flow-through reactors and carbon paste-based integrated biosensors. For use as a detector in a HPLC system, the tyrosinase carbon paste biosensor was the most convenient. The obtained detection limits were 0.072, 0.037, and 0.032 mg/L for hydroquinone, phenol, and catechol, respectively. Guerrieri and Palmisano (*H11*) developed a LC detector based on a fast response and sensitive bienzyme amperometric biosensor for acetylcholine (ACh) and choline (Ch). The detector fabrication consisted of glutaraldehyde co-cross-linking of acetylcholinesterase and choline oxidase with bovine serum albumin on the Pt working electrode of a conventional thin-layer electrochemical flow cell. Linear responses were observed over at least four decades, and absolute detection limits (at a signal-to-noise ratio of 3) were 12 and 27 fmol injected for Ch and ACh, respectively. After one month of intensive use in the LC system, the detector retained ~70% of its initial sensitivity.

The detection of proteins with enzyme-amplified biochemical detection (EA-BCD) coupled on-line with HPLC is demonstrated in this work by van Bommel et al. (*H12*). The EA-BCD system was developed to detect biotin-containing compounds. Hb, which was used as a model compound, was biotinylated prior to sample introduction. Several biotinylation parameters, such as pH and removal of excess biotinylation reagent, were investigated. The nonfluorescent substrate is converted to a highly fluorescent product by the enzyme label. A detection limit of 2 fmol of biotinylated Hb was achieved with good reproducibility and linearity. However, biotinylation at low analyte concentration suffered from low yield due to slow reaction kinetics. Finally, Hb was successfully extracted from urine with a recovery of 94%.

Immunochemical-Based Detection. Coupling immunoassays with HPLC separation techniques is becoming increasingly useful in the analysis of biological and nonbiological samples of both large and small molecules. This is because it provides both sensitivity and selectivity for molecular analysis at relatively low cost and low maintenance and with excellent potential for automation. Tang and Karnes (*H13*) reviewed the application of this hyphenated approach in both the precolumn immunoextraction and postcolumn immunodetection modes. Advantages and limitations for the various approaches were also discussed. Lee and Kennedy (*H14*) focused their review on immunoanalytical techniques for environmental monitoring. This paper showed these techniques can deliver rapid, accurate, and relatively inexpensive analysis with high throughput and have the capability to be field oriented.

An on-line LC-immunochemical detection (LC-ICD) system for the quantification of cytokines in cell extracts has been developed by Schenk et al. (*H15*) using a postcolumn continuous-flow reaction detection system with fluorescence-labeled antibodies. Cytokines eluting from the micro-HPLC column react with antibodies to form fluorescent complexes. In a second step, the excess of free antibody is trapped on a cytokine-bound support prior to fluorescence detection. The concentration detection limit of the flow injection-ICD system was 50 pM (20-μL injection volume) for interleukine 4 (IL-4). An absolute detection limit of 1 fmol was obtained for IL-4. The present ICD system for interleukines 4, 6, 8, and 10 was coupled to ion-exchange, size exclusion, and reversed-phase chromatography. Important parameters (reaction times, reaction conditions) were investigated to get a better understanding of postcolumn ICD systems for macromolecules. Graefe et al. (*H16*) described an on-line chromatography with immunochemical postcolumn fluorescence energy transfer detection for digoxin and its metabolites. (*R*)-phycoerythrin (PE) was used as the donor and an indodicarbocyanine dye (Cy5) as the acceptor label. These labels allow the detection in the far-red spectral region, which is more selective for biological samples. Hence, digoxin was labeled with PE using the activated digoxigenin-NHS-ester and monoclonal anti-digoxin antibody was labeled with Cy5. Digoxin and its metabolites were injected into the HPLC system followed by postcolumn injection of (*R*)-phycoerythrin-labeled digoxin and by Cy5-labeled anti-digoxin antibody. Incubation time was provided using an open-tubular reactor coil at room temperature. The dynamic range of digoxin spiked in 0.01 M phosphate buffer (pH 7.4) was 0.05−10 ng/mL with a correlation coefficient of 0.989. The limit of detection was 33 pg/mL. The

precision of two controls, 0.4 and 4 ng/mL, was found to be 2.2 and 8.7% RSD, respectively, and accuracy was 10.7 and 20.3% ($n = 6$ in each case).

Viscometry. Yi et al. (*H17*) combined three size exclusion detectors (i.e., refractive index, right angle laser light scattering, and differential viscometry detectors), or simply SEC^3, to study novel liquid crystal polyacetylenes. Based upon the data generated for these materials, the relationship between molecular size and the polymer structure was investigated, SEC universal calibration was shown to be valid, Flory's characteristic ratio was estimated, and the effect of the size of the side group on chain flexibility was elucidated. Baran and co-workers (*H18*) investigated the LC of polymers at the exclusion−adsorption transition point (LC EATP). A correlation between polystyrene dimensions and this chromatography mode was attempted by viscometry and light scattering measurements. It was demonstrated that EATP is obtained for specific values of polymer/adsorbent interactions, for a given eluent desorb/adsorb, and for a critical polarity of the eluent.

COMPUTATION

Simulation. The use of simulation software is increasing in popularity in order to decrease methods development time. Dzido et al. (*I1*) optimized the separation of 11 phenolic acids in reversed-phase HPLC systems by employing the Drylab software. The simulated and experimental retention data showed good agreement. Haber et al. (*I2*) described computer software that allowed the simulation of any chromatographic separation as a function of simultaneous changes in any one or two variables that can affect sample separation order (selectivity). For one example, an application was described for the simultaneous variation of the mobile-phase pH and gradient time in reversed-phase LC. The accuracy of such predictions was examined for a sample mixture of 17 substituted benzoic acids and anilines, and requirements for an acceptable predictive accuracy are summarized.

Beck and Klatte (*I3*) looked at computer simulations of interphases and solute transfer in liquid and size exclusion chromatography. Previous studies are reviewed concerning chain structure and dynamics and solute retention, and new results are presented from computer simulations of LC interphases of C18 chains in contact with three different water/methanol mobile phases. These simulations probe the particle densities and free volume profiles across the interface, solvent orientation passing from bulk into the stationary phase, and dynamical properties of the alkane chains and solvent. Discussion is given of preliminary studies of the partitioning of charged solutes in SEC. Double-layer effects are included by numerical solution of the nonlinear Poisson−Boltzmann equation which yields the potential of mean force between the charged dendrimer solute and the like-charged pore.

Software. Steger-Hartmann (*I4*) discussed computer programs utilized in LC applications, for example, the chromatography of proteins. A computer simulation program of reversed-phase HPLC was developed for training purposes by Reijenga (*I5*). Experimental retention values of 75 organic compounds on a reversed-phase column at four different percentages of organic modifiers were reduced to a two-parameter retention model with the modifier content as variable. Modifiers used were acetonitrile, methanol, and THF. Isocratic and programmed solvent composition were included together with the usual experimental parameters available in modern HPLC equipment, such as UV diode array and refractive index detection. Instrument specifications were made variable within wide ranges, and detailed dispersion data were made available as tabulated output.

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