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Liquid Chromatography in 1982

David H. Freeman

The liquid chromatograph has become the most powerful instrument for separations available in chemistry. At the center of its capability is an array of new selective sorptive effects which are being harnessed to solve many practical problems. Research in this area is yielding new insights into interactions in liquids. The advances in liquid chromatography are too numerous to summarize in a short article; instead, an attempt will be made to review some of the more interesting trends.

Liquid chromatography (LC) was first reported in 1906 by Michael Tswett, a Russian botanist. The technique was used to separate plant pigments. Tswett's ideas were expressed with polemic views that made the ideas unattractive to the chemical profession. In the early 1930's the technique was revived, and a growth period of refinement and inventiveness began (*1*). The improvement LC offered over other separation techniques was widely noted in 1971, when the Nobel laureate R. B. Woodward reported a definitive separation of remarkably similar vitamin B_{12} intermediates (*2*), as shown in Fig. 1.

Several years earlier, commercial LC instrumentation had started to become a growth industry. The popularity of the technique since then has grown enormously. Liquid chromatography now dominates the analytical chemistry market and may be a billion-dollar industry by 1985 (*3*). With LC, many chemical separation problems are now being solved, often quickly, by methods that nonspecialists can learn with a little practice. Its success is comparable to that of gas chromatography two decades ago and can be expected to continue.

Liquid chromatography has expanded into a wide range of scientific and industrial applications. It plays a major role in qualitative and quantitative analysis, is a powerful method for group separations, and is used to isolate or analyze products from the mixtures that form during chemical synthesis. It is applied, for example, for quality control of pharmaceuticals, to measure chemicals in foods, to investigate the chemistry and metabolism of biological systems, to aid forensic science, and to help measure unsafe chemicals in the environment. An illustrative application to clinical chemistry is shown in Fig. 2 (*4*).

Established LC separation procedures are available for amino acids, proteins, nucleic acids, lipids, terpenoids, steroids, carbohydrates, drugs, pesticides, petrochemicals, antibiotics, and inorganic and organo-inorganic compounds. The applicability of the technique is rarely limited. Technical and scientific articles on the subject are appearing at the rate of 3000 per year.

Apparatus

An LC separation begins when a small volume of liquid containing a dissolved sample mixture is injected into a moving liquid carrier. The apparatus is shown schematically in Fig. 3. The sample is transported by the carrier stream as it flows through the chromatographic column. This is a cylindrical bed packed with fine sorptive particles, averaging 10 micrometers or less in size. Because the particles resist the flow of liquid, carrier delivery and liquid sample injection must take place at back pressures up to a few hundred atmospheres. Sample injection is usually done with an injection valve, as shown in Fig. 4.

David H. Freeman is a professor in the Department of Chemistry and the Chesapeake Biological Laboratory, University of Maryland, College Park 20742.

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The LC conditions are designed so that each sample component can travel with a characteristic velocity through the column. The components are expected to form narrow concentration waves, known as chromatographic bands. The maximum band velocity is generally that of the flowing liquid. Sorption causes retention and a lower band velocity. Retention differences are due to the chemical selectivity of the system; they result in velocity differences that cause the bands to separate. The desired result is that the separated compounds elute (emerge) one at a time from the downstream end of the column.

Summary. Classical liquid chromatography gave rise 15 years ago to new ideas about high-speed separations. Today, difficult separations can be made almost routinely by use of liquid chromatography instruments with automated controls and sensitive detectors. Sorptive effects are often best achieved with small, porous, "bonded phase" particles. The trend is to control the chemical selectivity by means of the liquid phase. These techniques are easily learned, and they have been widely accepted throughout chemistry and its allied disciplines. As a result, liquid chromatography has become the most rapidly expanding branch of the chemical instrumentation field.

The carrier liquid then flows through a sensitive detector. This is a subject of intense interest, and more than 30 detection principles are currently feasible (*3*). The most commonly used are the measurement of a spectroscopic property (such as ultraviolet light absorptivity), an optical effect (refractive index), or an electrical one (conductivity). The choice depends on the type of compound, the liquid, and the requirements for detection sensitivity and selectivity. Sensitivity can be boosted by chemical derivatization—for example, by the addition of highly fluorescent dansyl groups to amino acid molecules. Laser fluorescence has been used to detect as few as 10 million molecules in the detection cell (*5*).

The detector signal responds to the sample concentration and indicates when to collect the separated bands as they emerge from the detector. The signals are sent to a chart recorder, and in the resulting plot of signal versus time the band profiles are represented by a series of peaks (the chromatogram). The peak width tells how well the instrument is performing, and the separation between adjacent peaks tells whether the selectivity goals have been reached. Detector signals can also be fed to a microcomputer for additional measurement or control purposes.

Peak Sharpness and Speed

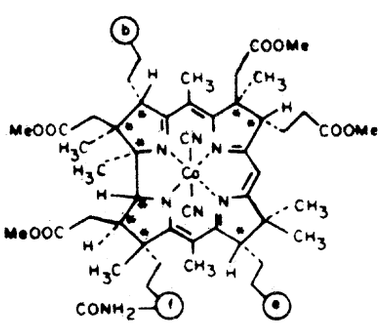
Efficient separation of a mixture depends on several crucial factors. I will start with band sharpness and the speed at which LC separations can be made.

An elaborate technology has evolved to prepare sorbent particles and packed column beds that give sharp peaks and the fastest separations practical (*6*). Commercially available (and costly) columns provide uniformly small sizes of rigid sorbent particles. Column packing of liquid-borne particles involves rapid flow into an initially empty column. At the downstream end of the column is a porous metal barrier (a frit) that retains the particles but not the liquid. After the column bed is prepared, the flow velocity through it must be kept below a critical maximum or the ability to form sharp peaks will be lost due to channeling or bed collapse.

Sharpness is defined in terms of theoretical plates. In general terms, the size of each sorbent particle is roughly the same as a hypothetical region, or plate, in which a repetitive sorption and desorption cycle occurs. Modern sorbent design seeks to minimize the time and distance needed for this repetition to occur.

The number of theoretical plates is measured on the chromatogram. To do this, a triangle is drawn with tangents to the two sides of a given chromatographic peak and with a connecting horizontal baseline. The retention time, t_R, is measured as the time difference between injection and the top of the peak (or the apex of the triangle). The peak width, W, is then measured along the baseline of the triangle. The number of plates, N, is calculated from $N = 16(t_R/W)^2$. Classical chromatography provided perhaps 100 plates, but commercially prepared LC columns now provide 3,000 to 15,000 plates. (A peak with a 12.5-minute retention time and a 0.5-minute base width corresponds to 10,000 plates.)

High-speed separations are valuable



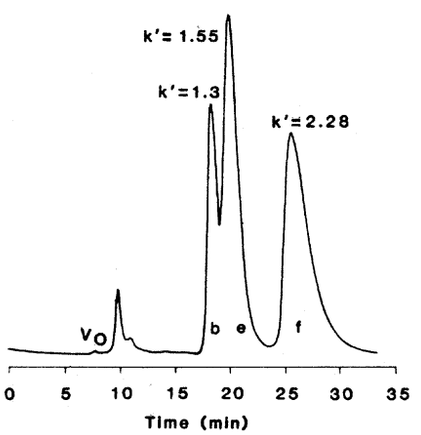


Fig. 1 (left). Isolation of the *f* amide (last peak) of cobester, a key intermediate in the synthesis of vitamin B_{12}, by Woodward (*2*).

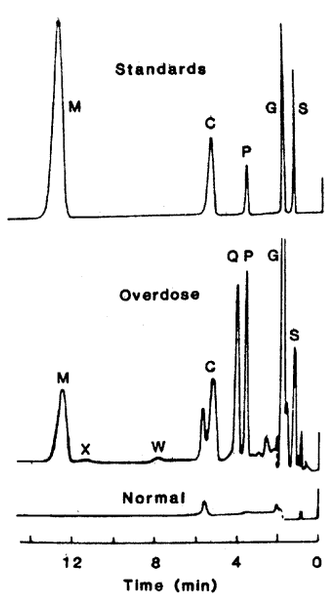


Fig. 2 (right). Comparison by LC of clinical standards with urine samples from normal and overdose patients. The basis for liver and kidney damage is indicated by high levels of the drug acetaminophen (paracetamol, *P*) and its major metabolites (mercapturic, *M*; glucuronide, *G*; cysteine, *C*; and so on) (*4*).

for routine chemical analysis. To some extent, speed and sharpness are conflicting goals, since peaks generally broaden as flow velocity increases. While peak sharpness improves with diminishing sorbent particle size, the resistance to liquid flow increases disproportionately. (The back pressure is related to the reciprocal square of particle size.) Another penalty as particle size decreases is that the chromatographic bed becomes increasingly prone to fouling by small impurity particles. Prior ultrafiltration of the dissolved sample and carrier liquids is the recommended routine.

Simplified Problem-Solving

Chemical selectivity is the logical basis for predicting and interpreting LC separations. In the past decade there has been increased utilization of the easily varied liquid properties that control selectivity. This has resulted in an increased understanding of the liquid phase with a wealth of scientific implications.

Consider the reasoning used when one encounters a new sample mixture that is to be separated. A mixture will usually include some chemical compounds whose molecular structures are either known or can be estimated. Direct approaches are now available to help in finding the column and liquid phase compositions needed to separate authentic samples of the likely knowns. This is a step toward verifying the presence of the knowns, and it simplifies the trial-and-error tactics used to separate the remaining unknowns.

The structure of each chemical is examined for molecular segments or functional groups that may have predictable sorptive behavior. For example, highly polar chemicals are often hydrophilic and water-soluble, whereas compounds structurally similar to saturated hydrocarbons are characteristically hydrophobic. Observations are also made to anticipate possible separations based on size differences between sample molecules.

For LC the sample mixture must be soluble in one or a combination of liquid solvents. The "like-dissolves-like" rule is applied to match the sample chemicals to a suitable solvent. While most salts and sugars dissolve in water and a petroleum sample usually requires a hydrophobic solvent, some experimental tests may be needed. For example, it is helpful to know the solubility of the sample in several common solvents, whether the sample is acidic, basic, or neutral, and whether the sample-solvent combination

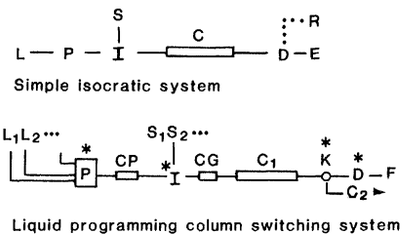


Fig. 3. (a) Isocratic LC system: *L*, liquid reservoir; *P*, pump; *S*, liquid sample; *I*, sample injection valve (see Fig. 4); *C*, chromatographic column; *D*, detector; *E*, liquid effluent containing separated samples; *R*, chart recorder. (b) Liquid programming LC system with column switching: L_1, L_2, . . . , selectable liquids available for blending in varying ratios as controlled by pumping system *P*; *CP*, precolumn to remove liquid impurities and, when needed, to presaturate carrier with silica as used in bonded phase chromatography; *CG*, guard column, which contains same packing material as in C_1 but traps irreversibly adsorbed impurities and impurity particles; *K*, switching valve so that effluent from column C_1 can be sent directly to the detector *D*, or vented, or switched to secondary column C_2 for additional selectivity [*K* can be a six-port valve (*41, 42*)]; *F*, fraction collector. The asterisk identifies microprocessor control points: for liquid gradient control at *P*, autosampler (of samples S_1, S_2 . . .) at *I*, column switching at *K*, data processor at *D*, and fraction collection at *F*. Modular components are assembled to provide the capabilities needed. Typical system complexity is usually between the two limits shown here, although the isocratic system is preferred for its simplicity.

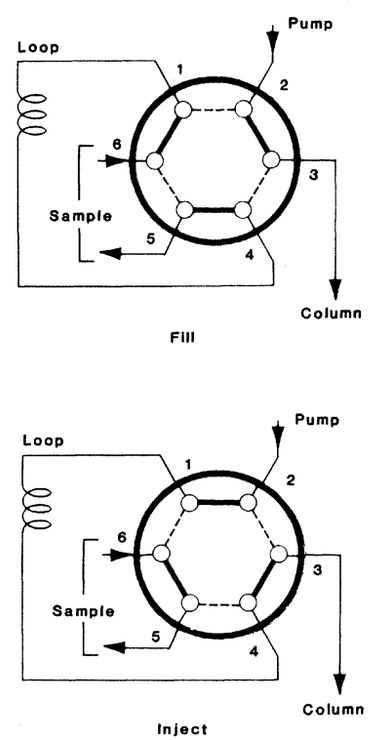


Fig. 4. The two steps of sample injection. The sample loop is filled with the dissolved sample. Then by rotating the valve the sample loop is inserted into the high-pressure liquid stream. [Drawings courtesy of Rheodyne Incorporated, Cotati, California]

is appropriate for use with a particular detector.

Selection of a suitable sorbent is next. Historically, LC separations have been classified according to sorbent types or sorptive processes. These include adsorption, partition, ion exchange, and molecular sieving or size exclusion effects known as gel filtration or gel permeation. Practical guidelines are available to help in searching for a suitable sorbent and estimating suitable chromatographic conditions (*6*, chapter 18). LC tests are made to find a desired level of sample retention effect. The liquid phase composition can be further adjusted until acceptable selectivity and speed are obtained.

Some situations are more difficult, particularly when individual chemicals contain more than one kind of functionality. Years ago, the search for selectivity in these instances was guided by few systematics and correspondingly high emphasis on trial and error. Today there are many sorbent and solvent choices and thousands of additives to modify the separation conditions. The number of plausible experiments is astronomical unless one has sufficient intuition, ingenuity, or experience. The available understanding of chemical selectivity is best when combined with practical experimental tactics. This is necessary to prevent separation problems from becoming research projects, at least until the need is forced.

The most efficient approach to finding LC selectivity is to assume that the conditions for separating the known compounds in the sample, or their analogs, are already available. A search, using common chemical sense with or without a computer, can be made for published LC separation conditions. The use of analogy is an established tradition in chromatography and is a frequently successful approach.

These tactics have become much easier to learn. For the beginner with some background in chemistry, short courses are available from the American Chemical Society, the LC industry, and educational institutions. In the 1980–1981 period more than 100 review articles and texts appeared (*3*). Professional chromatographers meet annually at the Pittsburgh Conference (*7*), the largest exposition of analytical chemical apparatus and suppliers in the United States. Industrial sales representatives are often well informed and contribute user support based on experience gained in applications laboratories. As a result, effective problem-solving techniques have diffused widely and practical approaches may be

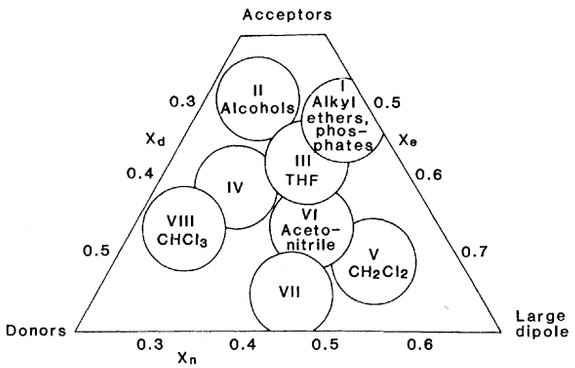


Fig. 5. Solvent selectivity triangle. Solvent interactions are subdivided into proton donor (Brönsted acid), proton acceptor (Brönsted base or Lewis base), and large dipole contributions (*18*).

readily learned and applied. Becoming an experienced chromatographer has come to depend less on genius than on interest and a properly equipped LC laboratory.

Simplified approaches to selectivity are now a major aspect of LC separations. There has been a major transition toward use of the more predictable tailor-made sorbents and correspondingly more rational choices of liquid phase composition. The harnessing of selectivity explains why LC now provides much better separations than could have been anticipated a decade ago.

The chromatographic column is the dominant source of peak sharpness and separation selectivity. The technology for forming sharp peaks has already been described. In the following sections I will discuss LC separations under the headings of the sorptive processes used.

Adsorption

Liquid chromatography began with liquid-solid adsorption chromatography (LSC). Adsorption results when a chemical binds with a presumed adsorption site. Adsorption effects range from the self-visualizing separation of pigments (*8*) to concentration of proteins from water onto porous glass (*9*). Many solid adsorbents have been tried, but more is known about silica (*10*). In its modern form (*11*) porous silica has become the predominant choice for LSC.

Silica gel is nominally SiO_2, but its adsorptive activity is due to surface silanol (SiOH) groups. These sites are hydrogen-bonding, weakly acidic, and therefore polar. It is characteristic of polar sorbents that the surface affinity for solvent, additive, or sample molecules varies directly with their polarity. Maximum sample adsorption with silica depends on minimum solvent adsorption; this occurs with nonpolar solvents such as hexane. Desorption is caused by liquids or soluble chemicals whose polar or hydrogen-bonding effects result in preferential evicting of the sample from its occupancy of the adsorption site.

Sample separation conditions are established by analogy or by trial and error. In LSC, selectivity is generally difficult to predict. Thin-layer chromatography is used for rapid scouting. Selectivity is often obtained with mixtures of weakly and moderately polar liquids containing small amounts of highly polar additives (*12*).

The literature is replete with successful separations on silica. Even so, the use of silica is not without problems. Surface hydration of the silica is crucial, and it is difficult to control the small amounts of water needed to muzzle the more aggressive adsorption sites. Silica gel is notoriously slow to equilibrate when the liquid phase composition is changed. Such separations are not precisely reproducible, and irreversible or degradative behavior is occasionally observed.

Modernized forms of silica have become available. In 1968, impermeable but carefully sized particles of silica gel gave sharp peaks at high carrier velocities; a separation of seven compounds in 4 minutes was demonstrated (*13*). Pellicular silica—multiple layers of colloidal silica bonded to an impermeable glass bead support and known as Zipax, gave improved speed and peak sharpness (*14*). The higher sample mass capacity of totally porous silica particles was established shortly thereafter. Although the improvement and taming of silica has been remarkable, it has attained unexpected popularity, not as an adsorbent, but as a base structure for preparing chromatographic derivatives, which will be described shortly.

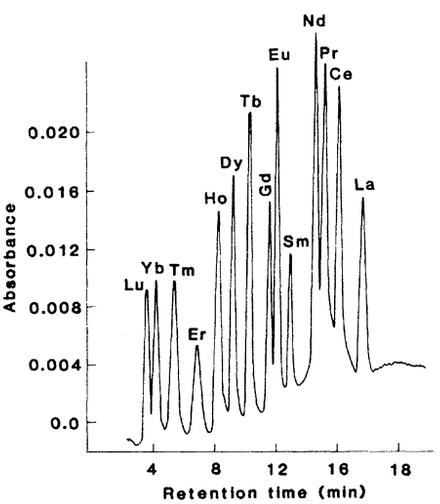


Fig. 6. Lanthanide separation obtained by high-speed ion exchange on silica containing covalently bonded ion exchange groups. A spectrophotometer at 600 nm was used to detect a color-forming reaction after the column (*23*).

Partition

Major advances have resulted from efforts to change the surface properties of adsorptive substrates. In 1952, A. J. P. Martin and R. L. M. Synge won the Nobel Prize in Chemistry for the invention of partition chromatography. A thin film of water was placed on silica particles, and a chloroform-butanol mixture was used as the liquid carrier to separate amino acid derivatives. The publication announcing this technique included a mathematical analysis that introduced plate theory in LC and placed the study of peak sharpness on its first rational footing (*15*).

Bridging plate theory, equilibrium partitioning behavior, and liquid chromatography was a major breakthrough. There is a nearly exact parallel between the selectivity effects in liquid-liquid extraction and those in liquid partitioning LC systems (*16*). Selectivity concepts based on chemical polarity are central to LC. Efforts have been made to adapt the solubility parameter theory of J. H. Hildebrand and G. Scatchard to LC, but the theory does not represent multifunctional chemicals, although the solubility parameter can be used to estimate relative retention for simple molecules. Exact predictions of gel partitioning were found to be blocked by the large influence of the liquid on the behavior of the stationary phase (*17*).

The pursuit of liquid phase contributions to selectivity has evolved beyond a unidimensional concept of polarity. Snyder (*18*) has subdivided polarity into proton donor (Brönsted) acidity and basicity and strong dipole interactions. Hydrophobicity is considered the opposite of polarity. It follows that partitioning effects should offer four nonredundant selectivity categories, but eight were iden-

tified by experimental correlation (*18*), as shown in Fig. 5.

Two opposing phase relations in liquid partitioning chromatography are easily recognized. A polar stationary film and a less polar carrier give conditions known as normal phase LC (NPLC), and the sample sorption effect increases with sample polarity. By contrast, a nonpolar film with a polar carrier gives the opposite retention order, and this is called reversed phase LC (RPLC). Partitioning effects are usually generated as the polarity sequence carrier-sample-sorbent increases (NPLC) or decreases (RPLC) in that order. Given a series of sample chemicals with estimated differences in polarity, one can predict the retention order for NPLC and its reversal with RPLC.

A major practical drawback to this early form of partitioning LC is that the film liquid and the carrier liquid should remain immiscible. It happens that changing the carrier composition toward maximum desorption usually dissolves the film liquid as a consequence of the like-dissolves-like rule.

Bonded Phase Chromatography

The problem mentioned above has been solved by chemical modifications that covalently bind functional groups onto the surface of porous silica. The initial goal of this work was to improve gas chromatography. Since then, many modifications of silica have been made and tested, and a wide range of surface polarities and bonded group functionalities is now available (*11*, chapter 3; *19*). These materials have other advantages, since they mask the silica substructure. They give reproducible sorptive effects, improve sample mass capacity, and permit the high diffusion rates needed for sharp peaks. These tailor-made materials are the basis for what is currently the most popular form of LC—bonded phase chromatography (BPC). More than 75 percent of LC applications now use the new bonded materials (*3*).

On the hydrophobic side are bonded octadecyl and other alkyl groups, with the carbon chain covalently linked to the surface by siloxane Si–O–Si–C bridges. Not all of the surface silanol groups are removed during synthesis, but their chromatographic perturbation is eliminated by "end capping" reactions. In order of increasing polarity, bonded RPLC sorbents are also available with octyl, methyl, and aromatic groups.

Many liquid phase additives are used to modify selectivity through secondary chemical equilibria (*20*). For example, the electrostatic charge state of certain chemicals (amines, carboxylic acids, and so on) is easily changed. Acid-base buffers are used to control ionic charge through gain or loss of protons. Confinement to the less polar uncharged state, known as ion suppression, boosts RPLC retention and improves peak sharpness.

Bonded amine or propylamine derivatives provide uniform basicity and possible hydrogen-bonding effects. These are commonly used for bonded phase NPLC. Bonded nitrile is somewhat less polar.

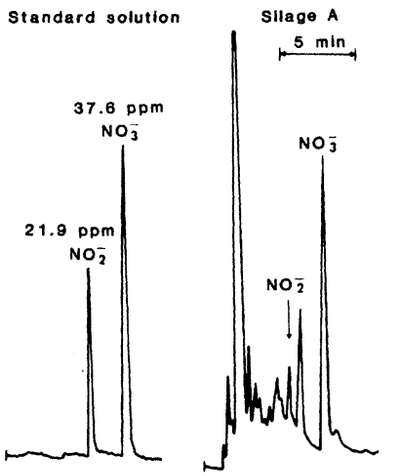


Fig. 7. Reversed phase LC separation of nitrite and nitrate anion standards. The liquid phase contains hydrophobic octylammonium cation, which is sorbed by the stationary phase. At elevated *p*H the hydrophobic reagent loses its cationic character and the anion retention behavior is lost. [Reprinted from (*28*) with permission from the American Chemical Society]

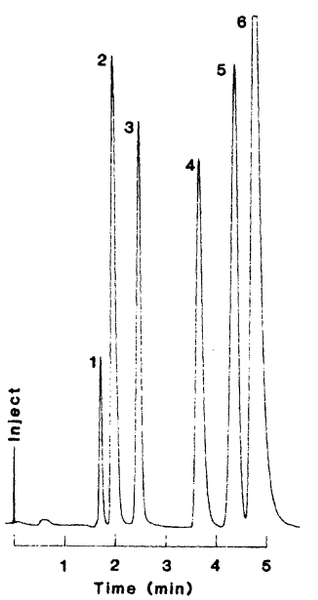


Fig. 8. Separation of a series of antihistamine and decongestant cations by the addition of pentane sulfonate salt during reversed phase LC. The hydrophobic reagent brings an anionic charge to the hydrophobic surface of the stationary phase. The resulting cation exchange affinity is similar to that in Fig. 7 but the charge relation is reversed (*29*).

The partitioning behavior of silica derivatives is not fixed by the structure of the functional group. Particularly with groups of intermediate polarity, such as nitrile or phenyl groups, NPLC or RPLC conditions depend on the relative polarity of the liquid chosen.

The commercially available phases used in BPC are stable provided they are used in nonreactive media. The silica substrate is only slightly soluble in water, but its solubility rises rapidly in basic media, and strongly acid conditions cause hydrolysis. Guidelines are available to help ensure column longevity (*21*).

Experimental approaches to finding optimal selectivity conditions can be made less tedious by automation. If the carrier has a fixed composition, its isocratic or fixed desorptive strength produces band spread that increases with increasing retention. Band narrowing occurs when the carrier is programmed so that the solvent strength is increased gradually or stepwise to promote desorption (*6*, pp. 662–719; *22*). An initial survey of sample retention is aided by microprocessor-controlled changes in the relative rates of pumping a pair of liquids so that the composition of the liquid blend entering the column follows a programmed change toward desorption. Similarly, the optimal carrier composition can be found by using automated LC instruments that are programmed to carry out repetitious experiments.

Ion Selectivity

The ion exchange resins now available have a high sample mass capacity and high chemical stability. In peak sharpness and speed, resin microparticles are now comparable to their silica-based BPC counterparts (*11*, chapter 8; *23*); an example of the use of the latter is shown in Fig. 6. Ion exchange has played a major role in the discovery of new elements and isotopes and in numerous separations of thermally labile biochemicals. The first LC instrument was the amino acid analyzer. Hamilton's separations of 175 ninhydrin-positive substances (*24*) was a major step toward modern LC.

Ion exchange requires the presence of a displacement electrolyte, which, until recently, swamped out the conductivity effect of the separated ions. This prob-

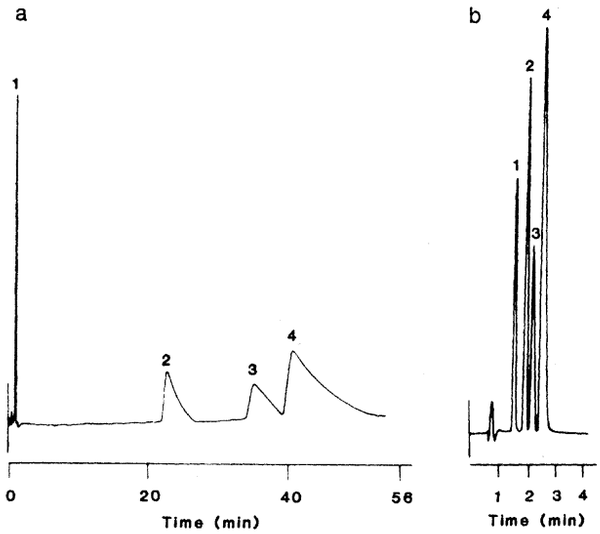


Fig. 9. (a) Reversed phase LC separation of benzocaine, lidocaine, tetracaine, and etidocaine cations, showing excessive retention. (b) Separation obtained when triethylamine is added to an acid carrier, forming the triethylammonium cation. This hydrophobic ion is sorbed, creating charge repulsion and lowering the partitioning effect to give the rapid separation shown (*29*).

lem was solved by the invention of ion chromatography, in which a suppressor column was used to remove the carrier electrolyte (*25*). This led to widespread interest in inorganic anion analysis. Improved designs now permit similar analyses by nonsuppressed use of low-capacity ion exchange resin (*26*).

In a more recent approach, ion selectivity has been achieved by adding an ionophilic reagent to the carrier; this has been demonstrated in both reversed phase and normal phase BPC. Partitioning effects can thus be superimposed on ionic selectivity, and hydrophobic and electrostatic interactions are combined. Simplification is possible, at least experimentally, because the selectivity depends on the carrier composition, which is controlled by the user. A number of selectivity effects are possible, analogous to those in the liquid-liquid systems studied by Schill (*27*). These effects are occasionally complicated and not yet fully understood. Their possible uses in LC are described under the heading of secondary ion equilibria (*20*).

In a very recent example, an octyl ammonium cation in the carrier was used with reversed phase BPC to give the anion separations shown in Fig. 7 (*28*). The hydrophobic cation has its nonpolar face attracted to a hydrophobic octadecyl stationary phase while the other side of the molecule provides the hydrophilic anion exchange site. The resulting selectivity patterns are very close to those in conventional ion exchange (*28*). When the octyl ammonium cation is removed the ion affinity disappears.

The addition of a pentane sulfonate salt, also in reversed phase BPC, resulted in the ion exchange separation of antihistamine cations shown in Fig. 8 (*29*). Ion exchange affinity is again introduced by use of a hydrophobic reagent, but the charge relations are the reverse of those in Fig. 7.

The use of reversed phase conditions to separate hydrophobic as well as partly ionic organic compounds often involves partitioning. Figure 9 shows an inefficient reversed phase sorption of cations. The behavior is improved (Fig. 9b) by the presence of a second hydrophobic cation, the triethylammonium ion, which causes ion repulsion and less retention (*29*).

The resolution of optical isomers has tested the chemical selectivity of LC. The most general approach has been described by Pirkle *et al.* (*30*). Selectivity can be obtained through chiral functionality in the stationary phase or in the liquid phase. Earlier work in which metal ions were used as sites for ligand exchange (*31*) provided a general basis for the separation of optical isomers by chiral ligand exchange (*32*). The use of high-performance reversed phase BPC has provided high efficiency plus the selectivity of a hydrophobic chiral metal ligand reagent. Gil-Av *et al.* (*33*) reported the wide separation of each of 19 different amino acids into their D,L pairs by adding divalent copper and optically active proline to the carrier. The chiral resolution was reversed when the proline additive was changed from D to L and lost altogether when the D,L mixture of proline was used. Similar use of a coordinating ligand to resolve the fluorescent dansyl derivatives was reported by Karger and co-workers (*34*).

While LC selectivity is the historical descendant of the stationary phase, the current thinking and trend is to induce selectivity from the mobile phase. Carrier-based selectivity can be as easily induced as it is removed. In the examples above, selectivity is due to the use of specific reagents. When they are removed from the carrier, and the polarity of the carrier matches that of the sorbent surface, the chemical selectivity disappears and the size exclusion selectivity of the pore structure is obtained.

Size Exclusion

Size exclusion chromatography (SEC) is due to the sorbent pore structure (under magnification, the pores look like those in a sponge). Large molecules are excluded but small molecules are able to permeate and thus be retained. This is a purely noninteractive separation effect. It is greatest when the pores are about four times the average size of the sample molecules. This subject has been reviewed by Bly (*35*) and is more fully presented in a recent text (*36*).

Size exclusion chromatography has two major applications: separating molecules that differ in size by at least a factor of 1.1 or 1.2, and measuring the apparent molecular weights of industrial and biological macromolecules.

Faster sorbent materials for SEC have been sought. With many substances, acceptable SEC conditions are obtained by matching the carrier and sorbent polarities. With proteins and polyelectrolytes the situation is more complicated. Since the technique as defined does not involve chemical affinity, a critical problem in aqueous media has been the suppression of unwanted sorptive effects.

The use of porous glass for biopolymer separations was pioneered by Haller (*37*). Various slow organic gels have also been used. Efforts to suppress protein adsorption and denaturation while obtaining sharp peaks and fast chromatography are beginning to succeed (*38*). In general, polyelectrolyte chromatography is complicated by inter- and intramolecular interactions, among other effects, and continuing attention is needed in this area (*39*).

Progress in SEC will probably continue even when the ultimate speed and peak sharpness have been obtained. Solubility parameter theory predicts that partition should increase in affinity as molecular volume is increased. In a recent report (*40*) describing the use of reversed phase BPC, there was a pronounced retention effect for polystyrene that varied directly with polymer size—the opposite of the effect in SEC. The initial liquid composition was not far from polymer insolubility. It would be a surprise if this idea were not applicable to other kinds of polymers.

Looking Ahead

Chromatography seems to work best in combination with other techniques. For instance, nonredundant (that is, dissimilar) combinations of selectivity effects have the potential to make LC more powerful than any currently known limit. Examples include the use of varied LC interactions, known as column switching (*41*, *42*), or combinations of detection principles, known as hyphenated methodology. Automation has begun to build reliability into and to take the drudgery out of repetitive experiments.

A simpler and more reasoned implementation of chemical selectivity is being developed. There is a relation between LC control, its dependence on analogs, and the role of selectivity. The instrumentation in LC is suited to user-programmed operation. The column and liquid incorporate programming in the form of chemical software, interactive chemical codes that carry out a sorting operation on other codes borne by the sample chemicals. McCorduck's quotation (*43*) from physicist Donald MacKay seems appropriate: "Being an analog man myself . . . I started thinking what sort of general mechanisms one could conceive of, artificial mechanisms, which would handle information in a more general sense than a digital computer does. For instance, a digital computer is unable to represent the concept of in between." LC represents that concept surprisingly well.

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Multichannel Detection in High-Performance Liquid Chromatography

James C. Miller, Stephan A. George, Barry G. Willis

High-performance liquid chromatography (HPLC) has been the fastest growing technology in the analytical field since the early 1970's (*1*). Some of the more notable advances in HPLC have been improvements in detector technology. With the advent of integrated circuits and inexpensive but powerful microprocessors, HPLC detectors have become so refined that they are now commonly the most sophisticated equipment in an HPLC system. Yet even with major advances in detector technology, the technique of HPLC has suffered from lack of a universal detector that provides optimum sensitivity and flexibility.

Currently, the most popular detectors in HPLC are photometers that measure absorbed ultraviolet and visible radiation. Photometric detection is highly sensitive for many compounds, but the technique has been somewhat limited by the technology available in commercial detectors. In this article we describe the development, design, and applications of a new commercially available photometric detector that uses photodiode array technology and computer network concepts to overcome technological limitations. Although it is still limited to compounds that absorb ultraviolet and visible light, this detector allows simultaneous acquisition of light intensity data at all wavelengths between 190 and 600 nanometers and is a powerful addition to liquid chromatographic detection.

James C. Miller is product support manager, Stephan A. George is product manager, and Barry G. Willis is project manager in R & D for spectroscopy products at Hewlett-Packard Company, Palo Alto, California 94304.

Development

The first HPLC photometric detectors were fixed-wavelength ultraviolet detectors using the strong 254-nanometer emission line of low-pressure mercury lamps. The low cost and relative universality of these detectors have made them the most common type on the market today. However, these detectors have the severe limitation that they are committed entirely to one wavelength. This

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