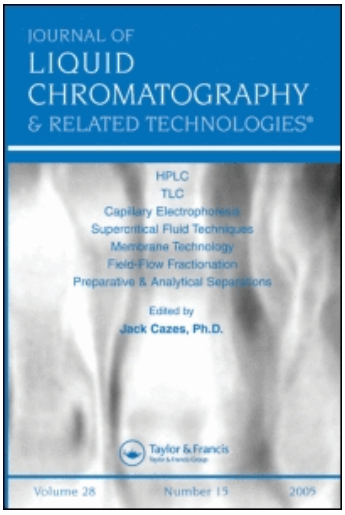

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History of Chromatography

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HISTORY OF CHROMATOGRAPHY

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INTRODUCTION

In an attempt to prepare a "History of Liquid Chromatography," it was evident, as is apparent to workers in the field, that one ends up with the generalized term "chromatography." None of the modes of chromatography covered here is without its liquid phase or mobile phase. Therefore, the title of this history is that of "Chromatography."

It must be pointed out that it would be impossible to include all the events which ultimately led to the technology as we know it today. There are instances in this review where many may disagree. With this warning in mind, the presentation which follows is an attempt to cover the highlights of the development of chromatography.

THE BEGINNINGS OF CHROMATOGRAPHY

It is interesting to note that, in spite of the general acceptance of the contributions of Tswett as being among the first to advocate adsorption in separation technology, this investigator is not the real discoverer of chromatography. Chromatography may have had beginnings in 1855 when Runge[1] published his work on dye separation on paper. Perhaps the real start of chromatographic analysis should be credited to Goppelsroeder, who presented some of his work in 1861[2] and Schonbein, who described separation of substances adsorbed onto filter paper[3].

Fischer and Schmidner[4] were aware of these works according to their reports which came out in 1892. However, the concept of separations on columns might be attributed to the work of Reed[5], who described some his early experiments using columns in 1893. Day, in 1897, demonstrated the use of columns for separation of petroleum fractions[6]. A short time later, Engler and Albrecht[7] followed with descriptions of fractionation by collecting eluants from a column. There have been other workers during this time who were practicing "chromatography" before Tswett[8,9] published his work in the separation of fragments. The following review presents further development of chromatography which had a renaissance in the 1930's.

The "rediscovery" of chromatography as it is now known dates from the reports of separations on columns by Kahn and Lederer[10,11]. Lederer separated carotenoids from egg yolk following perusal of Tswett's descriptions of chromatography.

PAPER CHROMATOGRAPHY

As was mentioned in the introduction, paper chromatography appears to be one of the earliest forms of chromatography. Paper chromatography was practiced by Pliny (23-79 AD) using papyrus impregnated with gall nuts for detection of ferrous sulfate. During the nineteenth century, vat solutions were checked for purity in the dyeing industry by spotting an aliquot on paper or cloth and observing the number of concentric rings formed as the liquid spread[12]. The method of spot testing of colored substances on paper was also investigated by Runge and Reed. These were predated, as mentioned in the introduction, by the reports of Goppelsroeder[2].

Progress was slow until Flood[13] described chromatography on impregnated papers. Brown[14] obtained separation using circular chromatography on paper. It was the work of Martin, Synge, Consden and Gordon[15] that advanced paper chromatography to the point where it could be considered practical.

Neuberger, who was interested in separating the neutral amino acids, observed that the partition coefficients of acetylated amino acids between water and an immiscible organic solvent differed for various amino acids. Martin and Synge[16], using this observation, attempted, in 1941, to separate amino acids by countercurrent distribution. This work was not successful, so they used silica gel as an inert support

to hold the aqueous phase and passed the immiscible organic solvent through a bed of the water-containing silica gel in order to conveniently bring about greater contact between the two phases.

The preparation of silica gel was tedious, so, to reduce the quantity of material needed, filter paper was substituted as the inert support. With the use of filter paper, the acetylation was no longer necessary, since these compounds could now be detected directly on the paper by treatment with ninhydrin. This idea led to "paper partition chromatography" as we know it today.

Until 1956, there were some 10,000 references dealing with paper chromatography, followed by 8,292 references in 1960. Several columns on paper chromatography were published, notably that of Hais and Macek[17] and that of Bush on the paper chromatography of steroids[18]. The book, "A Manual of Paper Chromatography and Paper Electrophoresis," appeared in 1958[19]. These were followed by numerous reports, but, because of its poor reproducibility and the advent of other methods, the technique began a decline.

By 1985, Index Medicus had only four references on paper chromatography. In the 1990 index, there were none. However, in 1986 Mattingly[20] reported the use of paper chromatography for screening amino acid abnormalities. The results of the screening of 800 pediatric and adult samples were presented.

GAS CHROMATOGRAPHY

The development of chromatography and its movement into gas chromatography (GC) owes much to the work of A. J. P. Martin and his coworkers. Martin was responsible for the emergence of liquid-liquid chromatography and advances in paper chromatography.

James and Martin published their first paper on GC in 1952[21]. In this report, they described the beginning of gas-liquid chromatography and the simple instrument which evolved. It is interesting that, in 1941, Martin and Synge[22] described their work on gas-liquid partition chromatography and gave, clearly, the concept od gas-liquid chromatography. It wasn't until 1949 that Martin and James resurrected the concept and subsequently published several papers on the subject[23].

However, the first published work on the use of gas-solid adsorption chromatography may have been that of Hesse et al.[24]. These investigators separated two hexanoic acids by passing their vapors, in a carrier gas of carbon dioxide, over a silica gel column. The first complete gas chromatograph probably came from Cremer's laboratory[25]. Thus, at this time, there appears to be concurrent development of gas chromatography, although in two different aspects, gas-solid and gas-liquid.

Applications for the use of GC for separations of various organic molecules began to follow rapidly, conditions for the column and requisite detection modules being available. Sweeley and Horning[26], in 1960, began their work with separation of steroids. Beerthuis and Recourt gave conditions for operating with some steroids[27]. But Sweeley and Horning developed thin films of siloxane coated onto inert supports. This was followed shortly by Touchstones report for separation of estrogens[28] and the use of electron capture detectors of Lovelock for sensitive detection[29].

The work of Claesson[30], followed by that of Ray[31] on detectors, resulted in considerable impetus when the thermal conductivity detector was described. Since it was largely a universal detector, many applications evolved from it. Lovelock[29] followed, in 1958, with the electron capture detector, using radium.

Flame ionization detectors were first described by Harley, Nel and Pretorius[32]. Not a simple as the thermal conductivity detector, it had a wide range of solutes for which it could serve as a detector, and it offered high sensitivity. This was followed by the argon ionization detector of Lovelock[29] based on earlier work of Jesse and Sadankis[33]. The hydrogen flame detector was described by Scott in 1955[34]. Perhaps a very significant development was the modification of a mass spectrometer to continuously monitor the effluent from a GC column, as described by Holmes and Morrell[35]. Gohlke[36] used a time-of-flight mass spectrometer to monitor the effluent gas from a GC. It has become a prime factor in GC development.

The above citations are by no means a complete chronology of the early history of GC. Those cited indicate that the beginnings of detector development followed shortly after the reports of the development of the mother instrument itself. Those cited, perhaps, were in the forefront of the early development of GC. This is to say nothing about the development of the numerous packings, coatings and other

column materials largely developed by the manufacturers of the polymeric substances and inert stationary supports.

Even at the time the packed columns were dominating the field of GC, Golay was already investigating the operating parameters of packed columns from the theoretical standpoint[37]. His data showed discrepancies between theoretical and experimental results. From these experiments, Golay designed an open tubular column[38], coated on the inside walls, which eventually evolved into the capillary columns in widespread use today.

More recent advances in GC will be illustrated briefly as they chronicle the steady growth of the technique since the 1970's.

Headspace analysis GC may be a very practical means of analysis, especially in the clinical laboratory. Robinson et al. reported the analysis of more than 200 volatile compounds from urine in an attempt to find differences between healthy and diseased subjects[39]. Indirect headspace analysis was applied, by Labows et al.[40] to species of pseudomonas growing on solid media in flasks. GC/MS was used to follow the release of volatile compounds.

Direct headspace analysis is now widely used. It has been automated for use in blood alcohol determinations[40]. Drasar et al. used direct headspace analysis to detect volatile acids from clostridium[41]. Larsson et al.[42] used this technique to detect growth in blood cultures.

GC/MS is one of the most universal techniques for analysis. It is also very sophisticated from the instrumentation standpoint. The mass spectrometer itself represents a very sensitive, selective detector. Along with this, it can provide spectra which are useful for identification. As noted above, this technique was in use as early as 1957.

THIN LAYER CHROMATOGRAPHY

The beginnings of thin layer chromatography can be attributed to the report of Beyerinck who separated sulfuric and hydrochloric acids as rings on a thin layer of gelatin[43]. Wijsman[44], using the same technique, separated the two enzymes present in malt diastase. As pointed out in the introduction, there was a lag phase in the early history of chromatography. Izmailov and Schraiber[45] used aluminum oxide, spread on

a glass plate. No binder was present and circular chromatography was performed by placing a drop of solution and development carried out by placing solvent drops in the center. They also mentioned the usefulness of this method for testing sorbents for column chromatography. Lapp and Erali[46] used a method of loose layer chromatography. Sorbent was spread as an 8 cm long layer on a glass slide. This was placed on an inclined aluminum sheet and solvent descending movement was used to develop and separate a sample placed at the upper end. This was followed by Meinhard and Hall[47] who used a mixture of aluminum oxide and Celite in a layer on a microscope slide to separate inorganic ions.

The real impetus for advancement of TLC started in 1951 when Kirchner and coworkers published their first paper showing the use of larger plates (5-1/4 x 5-1/4 in.)[48]. This was followed by the report of Stahl et al.[49] in which methods for preparation of more stable layers were described. These two workers are generally given credit for improving awareness of the advantages of TLC, especially since they published books on the subject.[50, 51]. Stahl's major contributions were the standardization of materials, procedures and nomenclature, as well as descriptions of selective mobile phases for resolution of major classes of compounds. The laboratory manual he produced popularized TLC. The aid of manufacturers at this time provided the groundwork for the growth of TLC which has continued since then. According to a review in Analytical Chemistry, 4300 papers were published between 1985 and 1987.

Quantitative TLC has experienced slow growth since it was introduced in the works of Kirchner et al.[52] who described an elution procedure for biphenyl in fruit. Densitometry had its beginnings in 1964 when Dallas and coworkers described their work with the Joyce Loebl Chromascan[53]. Densitometry moved forward with the appearance of the Schoeffel instrument, which may have been the first instrument which gave reproducible and practical quantitation of materials separated on thin layers. The use of this instrument and its application was described by Touchstone et al. in 1971[54]. Since that time, several instruments for direct scanning in TLC have been produced, notably those of Camag and Shimadzu, which were designed, primarily, for scanning TLC plates. The video scanner which was recently introduced by Analtech, should provide further impetus to quantitative methodology.

Another development in the field was the introduction of high performance TLC layers (HPTLC). These were produced commercially in the mid-1970's. Halpasf and Rippleahn[55] described the development of these in their review. In spite of the significant advantages of HPTLC, such as speed, smaller sample capability, as well as greater detection limits, conventional layers are still being used two to three times as much.

Manufacturers are increasingly producing layers with modified sorbents. Reversed phase C_2, C_8, C_{12} and C_{18} are chemically bonded. As the field advances further, more improved layers will become available. With the advance of TLC, instruments and improvements in usage became available. Some of these are adaptations of methods advocated in the early days of TLC. Although as early as 1962, Heyns and Grutzmacher[56] reported that analytes could be evaporated directly from silica gel into the Mass spectrometer source, [progress was slow utilizing direct MS of materials separated on TLC. In 1970, Hutzinger and Jamieson[57] demonstrated that indoles could be analyzed by this technique. The problem here was that the high temperatures required tended to decompose the analyte before MS took place. A number of workers removed the analyte from the plate and the mixture of sorbent and sample introduced to the MS by placing it on the direct insertion probe. Bay 1981, Unger et al.[58] described the analysis of TLC-separated components by secondary ion mass spectrometry. Until now, the methodology involves extraction of some form. Unger sputtered the sample directly from the TLC matrix. In 1985, Nakagawa et al.[59] were granted a patent for mounting small sections of plates (strips) that could be mounted directly to a movable direct insertion probe in the mass spectrometer. This was followed by the use of laser desorption for removal of the analyte into the MS probe as reported by Novak et al.[60]

Busch et al.[61] were the first to describe custom-built secondary-ion MS for analysis of TLC. The TLC is placed directly into the vacuum chamber. Several modifications and variations have been described by this group. In 1989, Busch's group reported replacement of the MS detector with a CCD optical camera detector[62]. The future of TLC-MS equipment may show improvements in the TLC systems.

Infrared TLC

Methods for direct transfer of analytes to KBr powders have been described. Most of the early work here involved elution of the analyte followed by adding the eluate, via solvent, to the KBr pellet.

The direct measurement of TLC fractions on the plate can be performed by diffuse reflectance or photoacoustic spectrometry[63]. However, in situ measurement can result in band shifts due to intermolecular interactions that occur between the analyte and stationary phase. The spectrum of the analyte will then appear different from the reference spectrum of the same compound obtained using conventional methods of sample p[reparation. The intensities of analyte bands are often strongly attenuated in regions where the silanol groups of the stationary phase absorb. The alternative to in situ measurement is to transfer the TLC fractions to an IR-transparent substrate prior to IR measurement. Although the transfer approach is less straightforward than the in situ measurement, better quality spectra are obtained.

The commercial accessory is based upon a sample transfer approach by which the mobile phase is made to flow sequentially in two directions as in two-dimensional TLC. The separation is performed in the first direction, followed by transfer in the second direction. The TLC fractions are thereby transferred simultaneously to an infrared-transparent powder for diffuse reflectance measurement. This procedure is done automatically with an instrument called the Optitrain. After the TLC is developed, the plate is removed, dried and placed in the instrument. Solvent is applied at right angles to the separation. This elutes the analyte into a chamber of stainless steel wicks. At the top of each wick is a cup filled with an IR-transparent diffuse reflectance powder. A controlled air flow is directed across the surface of the IIR, the Optitrain drawing the components through the wicks and powder until the have been concentrated at the tops of their respective cups. These are then inserted into the diffuse reflectance sampler and the measurement is made. These procedures are based on the work of Shafer et al.[63].

Overpressured TLC, or forced flow TLC is a system which can be analogous to HPLC. This could represent a "flattened column" under pressure. A membrane or pressure cushion covers the layer and is maintained under pressure with water or air. The key is that atmospheric vapors and contaminants are excluded. Variable flow is

established by controlling the pressure applied to the mobile phase. Linear, circular and anti-circular development can be performed. The basic instrument was first described by Kalasz and coworkers in 1980[64]. Since then, a number of reports have appeared which describe applications. Notable are those published by Tyihak[65] who separated thirteen sulfur-containing amino acids. Gulyas et al.[66] separated nine doping agents. By increasing the development distance, by using overrun or continuous development, flavonoids were readily separated[67]. Thirteen digitalis glycosides were separated in eight minutes by Mincsovics et al.[68] Although the instrument has shown some advantages, it has not seen general acceptance in the United States. Perhaps, when newer, improved models appear, this will change.

LIQUID CHROMATOGRAPHY

Liquid Chromatography had its origins in the early days of "chromatography." As outlined in the introduction, column chromatography was perhaps made practical by the work of Tswett. Twenty years passed since the initial steps were performed for the beginning of the analytical process that is used at present. Kuhn and Lederer[69] separated pigments following Tswett's procedures in 1931. It was not until the early 1940's that Martin and Synge published their classic paper on liquid-liquid partition chromatography.[70] In 1952, they showed the practicality of GC. They also indicated that fast analysis by LC would require small particle sizes for the column and use of pressure on the mobile phase. The advent of high speed liquid chromatography was realized in the late 1960's, when Kirkland[71], Huber[72], and Horvath[73] published their work on the development of high performance liquid chromatography as it is known today. Hamilton, Bogue and Anderson, in 1960 however, had already reported the use of HPLC for separating amino acids, but this received little notice. Credit for development of the system as it is now practiced goes jointly to Kirkland and Huber. Perhaps the real beginning of HPLC was when uniform small-particle sorbents were developed. The development of modern HPLC is linked to advances in silica technology.

Credit must be given also to Horvath for his early work, which undoubtedly was responsible for the success of HPLC as it is known today. In the early 1960's,

progress was hindered by the lack of suitable sorbents for use with HPLC. In the laboratory of Halasz, glass beads were prepared with a porous outer layer.[73] Since coating glass beads presented problems at that time, he switched to glass beads coated with styrene-divinyl benzene polymer. Using these as packings, which resulted in ion exchange characteristics, Horvath[74] was able to separate nucleotides. In this work, he also utilized, perhaps for the first time, elevated temperature in HPLC. With this, he was instrumental in the design of the forerunners of commercial HPLC.

The work of Kirkland, and the resultant special support particles consisting of impervious centers with thin, porous surface sorbents must be cited.[75] The duPont group was also instrumental in establishing parameters required for producing practical instruments for HPLC.[76] Snyder and Kirkland[77] greatly advanced HPLC when their volume, "Introduction to Modern Liquid Chromatography," appeared. The second edition was a much expanded version.

Beginning at this time, instrument manufacturers began serious work to develop a practical HPLC system. Much of this work was related to detector design. Most work, at present, employs ultraviolet detection. Refractometers and fluorometers emerged after design parameters related to the column were overcome. Electrochemical detectors are also being used.[78]

Fluorescence detection is being increasingly used.[79] Perhaps a significant advance in detectors should be noted, i.e., diode-array detectors, which are rapid-scanning ultraviolet detectors based on the linear photodiode. The resultant spectra, together with chromatographic characteristics provide dual criteria for component comparison identification.

Affinity chromatography is the most specific chromatographic method. Principles of affinity chromatography were discussed by Walters.[81] Isoenzymes of LDH were separated by affinity chromatography with Cibachron Blue F3GA on silica gel columns.[82] As the field grows, modifications in column design are developed. Open capillaries described by Kucera and Guiochon[83] in 1983 give improved separations. Packed capillaries have also been described.[84] Katz and Scott reported super-speed HPLC separations in which mixtures were separated in 4 seconds.[85]

As HPLC and capillary electrophoresis (CE) continue to grow, it would not be surprising to see the two fields merge. When we consider that packed capillaries are used in both HPLC and CE, it appears this will be the case.

SUPERCRITICAL FLUID CHROMATOGRAPHY - EXTRACTION

Since it was first described by Klesper[86] in 1962, supercritical fluid chromatography has seen sporadic development. This is probably due to the more rapid advances in high performance liquid chromatography. Once instrument manufacturers became involved, HPLC became very a very successful technique. However, the development of open tubular, fused silica capillary columns by Lee and coworkers[87] gave supercritical fluid chromatography its first real boost some 20 years later. This was followed by the introduction of a commercial instrument by Hewlett-Packard in 1982. Early work here by the two groups mentioned above focused on column technology and determination of operating parameters. Mass transport properties of SFC provided a basis for greater numbers of theoretical plates than with other chromatographic techniques. However, progress was slow until improved instrumentation became available in 1985. More applications were reported shortly thereafter.

Further growth in this area will depend upon successful preparation of suitable columns for specific applications. The technology of HPLC columns has been primarily applied for SFC. Injection systems are also open to improvement. Even more important is the need for immersed detection systems. The review of the current status of SFC, by Smith, et al.[88] offers a thorough assessment of what is needed for further growth of SFC.

Perhaps more important for supercritical fluid technologies is the growing emergence of sample preparation techniques. It is interesting that sample preparation has lagged far behind that of the chromatographic separation itself. The multitude of conventional extraction methods that are so time consuming and wasteful has stimulated interest in the use of supercritical fluid extraction. Technology here is only recent. Only 26 papers were published between 1986 and 1989. As more is learned, the field should grow rapidly. Solvent polarity modification and enhancement of selectivity in extractions will depend on a greater understanding of

separation mechanisms. An Excellent review was presented by Hawthorne[89] who has worked extensively in this field. Wheeler and McNally[90] have presented data for applications which should provide additional impetus for growth. The future of the instrumentation perhaps is dependent upon the ability to optimize desirable characteristics.

CAPILLARY ELECTROPHORESIS

Again, history of a technique appears to originate earlier than many might think. As early as the late 1800's, electrophoretic separations were attempted in solutions and gels. Many of these separations were performed in glass U tubes, with electrodes in the arms. An example is that of Hardy[91] who studied the movement of globulins in various U-tube designs. Problems of convective mixing were significant. In its early stages, capillary electrophoresis was described as a free solution electrophoresis in capillaries.[92] Hjerten gave the earliest demonstration of high electric field strength in 3mm ID capillaries in 1965.[93] Virtenen used smaller diameter columns in 1974.[94] Mikkers et al.[95] performed zone electrophoresis with 2 mm capillaries. These earlier studies did not produce satisfactory results because of poor instrumentation. The advancement of capillary electrophoresis can be attributed to Jorgenson and Lukacs[96] who reported experiments that showed theoretical and experimental approaches. Advances were slow until 1988, when commercial instruments first became available. CE has the advantage that selectivity, resolution and analysis time may be readily optimized. Progress has been rapid since commercial equipment provided a firm base of standardized instrumentation and replaceable components. Most workers prefer to buy components rather than to make their own for this reason. CE is no longer a scientific curiosity, and publications now focus on applications rather than theory.

Column technology has been a focal point of some recent reports. Tsuda et al.[97] described rectangular capillaries, which have the advantage of more efficient heat dissipation. Injection volume can also be increased. Detection across the long cross-sectional axis increases sensitivity.

A disadvantage of uncoated fused silica capillaries is poor reproducibility of electro-osmotic flow and adherence to the capillary wall. Lee et al.[98] used an external

electric field to control electro-osmotic flow. Another means of controlling wall effects was reported by Cobb and Novotny[99] who offered procedures for coating the capillary wall. This technique was also used by Towns and Regnier, who used polyethyleneimine to mask the silanol groups on the silica.[100] This was followed by the use of gel-filled capillaries which were developed by Beckman Instruments. Polyacrylamide gels were used, but preparation of the capillaries was tedious. Novotny et al[101] described a procedure which overcame these problems.

These recent developments indicate that the future will see development of more practical applications. Until recently, CE applications have focused on protein separations and DNA analyses. The separation of smaller molecules, particularly pharmaceutical preparations, is receiving increased attention. Capillary electrophoresis instrumentation appears to lend itself readily to miniaturization. Harrison et al have reported capillary electrophoresis and sample injection systems integrated on a planar glass chip.[102] Although most applications to date involve liquids as the medium, continuing reports point towards the use of packed columns (capillaries).

COUNTERCURRENT CHROMATOGRAPHY

In spite of the early reports of Craig[103] on the countercurrent distribution extraction apparatus, this technique received only limited acceptance, then seemed to fade away from existence, perhaps due to its slowness, and its limited applicability. The glass apparatus was fragile and cumbersome to manipulate. It was not until Ito and Bauman[104] reported on their countercurrent chromatography that the technology received attention and more general acceptance. Highly specialized equipment was still needed and, although separations with the "Ito coil" was much faster than Craig's countercurrent extractor, it left much to be desired in the areas of ease of use and limited applicability. The increased speed was made possible by applying a planetary force to the separating coil device, thereby permitting operation at higher mobile phase flow rates. However, the continuous nature of a device based on a continuous coil, coupled with its limited retaining force limited its use only with two phase solvent systems for which the density difference between the phases was large. Several

versions of the "Ito coil" have been reported. For example, Sutherland and Ito described, in 1978, a high speed toroidal coil chromatographic device which exhibited improved operating characteristics.[105]

Recently, a countercurrent chromatograph was interfaced with a mass spectrometer. Thermospray technology was employed to enable this interface.[106] About eight years ago, a commercial centrifugal partition chromatograph (CPC™) was introduced in Japan, then later, in the United States. In the the CPC device, a circular rotor contains numerous discrete partition channels which are used to hold the chromatographic stationary liquid phase while the mobile phase passes through the stationary phase as minute droplets. A centrifugal force, generated by high speed rotation of the rotor, retains the stationary phase in the partition channels, even with two-phase solvent systems where the density difference between the two phases is very small. High pressure rotary seals are used to permit operation over a wide range of solvent compositions and operating conditions; high mobile phase flow rates are routinely employed to minimize separation times. Sanki Laboratories, Inc. has pioneered in the development of a high performance centrifugal partition chromatograph (HPCPC™). The Sanki design can be readily scaled up to permit isolation and purification of kilogram quantities of chemical substances. Cazes et al.[107-109] have reviewed the development and current status of this technology.

As advances in instrumentation evolve, countercurrent techniques will become more widely used. Since solid stationary phase supports are not used, adsorptive effects are absent, separations may be performed at any Ph and biological activity is retained. Utility of countercurrent chromatography in biotechnology will be realized.

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