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Capillary-channeled polymer fibers as stationary phases in liquid chromatography separations

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Abstract

A method utilizing capillary-channeled polymer (C-CP) fibers as stationary phases in high-performance liquid chromatographic separations has been investigated. Polymeric fibers of differing backbones (polypropylene and polyester) having nominal diameters of ~50 and ~35 μm and a channeled structure on their periphery were packed into stainless steel tubing (305×4.6 mm I.D.) for use in reversed-phase separations of various mixtures. The fibers have eight channels running continuously along the axis which exhibit very high surface activity. As such, solvent transport is affected through the channels through wicking action. Bundles of 1000–3000 fibers are loaded co-linearly into the tubing, providing flow channels extending the entire length of the columns. As a result, backing pressures are significantly lowered (~50% reduction) in comparison to packed-sphere columns. In addition, the capital costs of the fiber material (<US$0.25 per column) are very attractive. Flow-rates of up to 5 ml/min can be used to achieve near baseline separation of related compounds in reasonable run times, indicating very fast mobile phase mass transfer (C-terms). The polymer stationary phases demonstrate high selectivity for a wide variety of analytes with gradient elution employed successfully in many instances. Specifically, separations of three polyaromatic hydrocarbons (benzo[*a*]pyrene, chrysene, pyrene), mixtures of both organic and inorganic lead compounds [chlorotriethyllead, chlorotriphenyllead, lead nitrate, lead(II) phthalocyanine], and a lipid standard of triglycerides were accomplished on the polymeric stationary phases. Other species of biological interest, including groups of aliphatic and aromatic amino acids have also been effectively separated. The reversed-phase nature of the fiber surfaces is supported through atomic force microscopy measurements using hydrophilic and hydrophobic functionalized polystyrene beads as the probe tips. Separations of the various analytes demonstrate the feasibility of utilizing C-CP fibers as stationary phases in reversed-phase LC. It is envisioned that columns of this nature would be particularly useful in prep-scale separations as well as for immobilization matrices for organic constituents in aqueous environments.

Keywords: Stationary phases, LC; Capillary-channel polymer fibers; Atomic force microscopy

1. Introduction

The development of new support and stationary phases for ''analytical'' liquid chromatography (LC) separations continues to be an active area of research. Silica and polymeric beads of 5–50 μm

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diameter have been the most widely employed support phases for many decades [1]. There are a large number of modifications available to enhance the selectivity of the surface of these supports that allow separations based on hydrophobicity, electrostatic, and molecular recognition interactions. These modifications have been extended to the coating of column walls to effect open-tubular separations for capillary-scale LC and electroosmotically-driven experiments. On the other end of the application spectrum are the high volume columns employed for ''prep-scale separations'' [2]. The relatively high efficiency afforded by spherical supports of small diameter used in ''analytical'' separations produces challenges with regards to the very high resultant backing pressures and subsequent high operation costs. As an alternative approach, the desire to effect high surface area-to-volume ratios (or specific surface areas) is a driving force for the development of monolithic support phases for all scales of high-performance liquid chromatography (HPLC) separations [3].

The development of open-tubular liquid chromatography (OTLC) has been a topic of high interest for over 20 years in hopes of attaining the highly effective surface area of stationary phases realized in capillary gas chromatography. While the underlying theory is well developed and has been experimentally verified [4–6], the combination of high backing pressures, difficulties in the application of uniform wall coatings, and extremely small sample volumes has limited their commercial implementation. An interesting approach to OTLC is the use of bundles of hollow polymer fibers as the solid phases. In these instances, pores within the tube walls are impregnated with organic modifiers that act as the stationary phase on the polymeric support. For example, Ding and co-workers used bundles containing 240 microporous polypropylene hollow fibers that were 61 cm long, having 100 μm I.D. [7,8]. Reverse micelles incorporated within the fiber pores were used to selectively retain proteins, which were subsequently eluted by changing the pH of the mobile phase. Yang et al. [9] have extended that work in the use of a single 250 μm I.D. polypropylene fiber treated with organic solutions acting as stationary phases for the separation of lanthanides. Here too, the organic solutes immobilized in the fiber pores serve to extract the metal ions from solution, which are then eluted as a function of mobile phase pH. Lamb et al. have also employed native polypropylene hollow fibers as support and stationary phases (combined) for anion separations by capillary electrophoresis [10]. In this application, the ''neutral'' polymer surface acquires a slight negative charge under high voltage application to effect solution electroosmotic flow. It should be noted here that each of the cited hollow fiber applications employed fibers of inner diameters far greater than the 10–20 μm diameter values generally acknowledged as being ideal for high efficiency OTLC separations [11].

Another approach to the use of polymer fibers as support/stationary phases for HPLC involves either solid-form continuous fibers (i.e., running the entire length of the column) [12,13] or ''packed'' fiber segments [14]. In these instances, the native chemistry of the fiber surfaces is employed to effect the separations. As such, the fibers serve as both the support and stationary phases. Chen et al. employed 1 mm segments of ion-exchange fibers (IFs) in the separation of anions [14]. Very symmetric peaks were produced for F^- and Cl^- at flow-rates of 1–3 ml/min, with backing pressures reported to be one-tenth those expected from packed-bead columns. Most relevant to the work described here, Jinno and co-workers have described the use of 250 μm I.D. capillaries in which bundles of cellulose acetate fiber running the entire length of the column (50 cm) are inserted in fused-silica capillaries [15–19]. In their work, the fiber surfaces have served as the stationary phases for reversed-phase capillary HPLC, capillary electrochromatography (CEC) and gas chromatography. The authors note that the columns are easily prepared (two fibers of 35 filaments each in a 200 μm I.D. capillary), and while the efficiencies are not as high as packed columns, fibers of different composition could yield dramatic improvements [17]. In a related body of work, Ladisch and co-workers have demonstrated the use of woven textiles for the separation of protein and polymer mixtures [20,21]. Fiber sheets of cotton/polyester and Nomex/Kevlar blends are rolled and inserted coaxially in the chromatography column tube. Important-

ly, the authors demonstrate the utility that can be realized by chemically modifying the textile materials.

We describe here the use of a novel form of polymer fiber as a support and stationary phase for liquid chromatography separations. Polypropylene and polyester fibers having nominal diameters of ~50 and ~35 μm (respectively) that are created by a spin-melt process and yielding eight ''capillary channels'' along the fiber axis, are presented. Fibers formed in this manner have capillaries that range from ~5 to 20 μm in diameter. Due to the high activity of the channel surfaces, the fibers exhibit a strong wicking action via a spontaneous wetting mechanism [22]. The fibers were initially developed for use in textile applications where fluid transport is desired, such as in disposable diapers. Chromatographic columns are prepared simply by pulling the fiber strands through 4.6 mm diameter stainless steel tubing typically used in liquid chromatography. In this manner, the fibers run the entire length of the column, and as the channels run co-linear with the mobile phase flow the analytes should tend to follow a single flow path. As such, a high degree of surface interaction can take place with a minimum amount of resistance. In addition, intra-channel flow might be expected to alleviate substantial contributions from eddy diffusion that is typical in packed-bead columns. Greater detail about the properties of these fibers is presented in subsequent sections. The use of the capillary-channeled polymer (C-CP) fibers as combined support and stationary phases for liquid chromatography is illustrated in separations of mixtures of polyaromatic hydrocarbons (PAHs), organic and inorganic lead compounds, amino acids, and triglycerides. The predominately non-polar nature of the fiber surfaces is exhibited in each separation as the test compounds elute in order of polar-to-non-polar character, indicative of reversed-phase (RP) behavior. In the majority of the demonstrations, gradient elution is employed to effectively achieve separations. The RP (hydrophobic) nature of the C-CP fibers is verified through atomic force measurements using chemically-modified probe tips. The combination of high flow-rates with high capacities suggest that columns prepared from the capillary-channeled polymer fibers could find application in prep-scale liquid chromatography and as immobilization media for organic waste in aqueous streams.

2. Experimental

2.1. Column preparation

Chromatographic columns were prepared by pulling strands of the polymer fibers through 4.6 mm I.D., 305 mm long stainless steel tubes (Valco Instruments, Houston, TX, USA), with the fibers extending the entire length. Polypropylene (PP) and polyester (PES) fibers (Eastman Chemical, Kingsport, TN, USA) were obtained from bobbins having total lengths of >1000 m. In the case of the PP, the fibers were processed as bundles of 45 fiber filaments, while in the case of the PE, the bundles consisted of 30 fibers. The strands were wound onto a ~30 cm diameter wire frame spool to accumulate enough strands to tightly fill the columns. The windings were removed from the spool and a single cut made in the fiber loop. As shown in Fig. 1, a metal wire ''hook'' was placed at approximately the middle of the fiber lengths and the wire pulled through the column tubing. Following a trial and error series of packing densities, the PP fiber column consisted of a total of ~1200 fibers, while the PES column consisted of ~3000 fibers. Table 1 summarizes some of the general characteristics of the C-CP fibers and columns. Based on commercial pricing, the cost of the fiber content of each column is <US$0.25. Fig. 1 also depicts the general alignment of the fibers within the column along with the direction of the mobile phase flow. Ideally, all of the fibers would be perfectly aligned along the tube axis without twisting, and should be uniformly distributed in the cross section of the tubing. Surely this is not the case in the simplistic loading process employed here, though future work will focus on the uniformity and reproducibility of the packing process. The fiber lengths were trimmed to be flush with the tubing ends using a razor blade and the tubes sealed with 0.75 mm thick, 6.35 mm diameter frits having 10 μm porosity and column end fittings (Valco Instruments). Before use in the separations, the fibers were washed by running distilled/deionized water

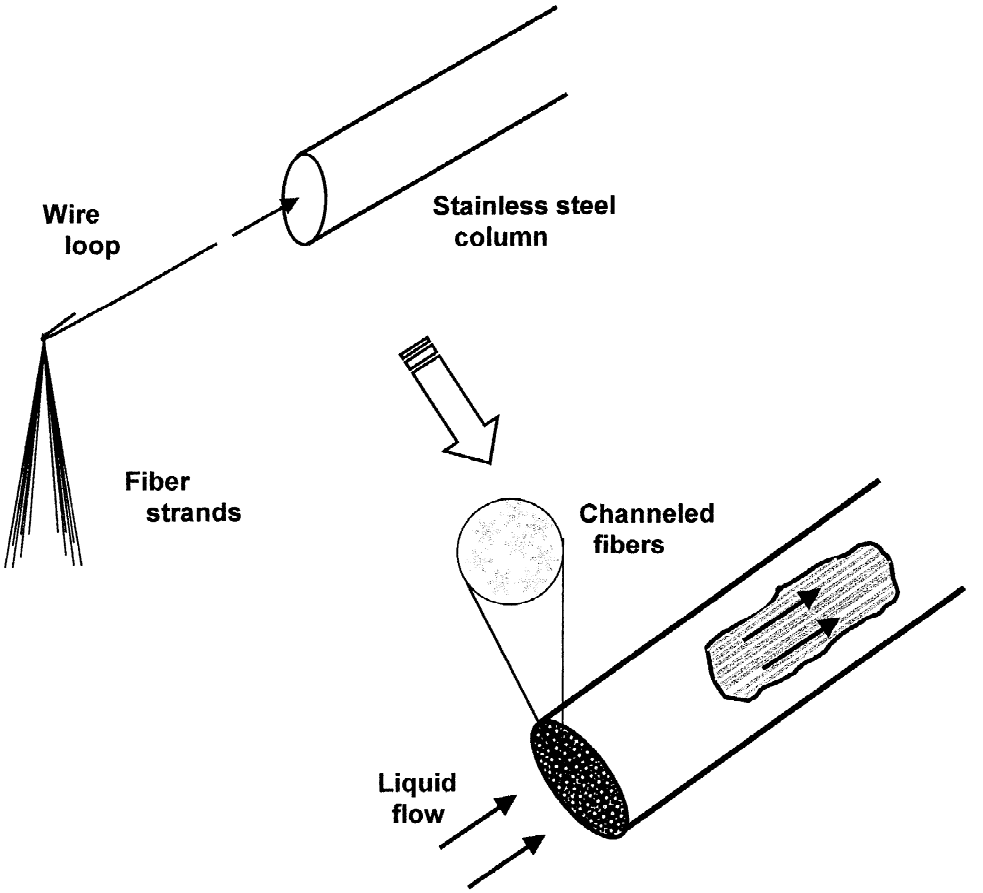


Fig. 1. Illustration of method employed for pulling capillary-channel polymer fibers into 4.6 mm I.D. stainless steel column structures and arrangement of fibers within the housing.

and organic solvents (methanol, MeOH or acetonitrile, ACN) through the column to remove residual anti-static detergent coatings from the manufacturing process. The fiber-filled columns were stored in a solution of water–methanol (50:50).

2.2. Sample preparation and solution delivery

Stock solutions of reagent-grade polyaromatic hydrocarbons (Sigma, St. Louis, MO, USA) in ACN–water (90:10) organolead compounds (Al-

Table 1
General properties of capillary-channeled polymer (C-CP) fibers and columns

Fiber type	Fibers/ bundle	Denier/ fiber	Average mass/ column (g)	Column porosity (ϵ_T)		
				ϵ_T (calculated)	ϵ_T (water)	ϵ_T (MeOH)
Polyester	34	4.4	2.5	0.49	0.55	0.53
Polypropylene	45	7.5	1.8	0.71	0.67	0.65

$\epsilon_T = V_0/\pi r^2 L$.

drich, Milwaukee, WI, USA) in MeOH–water (90:10) amino acids (Aldrich) in MeOH–water (30:70), and lipid standards (Aldrich and Alfa Aesar, Ward Hill, MA, USA) in ACN–water (95:5) were prepared with HPLC-grade solvents (Fisher Scientific, Pittsburgh, PA, USA). The separations presented here have been derived from previous ones performed in this and other laboratories on standard, C_{18}, reversed-phase packed columns. This was done simply as a convenient starting point for the evaluations. The separation of PAH compounds performed on the polypropylene polymer fibers was performed at a flow-rate of 1.5 ml/min by a combination of isocratic and gradient elution from water–ACN (90:10) to 100% ACN over 20 min. The PAH separation on the polyester fiber column was initiated under isocratic conditions of water–ACN (70:30), with a gradient change to a final value of 100% ACN over the ensuing 20 min. Separations of the inorganic and organic lead compounds were performed on both polymer fiber columns by a combination of isocratic and gradient elution from 100% water to 100% MeOH over 25 min at a flow-rate of 1.0 ml/min. The two amino acid mixtures were separated under different chromatographic conditions. The aliphatic amino acids were separated under isocratic conditions water–MeOH (70:30) at a flow-rate of 1.5 ml/min, while the aromatic species were eluted over a gradient of water–MeOH (97:3) to 100% MeOH. A lipid standard mixture was separated on the polyester fiber column by gradient elution from 95:5 to 5:95 water–ACN over 25 min at 2.0 ml/min.

2.3. Chromatographic system

The chromatographic system consisted of a Waters (Milford, MA, USA) Model 600S high-performance liquid chromatography pump with a Rheodyne (Rohnert Park, CA, USA) six-port injector valve fitted with a 20 μl injection loop. The C-CP fiber chromatography columns were simply mounted in the place of the conventional LC column. A Waters 2487 dual-wavelength absorbance detector was used for the separation evaluations and the determination of correct mobile phase compositions. The chromatographs (absorbance vs. elution time) were obtained from the Millennium 32 Chromatography Manager and further processed and managed in the form of Microsoft (Seattle, WA, USA) Excel files. In each of the presented chromatograms, the position in time where the solvent front should elute is labeled t_0.

2.4. Scanning electron micrographs

Micrograph images of the polymer fibers were taken with a Hitachi S-3500-N (Hitachi Scientific Instruments, Pleasanton, CA, USA) scanning electron microscopy (SEM) system. The fibers were mounted on standard stainless steel SEM platforms using double-sided graphite tape. In the case of the cross-sectional micrograph (Fig. 2b), a hemispherical steel platform was employed to mount the fiber in a near-vertical position. The images presented here were taken at accelerating voltages of 15–20 kV, yielding image magnifications of 600–700×.

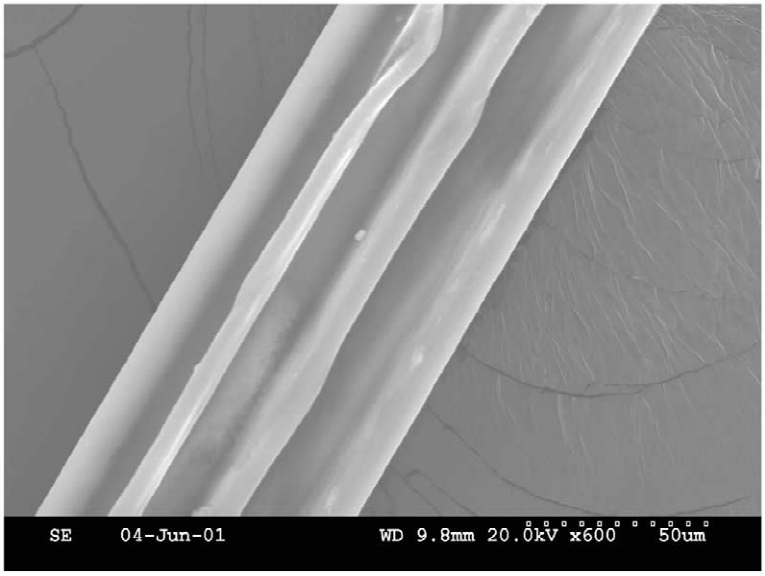
2.5. Atomic force microscopy

Assessment of the chemical reactivity of the polymer surfaces is fundamental to understanding the separation characteristics of the C-CP fibers. Topographic imaging and force measurements were performed using contact mode atomic force microscopy (AFM) on a Digital Multimode Nanoscope IIIa AFM scanner head E. The SiNi tips were purchased from Digital Instruments and the modified tips were purchased from Novascan Technologies. Force measurements were carried out using three different tips: a standard 100 μm narrow SiNi tip with a spring constant of 0.58 N/m and two different 200 μm wide modified tips with a spring constant of 0.12 N/m; one with a 5 μm polystyrene bead attached, and the other with 5 μm polystyrene bead attached and the addition of a COOH group. The setpoint for the measurements ranged from −3.00 to 0.200 V. Scan sizes ranged from 0.500 to 12 μm. The images presented are not filtered. Image analysis was performed using Nanoscope IIIa software (version 4.23) and Nanotec Software WSxM (1.1 Beta 5.1).

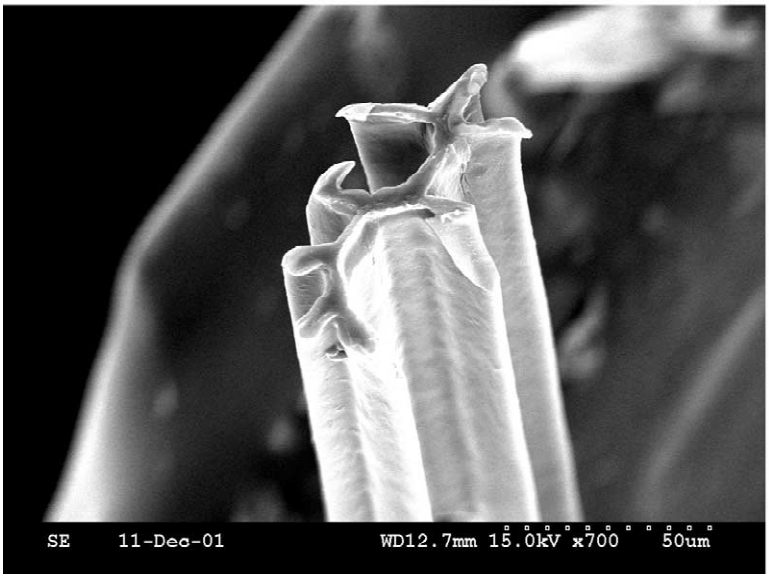
3. Results and discussion

3.1. Polymer fiber characteristics

The fibers employed here have four key aspects



(a)



(b)

Fig. 2. SEM micrographs of capillary-channeled polymer fibers (a) perpendicular image of polypropylene—magnification = 600, accelerating voltage = 20 000 V, (b) cross sectional image of polyopropylene—magnification = 700, accelerating voltage = 15 000 V.

which suggest their application as support/stationary phases for liquid chromatography: (1) large surface area-to-volume ratio, (2) strong wicking action (i.e., surface activity within the exposed channels), (3) collinear, continuous channel geometry and (4) low capital (materials) costs. Presented in Fig. 2 are representative scanning electron micrographs of the fibers employed in the separations described here. As can be seen in Fig. 2a, the polypropylene fibers appear to have a nominal diameter of ~50 μm with the walls of the channel clearly seen running along the fiber axis. It must be kept in mind that neither the

bulk fibers nor the channel walls are rigid, as seen in the slight bends along the channels. This flexibility is an asset in terms of fabrication of the columns, though may lead to some irreproducibilities in the packing process. An analogous micrograph taken of the polyester fiber is very similar in appearance, showing a nominal diameter of ~35 μm and channel wall thicknesses of ~3 μm, as seen for the polypropylene.

Fig. 2b is a scanning electron micrograph taken of a cross-sectional region of a polypropylene C-CP fiber. Seen more clearly here is the profile of the channel structure produced in the spin-melt processing of these fibers. The fibers are not actually a hub-and-spoke geometry, but more of a rectangular profile having a width (not diameter) of 50 μm, and a height of ~25–30 μm. The micrograph shows eight capillary grooves having diameters of 5–15 μm. The channel walls are very well defined, with sharp tips. Clearly, the active surface area of the fibers is far greater than that realized for solid fibers of circular cross sections of the same nominal dimensions. The fiber surfaces exhibit some surface structure or roughness that would also contribute to the effective surface area, though no pores are observed per se. The unit of measure describing this aspect of the fiber structure is termed the ''shape factor'' in the fiber/textile vernacular. Based on manufacturer-provided information, these particular fibers are produced in such away that their shape factors are in the range of 2.8–3.0. With regards to chromatographic separations, this infers that the exposed surface area is ~three-times greater for these fibers than cylindrical ones. This micrograph in fact suggests that this may be an underestimate.

A more practical measure of the potential for LC separations of the CC-P fibers is the effective surface area; determined by so-called BET measurements. In the case of polymer fibers that may in fact have surface porosity that provides additional interaction area, the use of nitrogen fixation measurements is a useful tool. BET measurements performed on both types of fibers are somewhat of a disappointment as they yield values on the order of 3 m^2/g. This number pales in comparison to standard spherical bead column packing materials of 100–500 m^2/g [1] and monolithic columns having values of 300 m^2/g [3], but is in line with the pellicular Poroshell (3–21 m^2/g) from Agilent [23] and NPS Micra-ODS-1 (<3 m^2/g) from Eichrom [24]. The specific volume of the fibers is ~2 cm^3/g, wherein the channel volume is approximately one-third of the total volume. Interestingly, the open area of the channels represents about 40% of the cross sectional perspective, which is in the range of free (inter-particulate) volume associated with packed columns [1]. Based solely on considerations of the effective surface area, C-CP fibers would need to exhibit very high analyte/surface activity and perhaps other benefits to be seriously considered for use in analytical reversed-phase liquid chromatography separations.

The last of the more general attributes of the C-CP fiber columns is the total porosity of the columns (ϵ_T). In the general case ϵ_T is the ratio of the void volume (V_0) divided by the total column volume:

$$\epsilon_T = V_0/\pi r^2 L \quad (1)$$

In the case of the C-CP fibers, the total porosity is essentially the void space in the fiber channels and inter-fiber regions that can be occupied by solvent (mobile phase). Based on the SEM micrographs of Fig. 2, porosity in the fiber structure is not an appreciable contributor. Theoretical ϵ_T values have been computed based on the mass of fiber placed in the columns and the practical values determined experimentally using both water and methanol as solvents. The theoretical values were generated by dividing mass of fibers by their density to obtain the ideal volume occupied by the fibers. The practical values were determined by placing the columns vertically in known volumes of the two solvents and allowing the natural wicking action to fill them to the top. The difference in solution volume in the graduated cylinder was taken as V_0. As can be seen in Table 1, the agreement between the theoretical and practical column porosities is within 10%, relative. The proximity of the values determined for the water and methanol solvents adds validity to the method of V_0 determination as well as reflects fairly uniform wetting of the fiber surfaces (at least for these solvents). The total porosity values of the C-CP fibers shown here are well in line with standard, commercial packed columns.

As the separations presented here are reversed-phase (hydrophobic) in nature, potential analyte/surface interactions can be assessed to a first approximation through the use of AFM though the use of

chemically modified probe tips. Standard SiN tips, and those with ~5 μm diameter polystyrene beads attached were used to perform (attractive) force measurements of the fiber surfaces. Hydrogenated polystyrene (PS-H) tips were used to assess hydrophobic interactions with the polymer surfaces while carboxylic acid-modified beads (PS-COOH) were employed to characterize the extent of hydrophilic character of the polymers. Measurements were performed on C-CP fibers in their native state (i.e., as spun, having surfactant coatings) and after washing to remove the coatings. In this way, the possible contributions of the coatings can be distinguished from the chemical nature of the native fiber surfaces.

The results of the contact-mode AFM force measurements are summarized in Table 2, which are the average of five measurements at different locations for each interaction type. Seen first is the fact that an attractive force does indeed exist between the surfaces and the ''inert'' SiN tips. The values seen here (reported in units of nano-Newtons) are appreciable, with the un-washed fibers showing elevated values, particularly for the polyester fiber. This is not surprising, as the surfactant coatings (which are not the same for the two C-CP fiber types) have multiple chemical functionalities. In the first case of the polyester fiber, the surfactant coating yields a very high attractive force value of >200 nN. The washed fiber, though, still results in a force measurement of >150 nN. This value supports the propensity for very strong hydrophobic interactions between non-polar analytes and the fiber surfaces. The corresponding data for the polypropylene fibers is interesting from the point of view that the surfactant used for this fiber type yields a polar surface, having an interaction of <1 nN (i.e., virtually no interaction). The washed fiber surface, though, yields an attractive force of >200 nN. The data presented for the hydrophyllic bead surfaces (PS-COOH) consistently points to fact that the fiber surfaces are indeed non-polar in nature as the force values are all less than 1 nN. These AFM force measurements point clearly to the differences in surface activity between the as-spun and washed fibers and the fact that there should be strong interactions between non-polar species in solution and both of the fiber surfaces. A study comparing the force characteristics of commercial packing materials and the C-CP fibers using modified AFM probe tips in certainly in order.

Table 2
Results of atomic force measurements between C-CP fiber surfaces and inert (SiN), hydrophobic (PS-H), and hydrophilic (PS-COOH) AFM probe tips

Fiber type	Tip material		
	SiN	PS-H	PS-COOH
Polyester			
As-spun (nN)	58	210	0.15
Washed (nN)	8	166	0.26
Polypropylene			
As-spun (nN)	13	0.31	0.15
Washed (nN)	11	210	0.21

3.2. Separation of polyaromatic hydrocarbons

At a first glance, one might consider that polypropylene and polyester would not be very interesting from a chromatography point of view. The nature of the channel surface must be considered on a molecular level, including chemical and physical attributes. For example, PP is a continuous aliphatic hydrocarbon with a methyl group stationed on every other carbon. PES is a continuous chain having both aromatic and polar (ester) character. Based the ''seemingly'' hydrocarbon nature of the PP and PE polymers fibers revealed in the AFM measurements, it was anticipated that columns would likely have reversed-phase character (i.e., non-polar analytes would tend to be retained on the fibers). Due to their associated toxic/carcinogenic nature and widespread existence, polyaromatic hydrocarbons are some of the most heavily studied compounds with regards to HPLC stationary phase development. Wise and coworkers have evaluated a variety of hydrophobic (hydrocarbon) bonded phases on both silica and polymer bead supports in an effort to develop a systematic understanding of the selectivity afforded by those stationary phases [25–27]. They found that the length-to-breadth ratio (L/B) of the individual PAHs was a key factor in the inter-analyte selectivity, implying that analytes fit into ''slots'' in the immobilized alkane chains. The more closely related the L/B values, the more difficult two compounds are to resolve on a given column. As described previously, the C-CP fiber surfaces might be better considered in two dimensions than three as there is very little hydrocarbon structure perpendicular to the

fiber surface. Even so, PAHs serve as a very useful benchmark for column development.

Fig. 3 illustrates the respective separations of 20 μl injection of equimolar ($1 \cdot 10^{-4}$) mixtures of pyrene, chrysene, and benzo[*a*]pyrene on the C-CP fiber columns. In each case, a solvent gradient is applied at a 1.5 ml/min mobile phase flow-rate. It is important to note that each of the compounds is entirely retained on the respective columns at mobile phase compositions of greater than 90% water. Only after increasing the organic content of the mobile phase are the analytes eluted from the columns. The

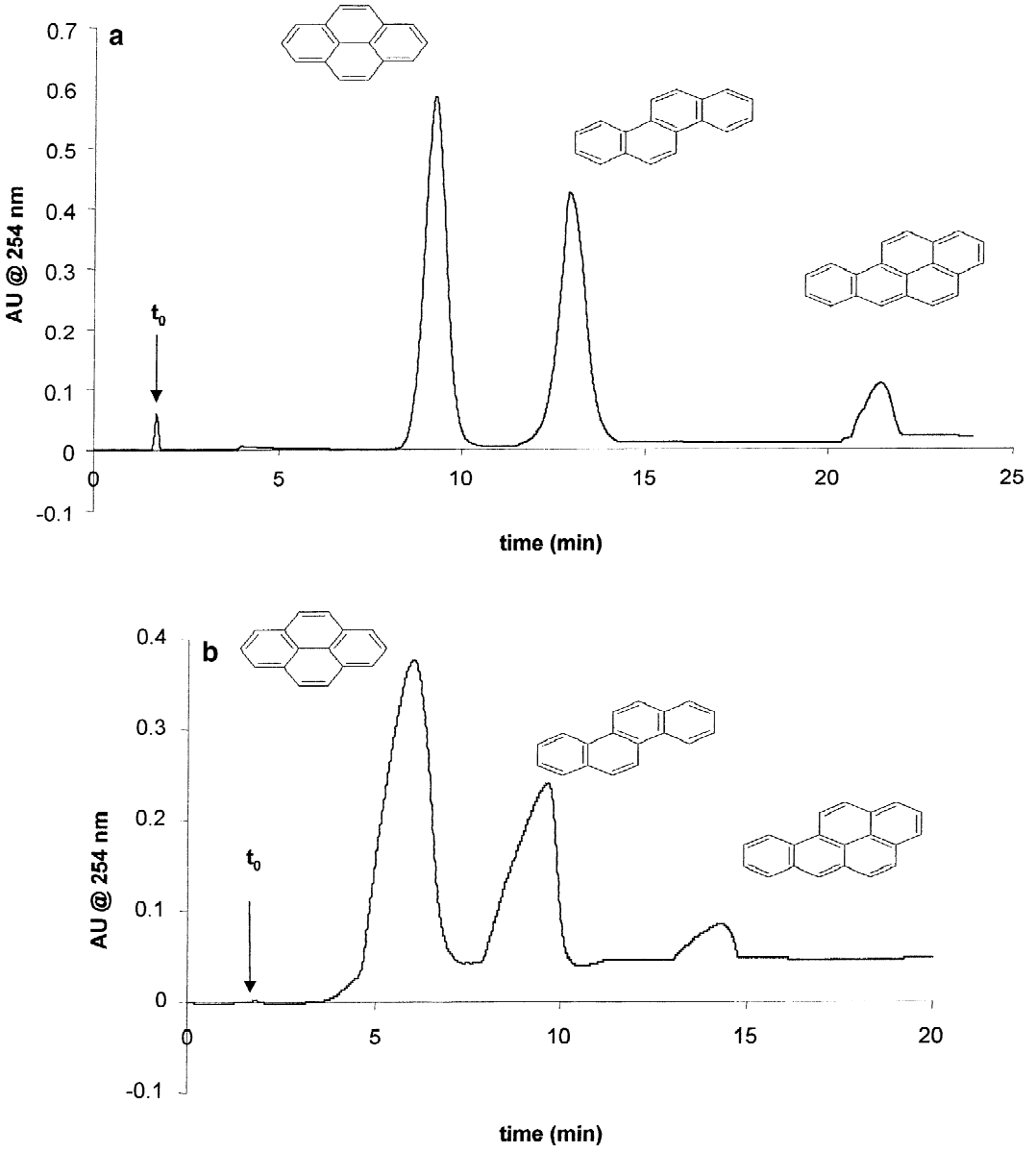


Fig. 3. Separation of $1 \cdot 10^{-4}$ *M* mixtures of polyaromatic hydrocarbons (PAHs) on (a) propylene and (b) polyester C-CP fiber columns. Elution order: pyrene, chrysene, and benzo[*a*]pyrene. Separation conditions: (a) gradient elution from water–ACN (90:10) to 100% ACN from 0 to 20 min, flow-rate = 1.5 ml/min; (b) gradient elution from water–ACN (70:30) to 100% ACN from 0 to 20 min, flow-rate = 1.5 ml/min. UV absorbance detection at 254 nm, injection volume = 20 μl.

chromatogram presented in Fig. 3a indicates that only when the composition of the mobile phase reaches a level of ~30% acetonitrile does the first of the analytes (pyrene) elute from the PP column. Increasing the organic content leads to the successive elution of chrysene and benzo[*a*]pyrene. The observed elution order is consistent with the reversed-phase separations of Wise and May in terms of increasing L/B ratios [25]. Interestingly though, the selectivity factor (α) between chrysene and benzo[*a*]pyrene is somewhat farther from unity than for C_{18} columns run under isocratic conditions, implying greater selectivity of the C-CP fibers. In fact, the benzo[*a*]pyrene is eluted at an earlier time here due to the higher organic content in this separation (i.e., without the gradient the time differential would be far greater).

The separation of the three PAHs on the PES column is presented in Fig. 3b. Different from the case of the PP stationary phase, a mobile phase composition of water–ACN (40:60) is required for the first component of the mixture (pyrene) to elute from the column. This indicates that the PAHs are more effectively retained on the polyester stationary phase, which is not surprising as the polymer backbone includes an aromatic ring (i.e., π–π interactions take place). In this particular separation, the solvent gradient was begun at the 70:30 value to shorten the analysis time, as such the time axes in Fig. 3a and b are not directly comparable. As with the case of the PP stationary phase, the elution order is the same as seen in conventional C_{18} reversed-phase separation, with baseline resolution realized for the three components. Different from the PP column, the peak shapes seen hear show a good deal of fronting, which is usually indicative of irregular column packing [1]. Studies are currently underway in this laboratory to further differentiate this phenomenon from possible π–π interactions between the PAH solute molecules in the mobile phase (i.e., a non-linear isotherm). Interestingly, in both of the PAH separations (Fig. 3) and all the subsequent ones presented here, elution peaks widths are constant (typical under gradient elution conditions) within a given chromatogram. This phenomenon is usually an indication that extra-column phenomena are the major contributors to band broadening. At this stage of development, dead volumes in the connections between the steel capillaries and the column ends are likely culprits. In addition, the breadth and non-ideal peak shapes (throughout these studies) are surely some reflection of the fact that each of the analyte solutions is prepared in solutions of greater organic content than the initial composition of the chromatographic solvent gradients. This situation inhibits the adsorption of analyte species at the head of the analytical column. A final cause for the broadening at present is overloading of the columns. Detailed studies are currently are currently underway to elucidate the capacity of the C-CP fiber columns.

3.3. Separation of lead compounds

One area of chemical analysis where liquid chromatography is seeing increased use is in the so-called speciation experiment [28,29]. In such a determination, the various chemical forms of a metal ion of interest are separated and hopefully identified. In the ideal case, the separation process would be able to differentiate between both inorganic and organic forms of the analyte. An investigation of the use of C-CP fiber phases to separate inorganic ions and organometallics is depicted in Fig. 4 for the case of lead compounds. The test mixture contained $Pb(NO_3)_2$, chlorotriphenyllead, and Pb(II)phthalocyanine, which are seen to elute in proper order for a reversed-phase mechanism. As in the case of the PAHs, the solutes are well resolved with the chromatographic peaks showing reasonable temporal structure (i.e., peak shapes). On the other hand, the chromatogram obtained from the PES column indicated that the inorganic and phenyl-lead compounds are barely retained and not resolved, with none of peaks showing good chromatographic quality. Different from the cases of the PAHs, each of the lead compounds has some ionic/polar nature, and so this is not a classical sort of reversed-phase separation. It is somewhat surprising that the inorganic Pb compound is retained to any extent, as it elutes after the solvent peak. This separation points to some other form of interaction, rather than one based solely on polarity. While no other evidence to this effect has been seen, this may be an indication of residual surface contamination from the surfactant coating.

3.4. Separation of amino acid mixtures

The previous separations have shown the ability to

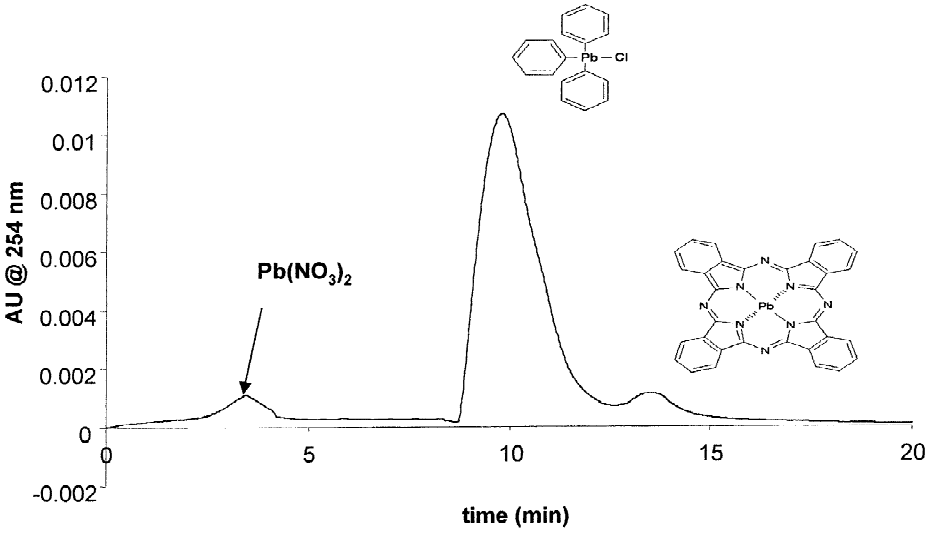


Fig. 4. Separation of a $1 \cdot 10^{-3}$ *M* mixture of lead nitrate, chlorotriphenyllead, and lead(II) phthalocyanine on a polypropylene C-CP fiber column. Separation conditions: gradient elution 100% water 0–3 min.; 100% water to 100% MeOH from 3 to 10 min. Flow-rate = 1.0 ml/min, UV absorbance detection at 254 nm, injection volume = 20 μl.

separate species according to molecular size/aromaticity (in the case of the PAHs) and polarity (in the lead speciation). Mixtures of amino acids pose a further challenge in that they have similar chemical functionality (amine and carboxylic acid) while having different hydrocarbon content. Separation of amino acids by liquid chromatography is usually performed by pH controlled reversed-phase or ion chromatography [30,31]. These two approaches rely chiefly on the manipulation of the charge states of the acidic/basic sites on the amino acids to affect the ionic character in the separation. Gradient elution with regards to solution pH is most common in ion chromatography separations. In many instances, the separation methods of amino acids and small peptides become driven by the subsequent detection means, as pre- or post-column derivatization becomes necessary [30]. Shown in Fig. 5a is resulting chromatogram from the isocratic (water–MeOH, 70:30) separation of the aliphatic amino acids glycine, alanine, and valine. As shown in the structures, these three species vary according to the number of methyl/methylene groups on the side chains on each molecule. The respective components separate cleanly with the retention order following the hydrocarbon content, as would be expected from a reversed-phase separation. The chromatogram shows very good resolution for the successive pairs of compounds, with the figures of merit for triplicate separations reported in Table 3. The separation quality and retention time precision values are quite encouraging given this early stage of development.

The use of a gradient elution approach to improve the separation speed is seen in Fig. 5b for the case of three aromatic amino acids (3,4-dihydroxyphenylalanine, tyrosine, and tryptophan). Here too, the molecular species are well resolved based on the respective polarity. In this case, the elution order follows the decreasing number of hydroxyl groups on the aromatic rings. Different from other amino acid/peptide separations, the solutions here are ''neat'', composed only of water and methanol. It is important to mention that the polyproylene C-CP fiber surface would likely be unaffected by the presence of buffers or differences in pH as dictated by particular biological specimens. As such, the actual separations would still occur based on the polarity of the components and not be subject to chemical effects that might deteriorate other stationary phase materials.

3.5. Separation of a triglyceride mixture

An interesting test of the separation character of the C-CP stationary phases with regards to biomolecule separations is a homologous series of

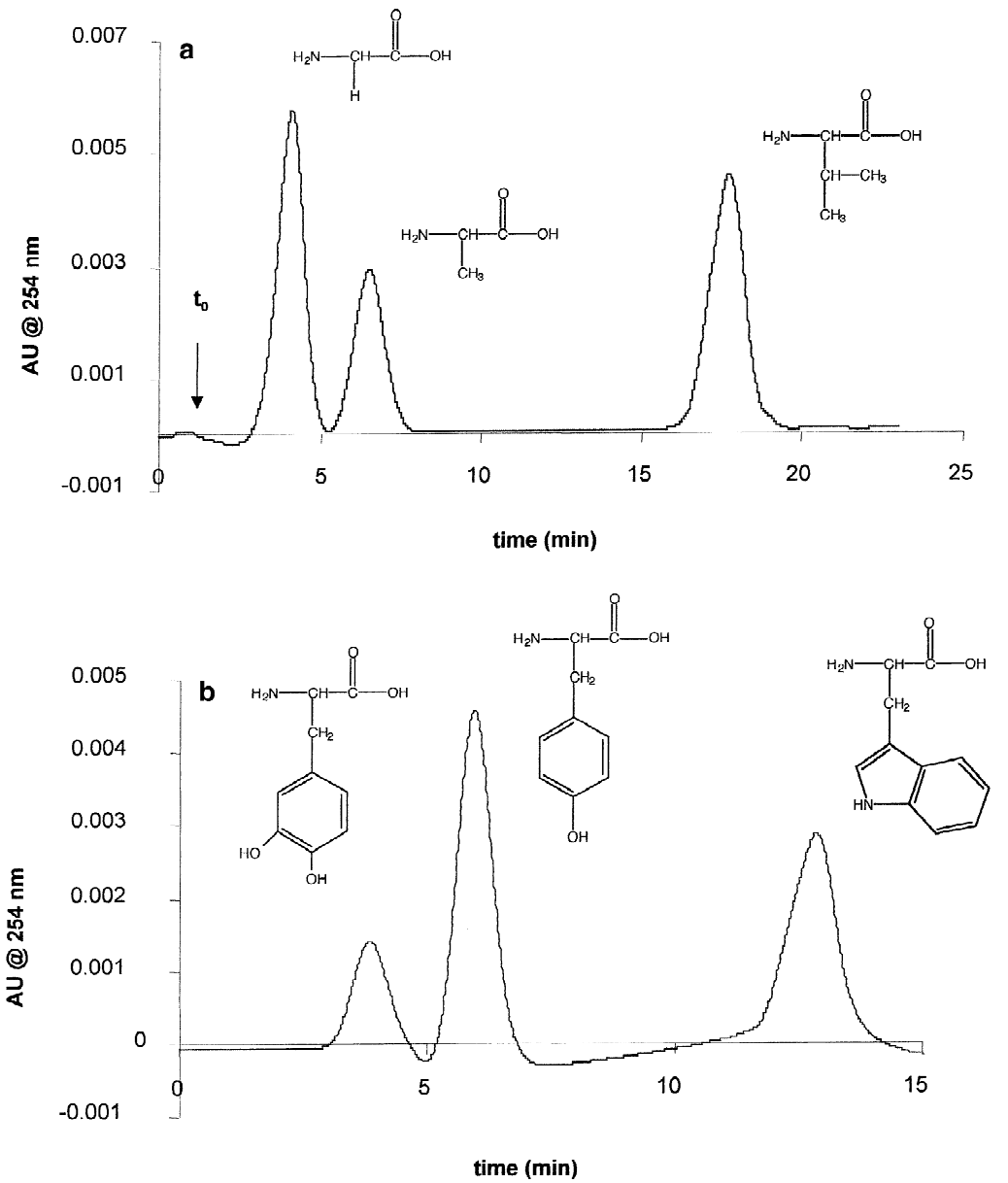


Fig. 5. Separation of $1 \cdot 10^{-4}$ *M* mixtures of (a) aliphatic amino acids and (b) aromatic amino acids on a polypropylene C-CP fiber column. Separation conditions: (a) isocratic elution at water–MeOH (70:30). (b) Gradient elution from water–MeOH (97:3) to 100% MeOH from 0 to 12 min. Flow-rate = 1.5 ml/min, UV absorbance detection at 254 nm, injection volume = 20 μl.

triglycerides. In general, the solubility of lipids is such that non-aqueous, reversed-phase and sub-critical separations are required, particularly for high molecular mass species [32,33]. The series tested here consists of tri-esters, which have successive additions of ethylene units alpha to the carbonyl carbons. As such, the triglycerides become sequentially more non-polar. The gradient elution (95:5-to-5:95) water–ACN chromatogram of the triglyceride mixture on the PES stationary phase shown in Fig. 6 clearly displays the expected trend in polarity as the analytes elute according to the length of the alkyl

Table 3
Chromatographic figures of merit for the isocratic separation of amino acids (glycine, alanine, and valine) depicted in Fig. 5a on C-CP fiber columns

Column type	Compound	Retention time (min)	Retention factor (k')	Selectivity (α)	Resolution (R)	Plate number (N)	Plate height (H, cm)	Repeatability (%, RSD, $n=3$)
Polypropylene	Glycine	4.5	2.0	–	–	56	0.54	5.0
	Alanine	6.9	3.7	1.8	1.8	120	0.25	6.8
	Valine	18.2	11.2	3.1	6.5	410	0.075	2.8

$k'=(t_R-t_0)/t_0$; $\alpha=k'_B/k'_A$; $R=2(t_B-t_A)/(w_{tA}+w_{tB})$; $N=16\cdot(t_R/w)^2$; $H=L/N$.

chains. The first of the analytes, triacetin, is in fact only weakly retained on the column as it is barely resolved from the solvent peak. While this is a rather limited suite of triglycerides, it clearly demonstrates the chemical nature of the polyester stationary phase with regards to the hydrophobicity of the test species. One other point of interest in this particular separation is the fact that the separation is undertaken at a mobile phase flow-rate of 2 ml/min. Preliminary studies indicate that flow-rates as high as 5 ml/min can be used in many of the separations shown here. Studies are currently underway to characterize the chromatographic quality achieved as a function of mobile phase velocity as a means of better understanding the operable mechanisms of the C-CP fiber systems as well as the tailoring of analytical and prep-scale separations.

4. Conclusions

The use of C-CP fibers as combined support/

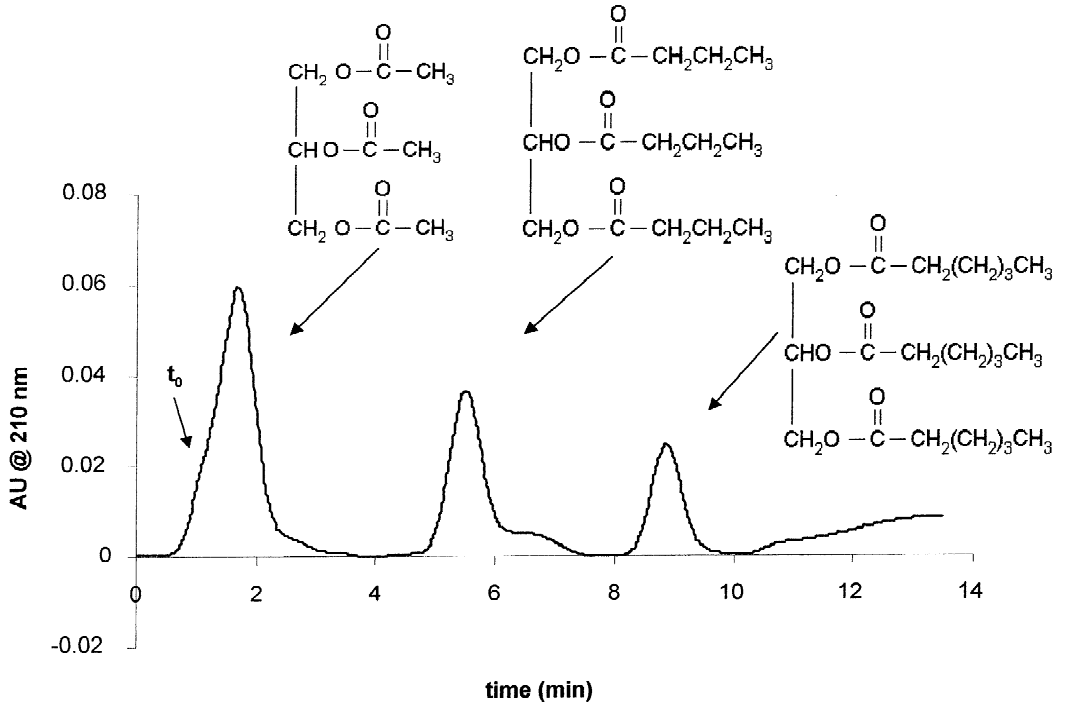


Fig. 6. Separation of a standard $1\cdot10^{-4}$ M lipid mixture containing triacetin, tributyrin, tricaproin on a polyester C-CP fiber column. Separation conditions: gradient elution water–ACN (95:5) to water–ACN (5:95). Flow-rate = 2.0 ml/min, UV absorbance detection at 254 nm, injection volume = 20 μl.

stationary phases in reversed-phase separations has been demonstrated. Separations of compounds including PAHs, organic and inorganic lead, amino acids, and lipids illustrate the possible implementation of fibers in diverse separation scenarios. Fibers with different polymer backbones (PP vs. PES) exhibit different separation characteristics based on surface chemistry. In each of the examples, the polypropylene fiber column consistently demonstrated better reversed-phase chromatographic (non-polar interactions) quality than the polyester column. This is perhaps not surprising given the totally hydrocarbon polymer structure versus the more functionalized backbone of the polyester. In the latter case, the aromatic character seems to come into play in some separations. Atomic force microscopy with the use of chemically modified tips reveals the very hydrophobic nature of the fiber surfaces and supports their reversed-phase separation character. The co-linear nature of the polymers within the steel tubing provides long, continuous flow channels stretching the length of the columns, resulting in significantly lowered backpressures (~50% reduction) when compared to packed columns. Based on a simple observation of the flow characteristics and the channel diameters, one might consider that the respective channels might act as individual ''open-tubes'' of near-optimum diameter and will be subjected to minimal eddy diffusion along the column length. Meyer et al. set out the operation principles of the use of such multicapillary columns in LC separations almost 2 decades ago [34].

There are a number of future avenues of development that are being considered. Certainly evaluation of the methodology for application in other chemical systems is suggested, with the separation of a mixture of proteins recently accomplished [35]. The column preparation procedure employed to this point is rather crude and is very likely to result in radial voids in the packing and some twisting of the fibers as they are pulled through the steel tubing. Even so, the precision in retention times for the analytes considered here were generally better than 10% RSD. Incorporation of a radial compression column may reduce the extent of voids left by the packing process while still maintaining a reduction in backpressure. Peak broadening due to column overloading is currently being investigated in detail. Based on the evidence of extra-column broadening, the plumbing of the system, including the header design, frit porosity, etc., will be examined [36–38]. There are chemical aspects of the use of the fibers that provide exciting opportunities. The basic manufacturing of the capillary-channel fibers is applicable to all spin-melt polymer systems (e.g., nylons, polylactic acid, and various ionomers). This presents a wide range of possible surface chemistries. In addition, chemical derivatization of the fibers could be employed as a means of tailoring the chemical selectivity of the fibers without the loss of the basic physical characteristics. We also envision further use of AFM as a tool in the chemical characterization of all forms of chromatographic stationary phases.

Based on the characteristics of selectivity, low cost, and low backing pressure, it is envisioned that the C-CP fibers could find application in preparatory-scale separations. One can also imagine the use of single-fibers laid in-channel for microfluidic separations. Finally, studies are currently underway as well in the use of the fiber as a sorbant material for waste remediation and for analytical solid-phase microextraction applications.

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