ELSEVIER

Journal of Chromatography A, 887 (2000) 277–285

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Characteristics of particle-loaded monolithic sol–gel columns for capillary electrochromatography
I. Structural, electrical and band-broadening properties

Chitra K. Ratnayake, Chan S. Oh, Michael P. Henry*

Beckman Coulter Inc., 4300 North Harbor Boulevard, Fullerton, CA 92834, USA

Abstract

Particle-loaded (3 μm, C_{18}) monolithic sol–gel columns have been prepared and selected characteristics measured. They have a surprisingly high permeability, allowing their operation in the microLC mode at pressures as low as 69 kPa where their efficiency is about 50 000 plates per meter and the CEC mode where efficiency is at least 106 000 plates per meter. These columns can withstand over 13.8 MPa pressure without compression or movement within the 75 μm capillary. Field strengths in the packed segments are approximately 50% greater than those in the open segments, due to the higher resistivity of the particle-laden regions. There is a relatively rapid loss of efficiency with increasing linear velocity in both the CEC and microLC modes, which may be due to a tortuosity effect in the inter- and intra-particulate voids. Chromatographic behavior is characteristic of conventional C_{18} particles, indicating that analytes have significant access to the surface within the pores of the immobilized bonded phase.

Keywords: Monolithic sol–gel; Band broadcasting; Stationary phases; Electrochromatography

1. Introduction

Particulate stationary phases for capillary electrochromatography (CEC) are retained within the column with frits [1–5], tapered inlet restrictions [6], a drawn pre-packed method [7] and gels [8–12] that adhere to the interior wall. The last three methods have the advantage that the problematic frits [1–5] are unnecessary. Placement of particulate solids within a CEC column using gels creates one of several types of so-called monolithic [13] devices. Its construction may involve the polymerization of monomers with cross-linkers to form a network, trapping sorbent particles that act as a prefabricated liquid chromatographic stationary phase. Such particle-loaded monolithic columns do not require frits and have chromatographic properties that derive from the nature of the original sorbent particles.

For example Lin et al. [8] have suspended molecularly imprinted polymer particles from 1 to 15 μm in diameter, in an acrylamide/bisacrylamide gel for enantiomeric separations of amino acids. They estimated that the pore size in the gel was about 500–600 nanometer for 4–7% acrylamide.

Dulay et al. [9] described a method for preparing particle-loaded monolithic columns in which a silica gel is formed around particles located uniformly throughout a portion of the length of a capillary. In principle the particles may be chosen from any type of material that is suitable for HPLC, such as 3 or 5 μm C_{18}, which these authors used. The silica sol–gel has large cavities that allow convection and diffusion of molecules to the surface of sorbent particles. A scanning electron micrograph of the monolithic

*Corresponding author.

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01142-5

material reveals that the supporting sol–gel matrix is formed throughout the entire width of the capillary. Column efficiencies of up to 80 000 plates per meter were obtained with 3 μm particles of C_{18} bonded phase. The authors attribute these modest plate numbers to the presence of inhomogeneities in their columns and some inaccessibility of analytes to the particles due to steric blocking by the sol–gel elements.

Chirica and Remcho [10] report the fabrication of silicate entrapped columns. After forming an inlet frit the capillary is pressure packed with 5 μm chromatographic particles, the column is filled with a potassium silicate solution that is subsequently gelled by careful heating to 160°C over several days. The inlet frit was removed leaving a silicate entrapped, monolithic, particle-loaded column. Scanning electron micrographs of the packed columns indicated that Chirica and Remcho obtained a porous, particle-packed solid that was held in place by small amounts of irregularly shaped silica. This is quite unlike that of Dulay and coworkers [9] where much more sol–gel is present between the particles. Separations of PAH's were accomplished producing smaller capacity factors, relative to conventionally packed capillary columns which contain no silicate binder. The authors ascribe this observation to a combination of effects including partial occlusion of pores by the silicate and possible hydrolysis of the C_{18} bonded phase during column preparation.

Tang et al. [11] describe a similar method of preparing a monolithic column. A capillary attached to a removable external frit is packed with stationary phase using supercritical carbon dioxide. A solution consisting of tetramethoxysilane, ethyltrimethoxysilane, methanol, trifluoroacetic acid, water and formamide was pumped into the column to encapsulate the particles to the desired length. The gelling process, that involved the hydrolysis and polymerization of the silanes to create a hybrid organic/inorganic composite, took 24 h at room temperature, and several hours at various temperatures up to 250°C in supercritical carbon dioxide. The final monolith consisted of particles of sorbent that were densely packed, and bound together and to the capillary wall by the gel network.

Dittman et al. [12] have described another method of creating an immobilized chromatographic bed inside a capillary. After slurry packing a capillary in the traditional way [14] the bed was immobilized over its entire length by an externally applied, high temperature (580°C) treatment. This involved moving a heated coil of Ni–Cr wire along the capillary held at 62 MPa causing a mild sintering of the packing material and bonding the particles together. This process resulted in a monolith that could withstand a pressure differential of about 6.2 MPa while maintaining the physical integrity of the column.

We have developed monolithic columns for CEC, adapted from the method of Dulay et al. [9]. For these columns it is important to understand how the nature of the supporting gel, packed bed permeability and electrical resistivity, band-broadening mechanisms, partitioning processes, electrophoresis and electro-osmosis contribute to their chromatographic properties and stability. This will enable us to control the primary function of such columns, which is to resolve complex mixtures in the shortest time possible in a reproducible fashion.

In Part I of this work we discuss and characterize the basic construction [9], electrical properties [16,17] and specific permeability of these columns. We also briefly examine band-broadening effects and certain aspects of their chromatographic nature. In Part II [18] we will investigate the nature of the supporting gel and discuss its effect upon electrophoresis, electro-osmotic flow, chromatographic efficiency, selectivity and capacity factors.

2. Experimental

2.1. Instrumentation and analysis

- *Instrument*: Beckman Coulter's P/ACE™ MDQ instrument was used for all measurements. Beckman Coulter's System Gold® high-performance liquid chromatograph was used for the pressure resistance tests.
- *Current and voltage*: Current was measured in μA with the P/ACE MDQ instrument, and is stabilized when pressure (usually 276 kPa) is applied at both ends. Voltages from 5 to 30 kV were preset.
- *Pressure*: The 10-cm column (A) was operated in

the microLC mode on the P/ACE MDQ instrument at pressures from 69 to 690 kPa.

- *Columns*: Experimental, monolithic particle-loaded capillary columns (containing particles of porous silica, 3 μm, C_{18}, 30-cm total length) were used. The lengths of packed segments were: Column A: 10 cm (packed from the outlet to the window). Column B: 10 cm (leaving the first 10 cm open). Column C: 21.5 cm (packed from the inlet to the window). Column D: Zero cm (empty capillary). The outside diameter was 375 μm with an inside diameter of 75 μm. The locations of the packed segments are illustrated diagrammatically in Fig. 1.
- *Mobile phase*: 70/20/10 acetonitrile/25 m*M* morpholine ethane sulfonic acid, pH 6/water and 95/5 acetonitrile/MES buffer (25 m*M*, pH 6.2).
- *Temperature*: 25°C.
- *Injection mode*: Electrokinetic injection (5 kV, 2 s) or pressure injection (3.5 kPa, 2 s)
- *Retention times*: These are given as the mean of five consecutive runs.
- *Linear flow velocity*: This was measured for the different segments by injecting thiourea as the unretained compound, either from the inlet with normal polarity, or from the outlet with reversed polarity.
- *Specific permeability, electrical properties and peak widths*. The mathematics for these properties are developed in Section 3.
- *Specific permeability and electrical properties* were calculated using the 70/30-acetonitrile/MES buffer.
- *Peak widths* (as height equivalent to a theoretical plate or HETP) were measured in the 95/5 acetonitrile/MES buffer. HETP values were calculated at several values of linear velocity for the 10-cm column operated in the CEC and microLC modes.

2.2. Chemicals

Thiourea, naphthalene, phenanthrene, pyrene and morpholine ethane sulfonic acid (MES) were obtained from Aldrich (Milwaukee, WI, USA). Acetonitrile was obtained from E. M. Science (Gibbstown, NJ, USA) and Mallinkrodt Baker (Phillipsburg, NJ, USA). The pH of the MES buffer was adjusted with sodium hydroxide.

3. Results and discussion

3.1. Structural properties

3.1.1. Construction

A slurry segment of gelling reagents and particles was moved to the desired region of the capillary lumen and gelled in place. No frits were used or required in the construction of these columns. Any length can be prepared at any location. Columns A, B, C and D (see Fig. 1) were prepared in order to measure the effect of bed length and its location in the capillary, upon electrical and chromatographic properties of the CEC column. The empty capillary was needed to determine the resistivity of open

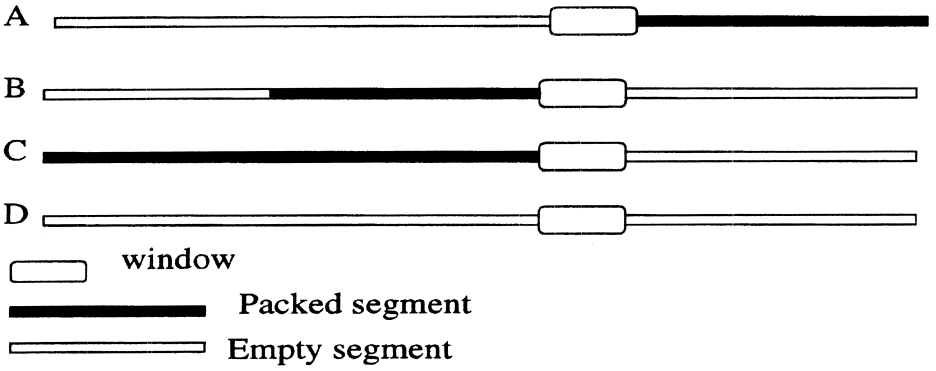

Fig. 1. Schematic diagrams of columns A through D illustrating locations of packed and empty segments.

segments, ρ_{open} (see Eq. (4), Section 3, below), which is assumed to be constant for any such region in any column (see Section 3.2), for a given mobile phase and temperature.

Columns A, B and C were subjected to an inlet pressure of 13.8 MPa with methanol for several hours with no visible sign of bed movement at any point. This result indicates that in 75 μm capillaries the gelled bed of particles is attached firmly to the wall and is strong enough to resist collapse at high pressures. The property is important when the columns are to be used in the microLC mode (see Section 3.4) and must withstand pressures up to 690 kPa continuously.

3.1.2. Specific permeability, B_o

Specific permeability, B_o can be calculated from the modified Eq. (1) below,

$$B_o = u \times \eta \times L/P \quad (1)$$

where u = linear velocity (m/s), η = viscosity (Pascal s), L = bed length (m) and P = pressure (Pascal), that quantifies Darcy's law [15] and is a measure of the resistance to fluid flow in a packed bed. It is independent of bed dimensions, the nature of the fluid, pressure and temperature, and depends only upon the particle size distribution and the structure of the bed.

When a sol–gel column (A, B or C) is operated in the microLC mode, a plot of pressure versus linear velocity should give a straight line whose slope is $\eta L/B_o$. This assumes that there is no pressure generated by any open segment at any linear velocity. The resulting value of B_o using this method and the pressure/linear velocity data given in Table 1, is a surprisingly high 7.0×10^{-14} m^2, which is about eight times greater than the specific permeability of a standard 3 μm HPLC column. Thus satisfactory migration times of certain analytes through 3 μm 10-cm columns can be obtained at pressures as low as 69 kPa. (See Section 3.4 and Fig. 3).

The high value of specific permeability is due to the low-pressure method of preparation of the sol–gel columns. Furthermore the gel itself is highly porous and allows liquid to flow through the columns at low pressures.

A description of the porous nature of the gel is the subject of a future publication (Part II) from our laboratory [18].

3.2. Electrical properties

Rathore and Horvath [16] have developed mathematics to enable the field strength, E, to be calculated across a packed bed or empty segment anywhere in the capillary. Evaluations of this property require simple measurements of currents and segment lengths in two capillaries having different fractionally packed lengths. In the work in this paper we have chosen one of the capillaries to be empty, which simplifies the mathematics and electrical measurements as shown in the following discussion.

The application of Ohm's law to CEC column behavior results in the quantitative relationships given in Eqs. (2) and (3):

Table 1
Number of plates (N), HETP (H) and linear velocity (u) in CEC and microLC modes[a]

CEC Mode				MicroLC Mode			
V (kV)	N[b] (plates)	H (μm)	u (mm/s)	P (kPa)	N[b] (plates)	H (μm)	u (mm/s)
5	10 632	9.4	0.54	69	5018	19.9	0.09
10	6284	15.9	1.10	138	6795	14.7	0.20
15	3381	29.6	1.76	276	5197	19.2	0.40
20	2220	45.0	2.27	414	3103	32.2	0.59
25	1724	58.0	2.89	552	3099	32.0	0.76
30	1472	68.0	3.38	690	2544	39.3	0.93

[a] Mobile phase: 95/5 acetonitrile/MES (25 m*M*, pH 6.2).
[b] Column A, 10 cm.

$$V_{open} = \rho_{open}\, iL_{open}/A \quad (2)$$

$$V_{pack} = V - V_{open} \quad (3)$$

where V_{open} is the voltage drop across any open segment of resistivity ρ_{open}, length L_{open}, lumen cross-sectional area, A, and in which column current is i amps. V_{pack} and V are the voltage drops across any packed segment and the entire column length, respectively.

The resistivity of any open segment, $\rho_{open,}$ is calculated from Eq. (4) using the empty capillary:

$$\rho_{open} = VA/iL \quad (4)$$

where L is the total capillary length.

Field strengths, E, for any open or packed segment can be calculated from Eqs. (5) and (6):

$$E_{open} = V_{open}/L_{open} \quad (5)$$

$$E_{pack} = V_{pack}/L_e \quad (6)$$

where L_e is the equivalent length, as defined by Rathore and Horvath [16], of a packed segment.

L_e is the total length traveled by an unretained, neutral marker in a column of length L_{pack}. L_e is calculated [16] from Eq. (7):

$$L_e = L[i_{open}/i_{pack}]^{1/2} - L_{open} \quad (7)$$

where i_{open} and i_{pack} are the currents through the CEC column in the presence and absence of packing.

The measured values of current, i, and the length, L, for the entire capillary, whether packed or empty, of all columns A to D are given in Table 2 using the 70% acetonitrile mobile phase. The value of A was calculated from the known internal diameter of the capillary. Migration times of the unretained thiourea through a given type of segment were measured by operating the columns in a forward or reverse fashion depending on where the segment was located.

Table 3
Calculated values of electrical and chromatographic properties of columns A through D

Column parameter	Units	A	B	C	D
ρ_{open}	Ω m	[a]	[a]	[a]	52[b]
ρ_{pack}	Ω m	147	164	141	NA
V_{open}	kV	8.3	7.8	3.1	20
V_{pack}	kV	11.7	12.2	17.9	NA
L_e	cm	17.8	19.1	36.5	NA
L_{open} [c]	cm	20	20	10	30
L_{pack}	cm	10	10	21.5	NA
E_{open} [c]	V/cm	415	389	308	667
E_{pack} [d]	V/cm	657	640	491	NA
t_{Ropen}	s	184	204	128	331
t_{Rpack}	s	69	60	196	NA
u_{open}	cm/s	0.11	0.10	0.08	0.09
u_{pack}	cm/s	0.15	0.17	0.11	NA

[a] Resistivity of all open segments will be constant.
[b] ρ_{open} is calculated for an empty capillary and will be the same for all open segments in CEC columns.
[c] Length of segment refers to the total of all lengths of the same type.
[d] This is the summed retention times of the marker for open segments of length L_{open}.

Values of electrical and migration properties of the four columns (A, B, C and D) may be calculated from Eqs. (2)–(7) using the basic data from Table 2, and are given in Table 3.

3.2.1. Resistivity, ρ

This property of the packed segment should be a constant regardless of the length or internal diameter of the capillary [16]. The higher value for column B may indicate that it is slightly more tightly packed

Table 2
Measured values of i, V, A, L[a] and unretained peak migration times for columns A through D

Column	Current i (μA)	V (kV)	L (cm)	A[b] (cm^2)	$t_{R(open)}$ (s)	$t_{R(packed)}$ (s)
A	3.5	20	30	44×10^{-6}	184	69
B	3.28	20	30	44×10^{-6}	204	60
C	2.6	21	32	44×10^{-6}	128	196
D (empty)	5.6	21	32	44×10^{-6}	331	NA

[a] The current i, voltage V and length L refer to the whole capillary, whether packed or empty.
[b] A is the cross-sectional area of the capillary lumen.

(higher resistance) than column A or C. Agreement of A with C is excellent.

3.2.2. Voltage drop, V

For a specific type of segment, voltage drop should be constant for a given length. Agreement between the two packed 10 cm lengths is fair, indicating that the location of the segment does not substantially influence most of its electrical properties.

3.2.3. Equivalent length, l_e

These are significantly larger than the simple length of the capillary, as expected. The path of the thiourea marker is a tortuous one, including the inter-particle and intra-particle void spaces filled with liquid buffer.

3.3. Linear velocities and band broadening effects

3.3.1. Linear velocities, u, using 70/30 acetonitrile/MES

Linear velocities, u, are calculated from Eq. (8):

$$u = L/t_R \quad (8)$$

where t_R is the time taken for an unretained marker to travel a distance L through a specific segment.

These are in good agreement (see Table 3) for the two 10 cm columns, for both the packed and open segments. The smaller value of linear velocity for the longer packed segment, 21.5 cm, is due to the smaller field strength for the longer column (491 V/cm versus 640 V/cm for column B).

3.3.2. Band-broadening effects in 95/5 acetonitrile/MES

Height equivalent to a theoretical plate (HETP): This was calculated for thiourea from Eq. (9):

$$\text{HETP} = L/N \quad (9)$$

where L = is the packed bed length, and N = the column plate number, given by Eq. (10):

$$N = 5.54[t_R/w_{1/2}]^2 \quad (10)$$

where t_R is the peak migration time and $w_{1/2}$ is the peak width at half height.

The height equivalent to a theoretical plate (HETP) of thiourea as a function of linear velocity is given in Table 1 for both the CEC and μLC modes.

The rate of change of H with linear velocity u of the mobile phase is significant in both separation modes. The relationships are approximately linear above 0.5 mm/s (microLC) and 1.5 mm/s (CEC).

Resolution is limited by the modest value of plate number for these sol–gel columns, which are approximately half those of conventionally-packed CEC columns. It is believed that the possible presence of tortuous paths originating from gel structure within or at the mouth of pores in the particles may play a role in limiting peak efficiency.

3.4. Chromatography of thiourea and polynuclear aromatic hydrocarbons (PAH's).

The separation of thiourea, naphthalene, phenanthrene and pyrene is given in Fig. 2 for the CEC mode and Fig. 3 for the microLC mode, for the 10-cm column (column A). The data are for voltages of 5, 15 and 30 kV and pressures of 69, 138 and 690 kPa. The mobile phase here was 95/5 acetonitrile/MES buffer, which was chosen to reduce retention times as much as possible. The data indicate the high speed of separation with a 10-cm column. The maximum voltage of 30 kV across the 30-cm capillary and the maximum pressure of 690 kPa were used for the CEC mode and microLC mode respectively in achieving the fastest chromatography.

Peaks are observed to be quite symmetrical, indicating a degree of homogeneity in the packed bed. The columns exhibit a degree of hydrophobicity characteristic of C_{18} columns, indicating that analytes can access the pores of the bonded phase particles held in the gel and interact with the nonpolar surface therein.

4. Summary

Particle-loaded monolithic sol–gel columns have been prepared and selected characteristics measured. The particles are conventional 3 μm C_{18} porous spheres used in HPLC. The open structure of these gelled columns confers on them a surprisingly high permeability, allowing their operation in the μLC mode at pressures as low as 69 kPa where their

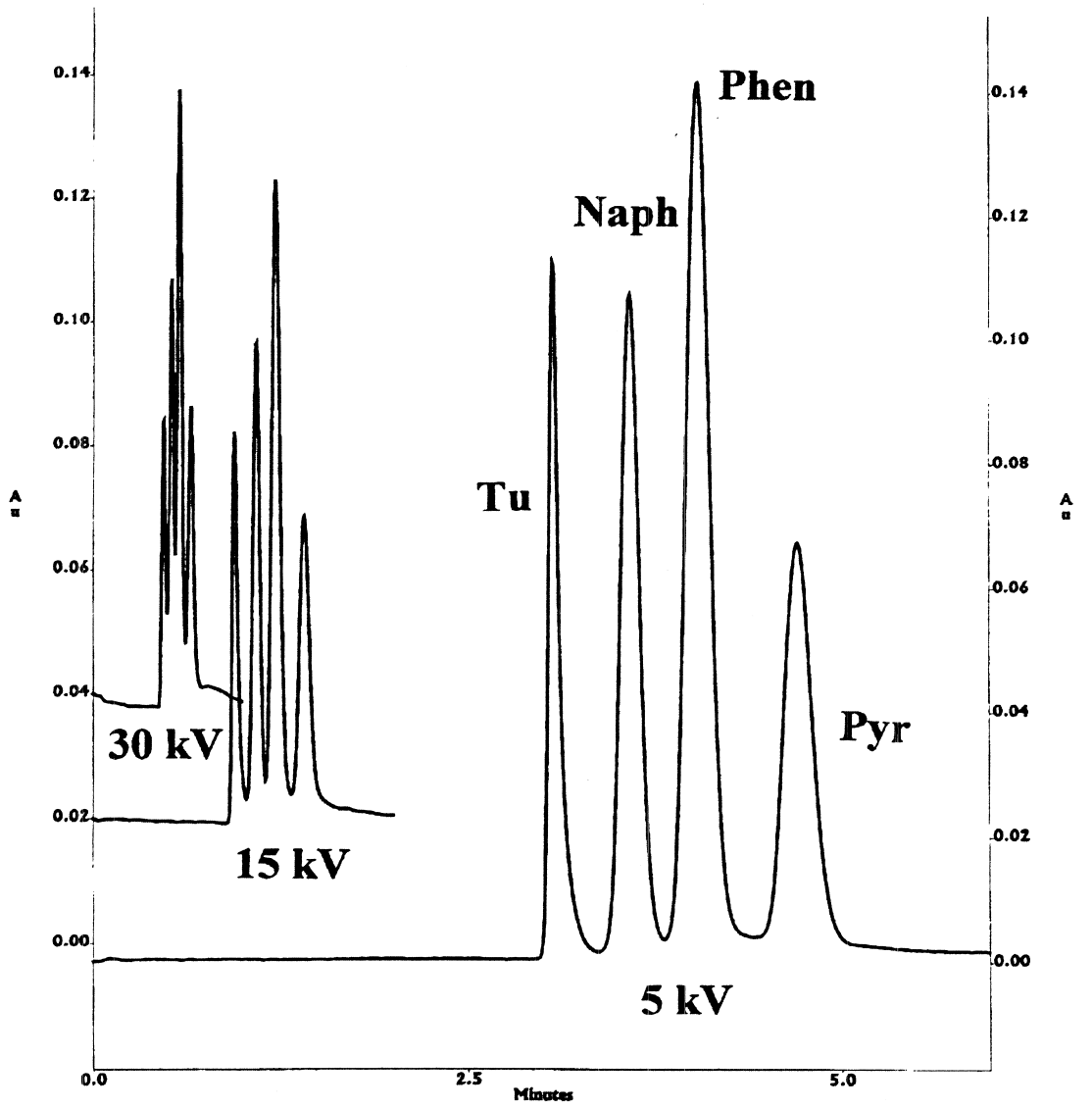

Fig. 2. Electrochromatogram shows the separation of thiourea from three polynuclear aromatic hydrocarbons at three different voltages. Column: 10 cm (packed from inlet to window), 30 cm total length, 75 μm I.D. Buffer: 95/5 acetonitrile/morpholino ethanesulfonic acid, 25 m*M*, and pH 6.2. Temperature: 25°C. Compounds: Tu = thiourea, Naph = naphthalene, Phen = phenanthrene, Pyr = pyrene. Samples dissolved in 80/20 acetonitrile/morpholinoethanesulfonic acid, 25 m*M*, and pH 6.2.

efficiency is about 50 000 plates per meter. These columns can withstand over 13.8 MPa pressure without compression or movement within the 75 μm capillary.

Field strengths in the packed segments are approximately 50% greater than those in the open segments, due to the higher resistivity of the particle-laden regions.

There is a relatively rapid loss of efficiency with increasing linear velocity in both the CEC and microLC modes, which may be due to a tortuosity effect in the inter- and intra-particulate voids.

Chromatographic behavior appears to be characteristic of conventional C_{18} particles, indicating that analytes have significant access to the surface within their pores.

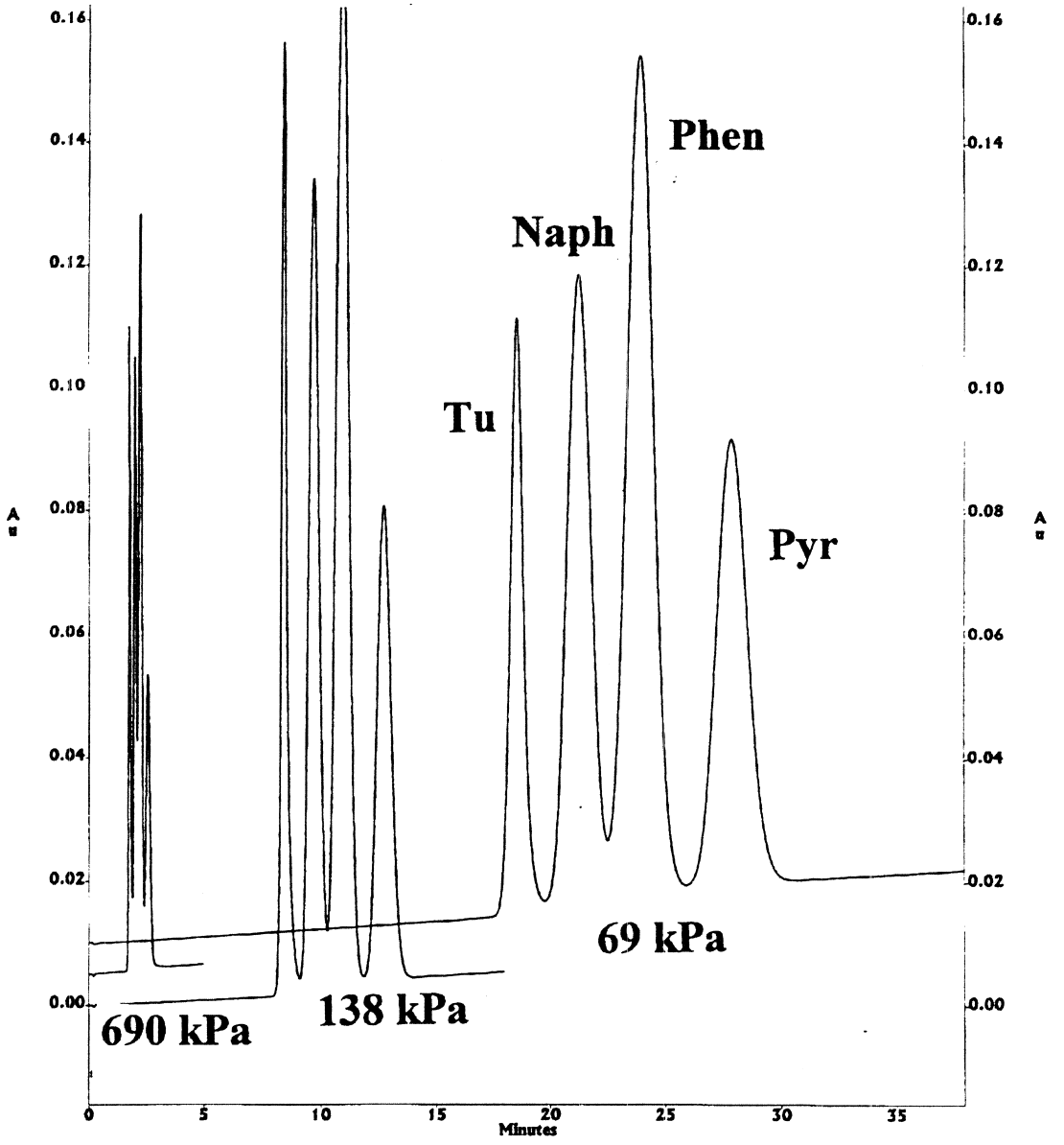

Fig. 3. Liquid chromatogram shows the separation of thiourea from three polynuclear aromatic hydrocarbons at three different pressures. Column: 10 cm (packed from inlet to window), 30 cm total length, 75 μm I.D. Buffer: 95/5 acetonitrile/morpholino ethanesulfonic acid, 25 m*M*, and pH 6.2. Temperature: 25°C. Compounds: Tu = thiourea, Naph = naphthalene, Phen = phenanthrene, Pyr = pyrene. Samples dissolved in 80/20 v/v acetonitrile/morpholinoethanesulfonic acid, 25 m*M*, and pH 6.2.

Acknowledgements

We are indebted to Richard Zare, Maria Dulay and Rajan Kulkarni of Stanford University, for the invention of the particle-loaded, sol–gel monolithic column technology described in this work. We wish to acknowledge the stimulating conversations with Dr. John Fassett, of the Advanced Technology Center, who brought professional electrical engineering insight to this work. We wish to thank Dr. James

C. Osborne whose support has enabled this research to continue from its inception.

References

[1] R.J. Boughtflower, T. Underwood, C.J. Paterson, Chromatographia 40 (1995) 329.
[2] C. Yan, R. Dadoo, H. Zhao, Richard N. Zare, D.J. Rakestraw, Anal, Chem. 67 (1995) 2026.
[3] K.D. Atria, N.W. Smith, C.H. Turnbull, Chromatographia 46 (1997) 664.
[4] B. Behnke, E. Grom, E. Bayer, J. Chromatogr. A 716 (1995) 207.
[5] J.H. Knox, I.H. Grant, Chromatographia 24 (1981) 135.
[6] M. Mayer, E. Rapp, C. Marck, G.J.M. Bruin, Electrophoresis 20 (1999) 43.
[7] C. Stathakis, C. Warburton, R.M. Cassidy, J. Chromatogr. A 659 (1994) 443.
[8] J.M. Lin, T. Nakagama, K. Uchiyama, T. Hobo, Chromatographia 43 (1996) 585.
[9] M.T. Dulay, R.P. Kulkarni, R.N. Zare, Anal. Chem. 70 (1998) 5103.
[10] G. Chirica, V.T. Remcho, Electrophoresis 20 (1999) 50.
[11] Q. Tang, B. Xin, M.L. Lee, J. Chromatogr. A 837 (1999) 35.
[12] M.M. Dittman, G.P. Rozing, G. Ross, T. Adam, K.K. Unger, J. Cap. Elec. 4 (5) (1997) 201.
[13] E.C. Peters, M. Petro, F. Svec, J.M.J. Frechét, Anal. Chem. 69 (1997) 3646.
[14] M.M. Dittman, K. Weinand, F. Bek, G.P. Rozing, LC–GC 13 (1995) 800.
[15] J.C. Giddings, Dynamics of Chromatography, Part 1, Principles and Theory, Marcel Decker, New York, 1965, Reprinted (1997), 201.
[16] A.S. Rathore, Cs. Horváth, Anal. Chem. 70 (1998) 3069.
[17] G. Choudhary, Cs. Horváth, J. Chromatogr. A 781 (1997) 161.
[18] C.K. Ratnayake, C.S. Oh, M.P. Henry, in preparation.