Analysis of Complex Samples by Solvating Gas Chromatography (Supercritical Fluid to Gas Transition)

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Abstract

The various forms of chromatography are primarily determined by differences in the physical state of the mobile phases. The main chromatographic categories include gas chromatography (GC), liquid chromatography, and supercritical fluid chromatography. Adjusting a temperature and pressure will change the mobile phase from liquid to supercritical fluid to gas, with concomitant changes in their physical properties. In this paper, the technique transition-phase chromatography (TPC) is described. In TPC, different mobile phase conditions exist inside the column. This phase transformation within the column results in huge differences in density, solvating power, viscosity, diffusivity, and, as a consequence, in the chromatographic properties of the mobile phase. TPC experiments using capillary columns packed in our laboratory have shown that when the mobile phase is transformed from supercritical fluid to gas, high column efficiencies can be achieved. The transition from supercritical fluid to gas (also called solvating GC), a particular case of the TPC, is evaluated for the separation of complex real samples (environmental, food, and fuels).

Introduction

Microcolumns (as compared with conventional columns) are essentially characterized by higher efficiencies and lower flow rates and require minimum sample sizes. They are therefore particularly suitable for the analysis of very complex mixtures and direct interfacing to mass spectrometers (MS) (1,2) and flame-based chromatographic detectors (3–5). These columns provide a great potential for the analysis of biological fluids, in which the manipulation of very small sample volumes is often required.

Packed-column efficiency directly depends on the particle size in the column. However, the efficiency per column length or the reduced plate height (h) has never achieved in gas chromatography (GC) the same high-performance as in liquid chromatography (LC) (h = 2) (6), especially when using long, packed columns. Currently, packed-column GC does not approach the high separation capability of open tubular GC, although high-speed separations have been demonstrated with short (3 cm) packed columns (7).

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Earlier, Lee et al. introduced a technique called solvating GC (SGC) in which different mobile phase conditions exist in the column. The idea behind this name was that, because of the experimental conditions selected for the mobile phase (mainly CO_2), it will act as a solvating compound instead of a traditional carrier gas (8). In this technique, high performance with a packed column (h = 1.3) was obtained (9). Recently, a broader definition of the transition-phase chromatography (TPC) was reported and justified by our research group (10–11). This involved any technique in which the transition of phase occurred inside the column; the SGC being a specific case. In SGC, supercritical or superheated fluids (superheated liquid) are introduced at the column inlet, and gases exit from the column outlet. In such situations, a phase transformation occurs in the column, which results in huge differences in density, solvating power, viscosity, and diffusivity.

The advantages of this technique are that packed columns can provide the same efficiency and separation capability as typical, open tubular columns. Sample can be successfully introduced into the column using the solvating power of the mobile phase at the column inlet, which is similar to sample introduction in supercritical fluid chromatography (SFC) and LC; and by adjusting the properties of either the packing material or the mobile phase, TPC can achieve higher selectivities than GC.

In this study, microparticulate porous silica was used as a packing material to perform SGC for the separation of complex samples. The results were compared with those obtained using an open, tubular column (25-m × 250-µm i.d.) coated with 0.30 µm of poly(5% diphenyl–95% dimethylsiloxane) (CROMA-5). Samples used to carry out the separations included diesel fuel, oxidized essential lemon oil, and a mixture of polycyclic aromatic hydrocarbons (PAHs).

Experimental

Apparatus and materials

Spherical porous (300Å) octadecyl bonded silica (ODS) particles (Nucleosil) with diameters of 10 µm were purchased from Alltech (Avondale, PA).

The apparatus for column preparation consisted of a Varian 8500 syringe pump (Walnut Creek, CA), CO_2 cylinder (White

Martins, São Paulo, Brazil) that supplied carbon dioxide via a needle valve, ultrasonic bath from UNIQUE (São Paulo, Brazil), homemade stainless steel cyclone reservoir, and primary reservoir fitted with a stainless steel frit (pore size < 0.2 µm) (2).

Chromatographic columns were evaluated using a GC oven modified inhouse for SFC (12). A 4-port Valco valve (Houston, TX,) with a 60-nL internal loop was used for sample introduction. A Shimadzu 17A and GC-2010 series GC (Shimadzu, Kyoto, Japan) was used for open tubular GC experiments. Other chemicals used were purchased from Merck (Darmstadt, Germany).

Column preparation

Each column was packed by following a procedure described in the literature (13), which was modified by replacing the cylindrical reservoir by a primary reservoir, followed by a cyclone one (11). The following is a brief description of the procedure for pack capillary columns.

The packing material and the cyclone-packing device were dried at 110°C for 12 h. The materials were placed in a desiccator to cool down. An appropriate amount of packing material was put into the cyclone reservoir, depending on the volume of the column being packed.

The end of the capillary tubing to be packed was put into the reservoir. The other end of the tubing was connected to a fused-silica capillary linear restrictor (25-µm × 60-cm i.d.) by means of a Valco union. The column tubing, restrictor, Valco union, and cyclone reservoir were placed underwater in the ultrasonic bath. Liquid carbon dioxide was introduced to the primary reservoir. The column was then sonicated during the packing process, and the temperature of the ultrasonic bath was maintained at 50°C.

The carbon dioxide was maintained in the liquid state (room temperature, 20°C) in the primary reservoir and under supercritical conditions in the section beneath the warm water (50°C).

The carbon dioxide flowing through the section beneath the water, under supercritical fluid condition, has a faster velocity and a lower viscosity than in the liquid state, which accelerates the packing process and improves the formation of a homogeneous packing bed. The packing pressure of carbon dioxide was held constant (20.0 MPa ~ 200 atm).

After the packing bed in the column was formed, the packing pressure was held for 30 min before cessation of sonication and reduction of the packing pressure. The column was left at the bath temperature (50ºC) for 2 h. The pressure had then fallen to approximately 5.00 MPa (~ 50 atm).

The restrictor connected to the end of the column was removed for an easier depressurization. When the pressure had fallen to zero, the column was disconnected from the reservoir and the other restrictor (50 µm × 20 cm) was connected in its place.

Results and Discussion

In TPC, the solvating power of the mobile phase can decrease the retention of solutes in the column. Interaction between the mobile and stationary phases usually reduces interactions between the solutes and the packing material using capillary columns packed with 10-µm C18 particles.

A previous study showed that a low minimum reduced plate height (h_{min}) close to two could be obtained when using a CO_2 mobile phase that changed from supercritical fluid to gas between the column inlet and outlet (10,11). A high column efficiency was obtained when using a mobile phase that changes from supercritical fluid to gas because of the linear change in density with changing pressure.

Separations of complex samples

In chromatography, the separation can be performed using temperature, pressure (or density), and mobile phase composition programming. Temperature programming is primarily used in GC, pressure (or density) programming is widely used in SFC, and solvent composition programming is mostly used in LC. In TPC, all of these methods can be used. A solvent composition gradient can be employed to adjust the solute retention factors. However, gas composition gradients are experimentally difficult to generate using readily available chromatographic instrumentation.

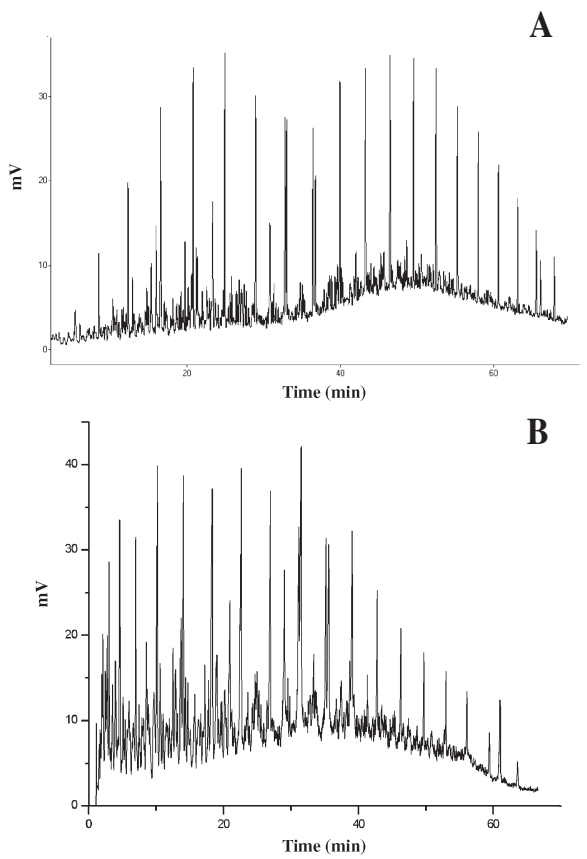

Figure 1. Open tubular column GC (A) and packed capillary TPC (supercritical to gas transition) (B) of a diesel fuel sample. Conditions: temperature program, 80°C/2 min to 3°C min–1 to 260°C/5 min; post temperature, 280°C; post time, 10 min; column, fused-silica capillary column (30-m × 250 -µm i.d.) coated with 0.30 µm CROMA-5 poly(5% diphenyl–95% dimethylsiloxane) H_2 carrier gas; and detector, flame ionization detector (FID). Conditions: column inlet pressure program, 12.0 MPa/10 min to 0.4 MPa min^{-1} to 18.0 MPa; temperature program, 110°C/5 min to 3°C min^{-1} to 160°C; column, 180-cm × 250-µm fused silica capillary column packed with 10-µm porous (300A) ODS bonded particles, CO_2 mobile phase; and detector, FID.

Currently, temperature and pressure (or density) programming can easily be accomplished. Packed capillaries are more effective than larger, conventional packed columns for temperature programming because of their better heat-transfer characteristics.

The separation of complex samples can be a challenge to any chromatographic technique. In GC, long open tubular columns are primarily used for such problems. However, the low-sample capacities of open tubular columns are a disadvantage. Packed capillary columns can provide much larger sample capacities than open tubular columns and would be desirable for many applications if they could provide similar resolution as open tubular columns.

An open tubular GC column (~ 25 m long) usually has a column efficiency of approximately 130,000 plates/column. In TPC, a packed capillary column of 2 m containing 10-µm particles can produce similar efficiency, as is demonstrated in this work.

Analysis of diesel fuel

Figure 1A shows a normal GC temperature-programmed analysis of diesel fuel. A TPC analysis is shown in Figure 1B. As can be seen, the excellent resolution obtained in open tubular GC column (30-m × 250-µm i.d.) is also obtained in TPC using a short packed capillary column (1.80-m × 250-µm i.d.).

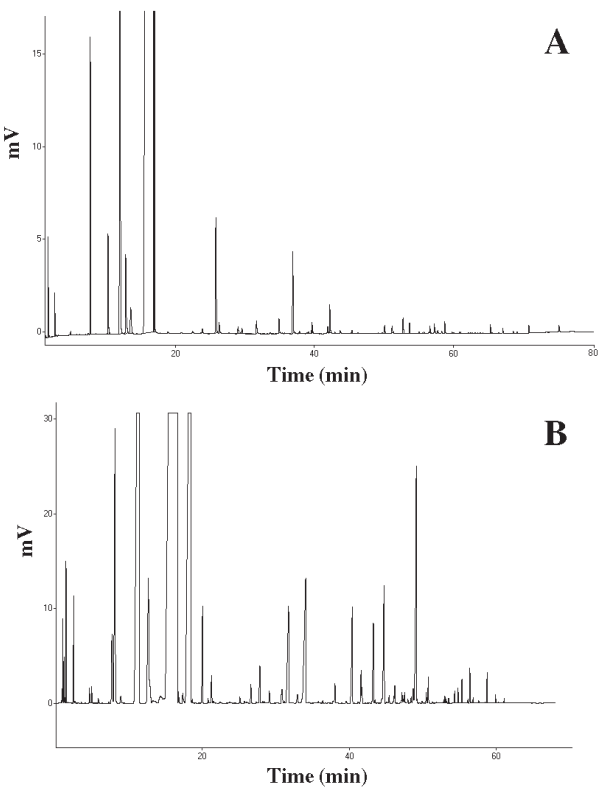

Figure 2. Open tubular column GC (A) and packed capillary TPC (supercritical to gas transition) (B) of an oxidized lemon oil sample. Conditions: temperature program: 45°C/2 min to 3°C min^{-1} to 180°C/2 min to 5°C; column, fused-silica capillary column (30-m × 250-µm i.d.) coated with 0.30 µm CROMA-5; poly(5% diphenyl–95% dimethylsiloxane); carrier gas, H_2; and detector, FID. Conditions: column inlet pressure program, 15.0 MPa/10 min to 0.2 MPa min^{-1} to 19.0 MPa; temperature program, 80°C/5 min to 2°C min^{-1} to 130°C; column, fused-silica capillary column (180-cm × 250-µm i.d.) packed with 10 µm porous (300A) ODS bonded particles with CO_2 mobile phase; and detector, FID.

Analysis of essential oils

Essential oils produced from fruit peels are widely used as flavors and fragrances in the perfume and cosmetic industries. Peel oils are mainly composed of a volatile fraction consisting of terpene hydrocarbons and their oxygenated derivatives, and of a nonvolatile residue, including waxes and pigments. Essential oil analysis has been a very active and productive area in capillary GC analysis for many years (14–17).

The complexity and fine detail of the chemical composition of such materials are normally realized only by the use of the most advanced separation techniques (18–20). The complexity of natural products can be seen in the literature (21–23), many of which report the identification of many of the components of essential oils by GC–MS.

TPC can offer the degree of resolution required for the analysis of essential oils, serving as an alternative for open tubular GC analysis.

Experimental results using a packed capillary TPC column for the analysis of an oxidized lemon oil are shown in Figure 2B. As can be seen, an excellent resolution was obtained in TPC analysis

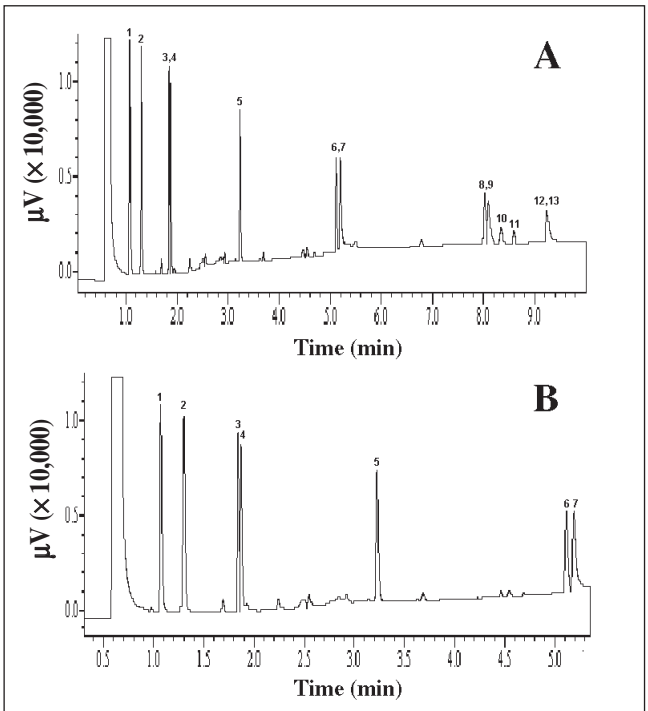

Figure 3. Packed capillary TPC (supercritical to gas transition) analysis of a PAHs mixture (A). Details of the separation of the critical pair (3-phenanthrene 4-antracene) (B). Conditions: column inlet pressure program, 16.0 MPa/2 min to 1.0 MPa min^{-1} to 22.0 MPa; temperature program, 100°C to 10°C min^{-1} to 160°C/10 min; column, fused-silica capillary column (40 cm × 250 µm) packed with 10-µm porous (300A) ODS bonded particles with CO_2 mobile phase; and detector, FID. Analytes: acenaphthylene (1); fluorene (2); phenanthrene (3); anthracene (4); pyrene (5); benzo(a)anthracene (6); chrysene (7); benzo(a)fluoranthene (8); benzo(k)fluoranthene (9); indeno(1,2,3-cd)pyrene (10); benzo(a)pyrene (11); dibenzo(a,h)anthracene (12); and benzo(ghi)perylene (13).

as compared to high-resolution GC on capillary fused-silica columns (Figure 2A).

Analysis of PAHs

Many compounds of environmental significance are nonvolatile molecules, and the number of environmentally related samples continues to increase; therefore, the development of fast separation methods is especially important. A typical chromatographic analysis time for separating PAHs ranges from 30 min to more than 60 min, depending on the complexity of the sample (24,25).

Recently, it has been found that when carbon dioxide is used in packed capillary column chromatography, fast separations can be obtained (9). In Figure 3, a fast separation of PAHs in packed capillary column TPC is illustrated as an example of the potential application of this technique in environmental analytical chemistry.

Conclusion

In the experiments described in this work, it was found that, as expected, by increasing the column inlet pressure, the analysis time was decreased. The separation of lighter components in the samples was better in the packed than the tubular column, as shown in Figure 1B. This resulted from the higher retention on packed capillary column, higher column efficiency when carbon dioxide was used as mobile phase, and higher sample capacity. In this study, pressure programming was used together with temperature programming. Figure 1 shows the separation of a diesel fuel sample using an open tubular GC column (25-m column) and a cyclone packed capillary column TPC (1.8-m column).

As can be seen, in approximately the same analysis time, the packed capillary column using the cyclone packing technique provided a similar resolution of sample components, especially for the heavier components present in the sample. This resulted from the higher retention in the packed bed than in the capillary column. As shown in Figure 2B, separation of the oxidized lemon oil sample components can be achieved on a packed capillary column employing the cyclone packing method. Excellent and fast separation of a PAH mixture was obtained by means of packed capillary column TPC.

Thus, the separation obtained using capillary open tubular GC can also be achieved in TPC using packed capillary columns. This technology has a potential in practical applications for the separation of complex samples. In addition, the cost to manufacture a packed microcolumn is lower than for the open tubular column because of the much shorter column length required to obtain similar separation in an equivalent time schedule.

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