FEATURING: CHROMATOGRAPHY

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Blurring the Boundaries

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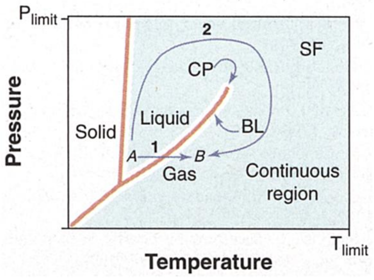
Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the most frequently used methods to separate complex chemical samples. Both employ differential transport of mixtures to achieve separation, but they barely resemble each other in practice or in instrumentation. Is there a set of rules that can explain the differences and similarities among diverse chromatographic methods? Would an understanding of such rules create new opportunities and capabilities not available previously? We think the answer to both questions is yes and leads us to the idea of Unified Chromatography (*1*).

The term Unified Chromatography may conjure visions of a single apparatus used for more than one technique. But the concept involves far more than just instrumentation. The approach challenges the apparent limits of conventional separations, integrates technique-specific theories, and potentially gains the most control and highest performance possible. The full implications of Unified Chromatography are still unknown; however, we are sure that its antithesis is the continued independent development of methods, instrumentation, and theories for individual techniques without regard to the bigger picture.



Continuous and discontinuous. Phase diagram for a pure fluid shows two paths for the transition from liquid *A* to gas *B*. Path 1, a temperature increase at constant pressure, is discontinuous. Path 2 avoids the boiling line and continuously changes liquid to gas without a phase transition. Pressure and temperature limits represent the limits of the apparatus, not the fluid behavior. Green, region of continuous one-phase fluid. CP, critical point; BL, boiling line.

Unifying the Behavior of Fluids

Because the physical properties of gases and liquids are so very different, GC and HPLC are practiced separately. Childhood experience with dirt, water, and air form our basic understanding of solid, liquid, and gas. These separate phases can meet at an interface like the beach, a riverbank, or the bathtub. At first, mud might seem like a new substance, but mud is just wet dirt; these three states of matter apparently do not mix.

As water boils, it undergoes a discontinuous change from liquid to gas. But for pure, stable fluids under certain conditions, continuity exists in all physical properties between the liquid and gas states (see the figure above). We do not often experience this continuity because we live at a relatively low, nearly constant pressure. The classic steam engine and a kitchen pressure cooker both illustrate that the boiling temperature of water can be varied over a large range by manipulating pressure. If equilibrium is maintained between a liquid and its gas while raising the temperature and pressure along the boiling line, the gas increases in density and becomes more liquid-like, whereas the density of the liquid decreases and becomes more gas-like. Eventually, as temperature and pressure continue to increase, the liquid and gas become identical at the critical point. At even higher temperatures and pressures, only one phase exists, supercritical fluid (SF), which can have the properties of a liquid, a gas, or something in between depending upon the pressure. Like a gas, the SF is compressible and will uniformly expand to fill its container, but like a liquid it can also interact with and dissolve other materials.

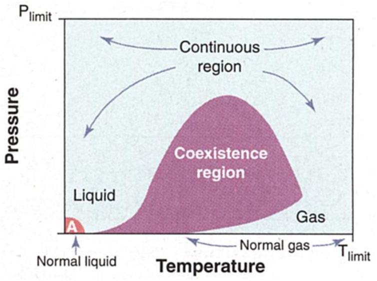
The ability of a solvent to dissolve a solute (its solvent strength) is determined by the forces between all the molecules involved. In turn, the strength of these intermolecular forces depends on the distances between the molecules. Ordinary liquids are nearly incompressible, so the intermolecular distances and the solvent strength are little affected by pressure changes alone. The distances between ordinary gas molecules near 1 atm are so large that the intermolecular forces are essentially zero and therefore are also unaffected by pressure. However, many SFs have large intermolecular forces and are still highly compressible. Their solvent strength is quite pressure-dependent and correlates with the density of the fluid. If the pressure of a SF is lowered toward ambient, the intermolecular distances increase, the intermolecular forces approach zero, and the fluid begins behaving like a gas. But at constant pressure, lowering the temperature of a SF continuously moves the fluid toward a liquid state. The key concept here is maintaining the continuity of all fluid properties while continuously changing liquids to gases and vice versa.

Naturally, the behavior becomes more complex as the number of components in the fluid increases, but the same type of continuity can exist for particular blends or mixtures of miscible solvents. The boiling line is no longer simple, as with a pure material; instead, there is a pressure-temperature region in which boiling might occur, depending on the ratio of the components. Still, continuity of all fluid properties between the liquid and gas states exists if the boiling region is simply avoided (see the figure below).

Unifying the Mobile Phases

Ideally, a GC mobile phase is chemically inert, exerting no intermolecular forces on the solutes or the stationary phase (*2*). The molecular interactions between solute and stationary phase determine partitioning, which is highly temperature-dependent.

In contrast, temperature was not considered over much of the history of HPLC, and ambient temperature was used simply by default. HPLC mobile phases are far from inert because the molecules must exert enough force on each other just to hold the liquid together at ambient conditions. Similar forces exist between solvent molecules and dissolved solutes. Partitioning of solutes between the stationary and mobile phases in HPLC depends on the competition among the various forces in each phase. When



Miscible liquids and the fluidity continuum. The composition dimension for the blend is perpendicular to the page and is not shown. The solid region to the left is also omitted. The blend depicted here is normally a liquid at ambient temperature and pressure (A). Purple, region in which mobile phase can coexist as liquid and gas.

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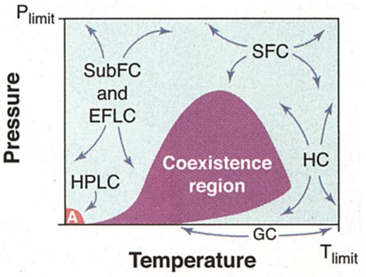
the stationary and mobile phases are liquids or liquid-like, changing the temperature has relatively little effect on the distribution of solutes. The more effective way to adjust solute partitioning, therefore, is by manipulating the mobile-phase composition to change the balance of intermolecular forces exerted on the solutes. In supercritical fluid chromatography (SFC), the interactions between solute and mobile phase can be altered by changing the temperature (as in GC), mobile-phase composition (as in HPLC), or pressure.

Controlling the temperature and pressure throughout the chromatographic instrument opens new possibilities in mobile-phase selection. Fluids that are normally gases under ambient conditions can be used, such as carbon dioxide. Because CO_2 sublimes at ambient pressure, its liquid state is not accessible unless the pressure is manipulated. However, if the outlet pressure of a column is kept above the vapor pressure to suppress boiling, CO_2 stays in a well-behaved liquid mobile phase from its freezing point to its critical temperature (31°C). Above the critical temperature, supercritical CO_2 can be used as a mobile phase at temperatures and pressures limited only by the physical constraints of the instrumentation.



Separation techniques. Different methods occupy distinct locations in the mobile phase and join continuously when boiling is avoided. A, ambient conditions.

By controlling the column outlet pressure, many familiar liquids can be used at temperatures well above boiling. Water continues to be a useful liquid to about 250°C if boiling is suppressed with pressure. When an aqueous mobile phase is heated, the viscosity drops, diffusion rates increase, and solvent strength increases for low-polarity solutes like oil. Controlling mobile-phase properties by using unusual temperatures is another basis for improving separations with the unified approach.

New Experimental Techniques

The figure above shows how several separation techniques relate to each other in the mobile-phase continuum, with HPLC at one end and GC at the other. HPLC is usually carried out at ambient temperature and outlet pressure, and thus occupies the portion of the diagram corresponding to ordinary liquids at ambient conditions. Subcritical fluid chromatography (SubFC) and enhanced-fluidity liquid chromatography (EFLC) (*3*) are both practiced at elevated pressure below critical temperature using fluids too volatile for conventional HPLC. Pirkle's work with chiral separations (*4*) using liquid CO_2 at subambient temperatures is just one practical example of SubFC. In EFLC, the more volatile mobile-phase component of a blend (such as CO_2 in methanol) reduces viscosity and enhances diffusion, leading to faster mass transport, shorter analysis times, and expanded possibilities for controls.

Higher temperature and pressure leads to the SFC region (*5*, *6*) in which temperature, pressure, and mobile-phase composition strongly affect the separation. Reducing the pressure while keeping the temperature above boiling leads to hyperbaric chromatography (HC) where faster diffusion and shorter analyses than in SFC are expected, but with weaker mobile phases (*2*). Solvating gas chromatography (*7*) is a subset of HC in which the column inlet is highly pressurized and the mobile phase dissolves solutes, but the outlet is maintained at atmospheric pressure. GC results when pressure is reduced so much that no intermolecular forces remain in the mobile phase, even at the column inlet. Pressure and mobile-phase composition have little or no effect on solute partitioning in GC, leaving temperature as the only effective parameter. HPLC and GC are simply the limiting cases of the Unified Chromatography that encompasses all the techniques mentioned here.

Solvation and Partitioning

Although it has recently attracted some attention, Unified Chromatography is not new. The late J. Calvin Giddings taught that many aspects are common among all separation techniques. He described the behavior of specific techniques from the fundamentals of equilibrium, transport, and peak broadening (*8*).

In the 1980s, Martire began developing a theory of solute distribution between two phases, and related it to LC, GC, and SFC (*9*, *10*). This was based largely on the lattice fluid model presented by Sanchez and Lacombe (*11*). Parcher and co-workers (*12*) have compared the lattice fluid model with experimental observation to characterize the interaction of CO_2 with polymeric and chemically bonded stationary phases.

Unifying Instrumentation

Continuity has been stressed in Unified Chromatography, but early instrumentation functioned discontinuously. Ishii and Takeuchi (*13*) built instruments to sequentially perform GC, SFC, and LC on the same sample, switching the mobile phase in discontinuous steps. Ishii used the image of a troika, a Russian carriage drawn by three horses, to present this approach (*13*, *14*). Bartle also developed a single instrument to perform multiple separations in sequence (*15*, *16*).

The best current example of a unified instrument may be the packed column–SFC (PC-SFC) (*17*), which can continuously vary mobile-phase composition between two fluids. Column temperature can be set from –60° to +200°C, and outlet pressures can be controlled from ambient to over 400 atm. Despite its somewhat misleading name, the PC-SFC can continuously move among all the techniques mentioned.

Conclusions

Much has been done to unify chromatography theory and practice. The prospect of novel mobile phases and an increased range of mobile-phase conditions provides new opportunities to refine separation selectivity. Lowering mobile-phase viscosity and increasing diffusion rates leads to higher flow rates and shorter analyses, or to the possibility of using longer columns when necessary. If we think about HPLC as a starting place and improve the pumps, control the column-outlet pressure, and broaden the temperature range, we will create an instrument like the SFC chromatograph: capable of performing a wide array of separation tasks, yet ready to perform conventional HPLC. True, the complexity of the technique and number of parameters would increase. But the enhancement of capability, speed, and effectiveness of separations are great rewards. They are, for separations, the next frontier.

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