The separation of complex mixtures is of great importance in the production of pharmaceuticals, fine chemicals, cosmetics and food products, among others. As a highly selective separation method, chromatography is often an essential step in the separation of mixtures of molecules of very similar structure. This article presents the key features and advantages of an unconventional chromatographic technology with a liquid stationary phase.

What is Liquid-Liquid Chromatography?

Liquid-liquid chromatography (LLC) combines the separation principles of liquid-liquid extraction and chromatography. As in extraction, a biphasic liquid system is used and the basis for the separation is the differing partitioning behavior of the mixture solutes between the two phases. As in chromatography, one of the two phases involved in the separation is kept stationary. Despite these similarities, several substantial differences set LLC apart from conventional chromatography with solid stationary phases (i.e. HPLC). In LLC the user prepares both phases, the mobile phase and the stationary phase, by mixing two or more solvents that form biphasic system. The liquid stationary phase is held in place during operation by application of centrifugal force and typically occupies 60-80% of the column volume. High sample loading is possible since the entire volume of the liquid stationary phase is accessible to the solutes. Irreversible adsorption cannot occur, allowing for complete sample recovery [1-3].

Since its early years, the isolation and purification of natural products has been the leading area of LLC applications [1,2]. The versatility of LLC is clearly demonstrated by the vast range of published applications, including separation of synthetic drugs, herbicides, pesticides, vitamins, amino acids, peptides, proteins, dyes and inorganic elements.
The “Column” in Liquid-Liquid Chromatography

In LLC, the “column” is a specially-designed housing mounted on the axis of a centrifuge.

This assembly is normally referred to as a “machine” and replaces the classical cylindrical column used in HPLC. Commercially-available machines can be grouped into two types based on their construction and the resulting centrifugal field: hydrodynamic and hydrostatic.

In hydrodynamic machines, a continuous piece of hollow tubing wound around a bobbin forms the “column”. The bobbin rotates around its own axis while simultaneously revolving around the axis of the centrifuge (fig. 1a) resulting in a variable centrifugal force across the length of the tubing. Mixing and settling zones are alternatingly distributed along the entire length of the column (fig. 1b). LLC performed using hydrodynamic machines is typically referred to as Counter Current Chromatography (CCC).

In hydrostatic machines, the “column” is made of several identical metal disks laid one above the other with annular Teflon plates in between (fig. 2a). Each disk has a series of circumferentially engraved cells, which are interconnected by ducts (fig. 2c). The last cell of one disk is connected to the first cell of the next by openings in the Teflon plates. The column is placed on a rotor of a centrifuge and has one axis of rotation, resulting in a constant centrifugal force profile. Separations using hydrostatic machines are referred to as Centrifugal Partition Chromatography (CPC) [2].

Column volumes range from 20 ml to 18 l and are available from Dynamic Extractions (UK), Armen Instrument (France) and Kromaton Technologies (France).
Selection of the Mobile and Stationary Phases

The first and most crucial step in the development of a chromatographic separation is the selection of the mobile and stationary phases in the form of a biphasic solvent system. The partition coefficient of the target component is used as the main screening parameter, with best performance achieved with a partition coefficient between 0.5 and 2.5. Due to the vast choice of available solvents, the selection of a biphasic solvent system for a specific LLC separation often takes up to 80% of the time spent to develop the entire process [3]. For this reason, several solvent system “families” using conventional solvents such as “HEMWat” (hexane/methanol/ethyl acetate/water) have been developed [1,2]. A wide polarity range can be obtained within one family by altering the global system composition. Biphasic liquid systems may also be “tailor-made” for a specific separation task. Polymer-based aqueous two-phase systems [4] and water-free deep eutectic solvent [5] or ionic liquid systems [6] have expanded the areas of LLC application.

The experimental effort needed to select a biphasic solvent system and its global composition can be significantly reduced, if not eliminated, by using thermodynamic models such as COSMO-RS for the prediction of the solute partition coefficients [7].

Continuous Separation

In LLC, either phase of the biphasic system may be assigned the role of the stationary phase, and the roles of the phases may be switched during operation. This opens the door to a wide variety of unique operating modes not possible using solid stationary phases. One such mode is the continuous process patented in 2005 [8]. Its principle of operation is schematically presented in figure 3 for the separation of a binary mixture of components A and B. It is assumed here that A and B preferentially distribute into the lower phase and upper phase of the biphasic system, respectively. This cyclic process is realized in two columns connected in series. The feed is continuously introduced between the two columns and the two products are collected sequentially at the opposite ends of set-up. One cycle consists of two steps: descending (Des) and ascending (As).

In the descending step, the lower phase acts as the mobile phase and is introduced into the unit through column 2. The feed stream is composed of components A and B dissolved in the lower phase and enters between the two columns. During the descending step, A travels with the mobile phase towards the left end of column 1 faster than B and can be collected in pure form. Just before component B reaches
the product collection point, the unit is switched to the ascending step. In the ascending step, the phase roles and flow direction are reversed. The upper phase is used as the mobile phase and as the basis for the feed. B travels faster towards the right end of column 2 and is collected in pure form. Before A leaves the column, the unit is switched back to the descending step, completing one cycle. The methods for selection of the unit operating parameters and simulation of the process can be found in [9].

The potential of continuous LLC separations is demonstrated in [10] for a binary mixture of capsaicin and dihydrocapsaicin. Both products were obtained at very high purity and yield despite differing in structure by only one double bond.

**Conclusion**

Liquid-liquid chromatography is a versatile technology combining the principles of extraction and chromatography. The presence of a liquid stationary phase presents many advantages over conventional liquid-solid chromatography techniques, including high sample loading capacity and lossless recovery. The wide range of available biphasic solvent systems and operating modes renders LLC applicable to a wide range of target components. Despite these benefits, LLC is an underutilized technology sure to increase in popularity in the coming years.

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**Quick introduction to LLC:** https://www.youtube.com/watch?v=MdD8dsGZzBo

**References**