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GILSON®

Introduction To Centrifugal Partition Chromatography

TECHNICAL NOTE (TN215)

Gilson Purification

WHAT IS CENTRIFUGAL PARTITION CHROMATOGRAPHY

Centrifugal Partition Chromatography (CPC), also known as Counter Current Chromatography (CCC) is a preparative, pilot and industrial scale liquid purification technique that does not require traditional solid supports. The main aim of this technology is to isolate the maximum amount of a specific molecule at the highest purity, in a minimum of time and without using any silica column or support media.



CPC and prep HPLC do have some similarities:

- Same objectives.
- Same fundamental chromatographic process.
- Identical peripherals: pumps, injectors, inline detectors and fraction collectors.

The heart of the LC system, in general, is the column where the separation occurs. The fundamental difference between LC, like Flash or HPLC, and CPC is the nature of the stationary phase.



The stationary phase in classical LC is made of coated or non-coated silica, where the skeleton of the particle is only a support and the surface chemistry generates chemical interactions with the mobile phase and compounds to be separated.

CPC does not require a solid support like silica; two immiscible liquid phases are used. One serves as the mobile phase or the eluent, and the other as the stationary phase. The stationary phase is retained in the column by a centrifugal field. The affinity of the solute for each phase can be measured by their partition coefficient, (Kd), that in turn dictates the order of elution for each compound.

HOW DO YOU MAINTAIN LIQUID STATIONARY PHASE INSIDE AN LC COLUMN?

Classical LC columns are simply made: a cylinder (stainless steel in HPLC and plastic or glass in Flash Chromatography) with an inlet and outlet at each end, filled with a solid stationary phase, usually silica.

The CPC column also has an inlet and outlet for the mobile phase, but that‘s where the similarities with an HPLC column end. To be able to maintain one phase of the biphasic system inside the column, a centrifugal field and specially designed CPC disks are used.

The CPC column consists of a number of CPC disks, (Figure 1, Picture 2) arranged on a rotor, (Figure 1, Picture 1). The rotor starts to spin to create the centrifugal force that retains the stationary phase. The disks consist of a number of cells linked together by a thin channel (Figure 1, Picture 3). A CPC column consists of over a thousand cells with a rotary seal at each end to connect the spinning column to the pumping system, detector, and other system components.

A valve allows for a change in the direction of flow and therefore the CPC system will work in either ascending mode, (Figure 1), where the lightest phase is the mobile phase or in descending mode when the heaviest phase is the mobile phase. This allows both normal and reverse modes without replacing the column.

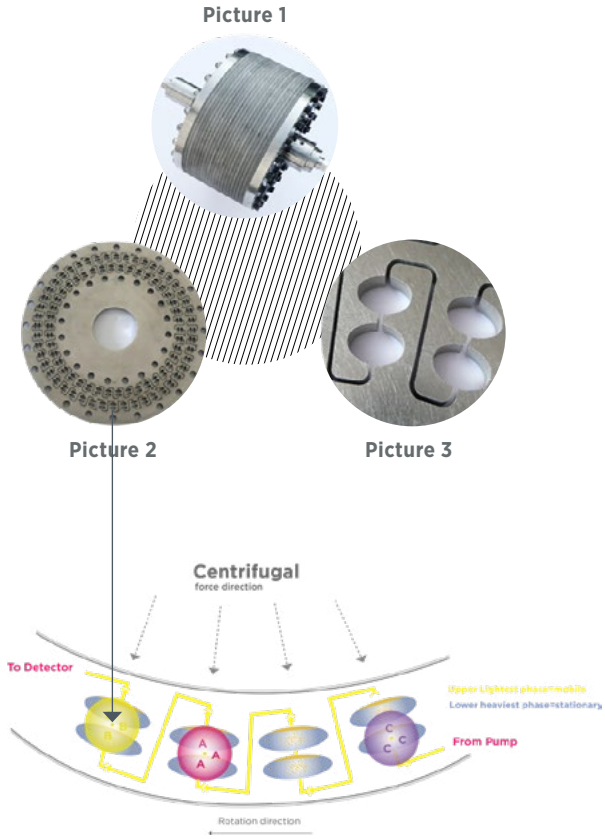


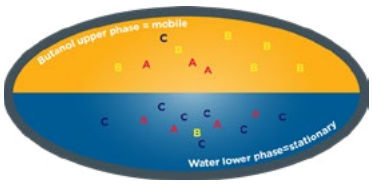
Figure 1

HOW DOES SEPARATION OCCUR INSIDE THE CPC "COLUMN"?

The chromatographic effect in CPC relies on the distribution constant, (or partition ratio, Kd). This is the equilibrium constant for the distribution of an analyte in two immiscible solvents. For a particular compound, it is equal to the ratio of its molar concentration in the stationary phase to its molar concentration in the mobile phase as per the equation Kd= [a]Stationary phase/[a]Mobile phase.

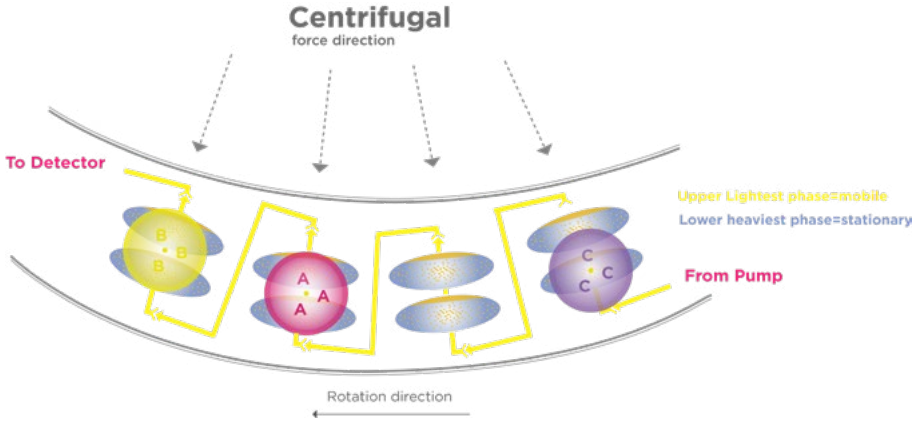
A Kd of 1 represents equal partition between the mobile and stationary phase. To effect a CPC separation the analyte of interest should be between Kd 0.5-5. If the Kd is too low, (below 0.5) the analyte is retained in the mobile phase and no separation occurs, if too high, (over 5) the analyte is retained in the stationary phase.

The solvent system can then be determined according to the partition coefficients of all molecules that need to be separated.



KdA= 1
KdB<1
KdC> 1

$$Kd = \frac{[A]stat}{[A]mob}$$



Choosing the solvent system in CPC is like choosing the column and the eluent in HPLC.

Figure 2

WHAT ARE THE ADVANTAGES OF CPC?

The volume of stationary phase available and the absence of silica means that there are a number of advantages for this technique versus traditional separation methods.

- No column to replace, no silica to recycle.
- Low solvent consumption.
- High flow rate for a low run time.
- High performance, high purity and high recovery.
- No sample loss.
- No denaturation, no irreversible adsorption of the sample.
- Huge application fields from natural products, essential oils, cannabinoids and petroleum extract to proteins.

Moreover, as there are no unwanted secondary interactions with silanols on the silica, this technique therefore preserves the integrity of fragile compounds without denaturation or loss by irreversible adsorption.

CPC is a viable alternative to classical LC purification with high loading potential and no on-going costs except solvents.

GILSON CPC SERVICES AND SUPPORT

In addition to “Batch” CPC systems, Gilson also provides a complete range of continuous CPC systems using a TMB concept. Batch CPC is similar to HPLC in that you inject at the head of the column and elute separated compounds at the end.

Gilson TMB systems consist of two CPC columns with two pumping systems. The process could be easily compared to SMB (Simulated Moving Bed) used in HPLC for continuous injection.

The sample is injected continuously at the junction of both columns and the separation effected by an alternating sequence of steps where the mobile phase is alternatively the upper phase and the lower phase. For a simple binary mixture it means that the molecule that has more affinity for lower phase will be eluted to the left of the system and the molecule that has more affinity for the upper phase will be eluted to the right.

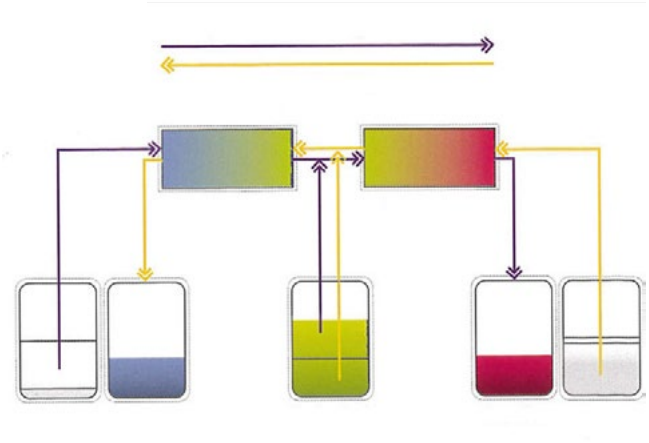


Figure 3

Figure 3 illustrates a continuous separation of two dyes. The green mixture is continuously injected inbetween both columns; pure red dye is recovered at the right outlet of the system and pure blue at the left.

The difference with a simple biphasic liquid extraction system in funnels, is that CPC TMB provides enough separating power in terms of plate efficiency, to separate very similar molecules, e.g. diastereoisomers. The size of the CPC TMB columns define the scale of productivity.

CPC TMB could be used as a continuous and high efficiency liquid extractor for the enrichment of natural extracts and to eliminate undesirable molecules, or in a binary mixture to eliminate molecules of secondary importance, or to concentrate a low content molecule in a crude mixture.

GILSON CPC SERVICES AND SUPPORT

Gilson has an extensive range of services that makes CPC an easy to use technology. Our fully equipped application laboratory in France allows us to offer:

- Custom training on CPC technology.
- Demonstrations.
- Custom Purification Services: feasibility studies, scale up studies and purification contracts.

Equipment installed in our laboratory includes:

- CPC 100 for method development.
- CPC 1000 for scale up.
- TMB-250.
- LaChrom HPLC with Diode Array Detector and auto sampler.
- Agilent GC system with auto sampler.
- Buchi Rotavapor® 1L and 20L capacity.

GILSON CPC PRODUCTS: EASY TO USE INTEGRATED SOLUTIONS

CPC Column Range

Gilson provides a complete range of stardard CPC systems with column sizes of 100mL, 250mL, 1L, and custom industrial systems designed to meet customer requirements, e.g. 5L column.



CPC SPECIFICATIONS

CPC 100: Part Number 21141001 (115V) **/ 21141006** (230 V)

Column Capacity	100 mL column capacity
Injection Range	Up to 1 g
Typical Flow Rate	Up to 15 mL/min
Maximum Pressure	100 bar (1450 psi)
Speed Range (Acceleration Range)	100–3000 rpm (1–728 *g*)
Weight	60 kg (132 lbs.)

CPC 250: Part Number 21141002 (115V) **/ 21141007** (230 V)

Column Capacity	250 mL column capacity
Injection Range	Up to 6 g
Typical Flow Rate	Up to 15 mL/min
Maximum Pressure	100 bar (1450 psi)
Speed Range (Acceleration Range)	100–3000 rpm (1–685 *g*)
Weight	70 kg (154 lbs.)

CPC 1000: Part Number 21141003 (115V) **/ 21141008** (230 V)

Column Capacity	1000 mL column capacity
Injection Range	up to 30 g
Typical Flow Rate	Up to 50 ml/min
Maximum Pressure	80 bar (1160 psi)
Speed Range (Acceleration Range)	100–1500 rpm (1–254 *g*)
Weight	120 kg (264 lbs.)
Maximum Rotation Speed	3000 rpm (100 mL column) and 1500 rpm (1 L Column)

CPC PRO SPECIFICATIONS

CPC 250 PRO: Part Number 21141004 (115V) **/ 21141009** (230 V)

Column Capacity	250 mL column with greater injection capacity
Injection Range	Up to 30 g
Typical Flow Rate	Up to 80 mL/min
Maximum Pressure	100 bar (1450 psi)
Speed Range (Acceleration Range)	100–3000 rpm (1–729 *g*)
Weight	65 kg (143 lbs.)

CPC 1000 PRO: Part Number 21141005 (115V) **/ 21141010** (230 V)

Column Capacity	1000 mL column with greater injection capacity
Injection Range	Up to 100 g
Typical Flow Rate	Up to 350 mL/min
Maximum Pressure	80 bar (1160 psi)
Speed Range (Acceleration Range)	100–2000 rpm (1–452 *g*)
Weight	115 kg (253 lbs.)

GILSON CPC BUNDLED WITH PLC PURIFICATION SYSTEM

MODEL	APPROPRIATE PLC CONFIGURATION
CPC 100 CPC 250	PLC 2050: Quaternary valve, Automatic Injection Valve, 5 or 10 mL loop, Automatic Backflush Valve, 200–600 nm UV/Vis detector
CPC 1000	PLC 2250: Quaternary valve, Automatic Injection Valve, 30 mL loop, Automatic Backflush Valve, 200–600 nm UV/Vis detector
CPC 250 PRO	PLC 2250: Quaternary valve, Injection Pump, Automatic Backflush Valve, 200–600 nm UV/Vis detector
CPC 1000 PRO	PLC 2500: Quaternary valve, Injection Pump, Automatic Backflush Valve, 200–600 nm UV/Vis detector

From a simple CPC column to a fully integrated and automated system

For a fully automated solution, connect a CPC column to our Personal Liquid Chromatography (PLC) system. This provides a complete solution in two modules able to perform CPC and prep HPLC by switching from one column to the other.



Gilson peripherals for CPC column



PLC system

Complete prep HPLC system that can be connected to classical LC column, Flash column and CPC column. Pump, automated loop Injector, UV-VIS detector, fraction collector, touch screen PC with GGC software. The whole system is integrated into one module.



Pump Range

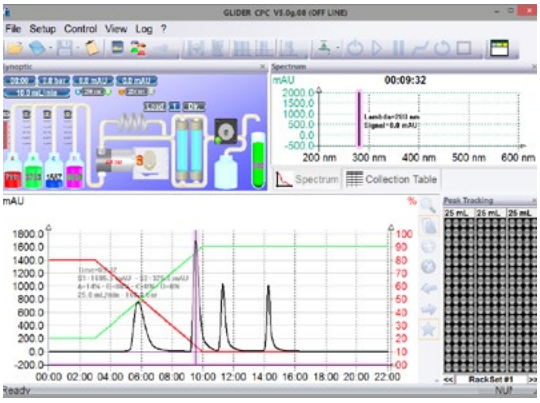
- 50 mL/min, 300 bar, Isocratic, Binary or Quaternary gradient
- 250 mL/min, 230 bar, Isocratic, Binary or Quaternary gradient
- 500 mL/min, 110 bar, Isocratic, Binary or Quaternary gradient

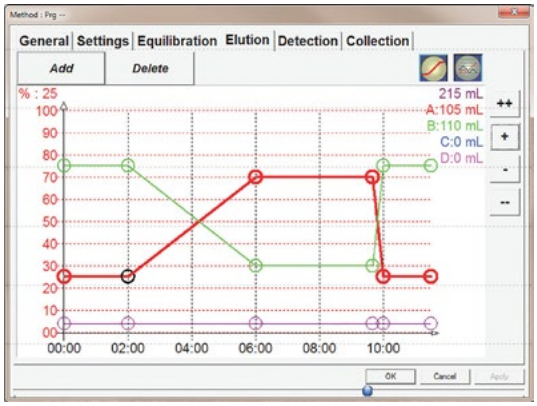
Gilson Glider CPC (GGC): Software

In addition to the development of enhanced CPC and prep LC hardware, Gilson develops software solutions designed to meet your specific needs. "Gilson Glider" software for prep LC and CPC is specially optimized for simple and intuitive access. All peripherals are under single point control from the CPC rotation to pumping, detection, and fraction collection.

With GGC software, you develop your complete method from stationary phase loading, equilibration, injection, elution, extrusion or dual mode, allowing you to perform your run in one click!

Fractions can be collected via time, volume, threshold and peak. Methods can also be modified on the fly, allowing real time control of your separation!





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