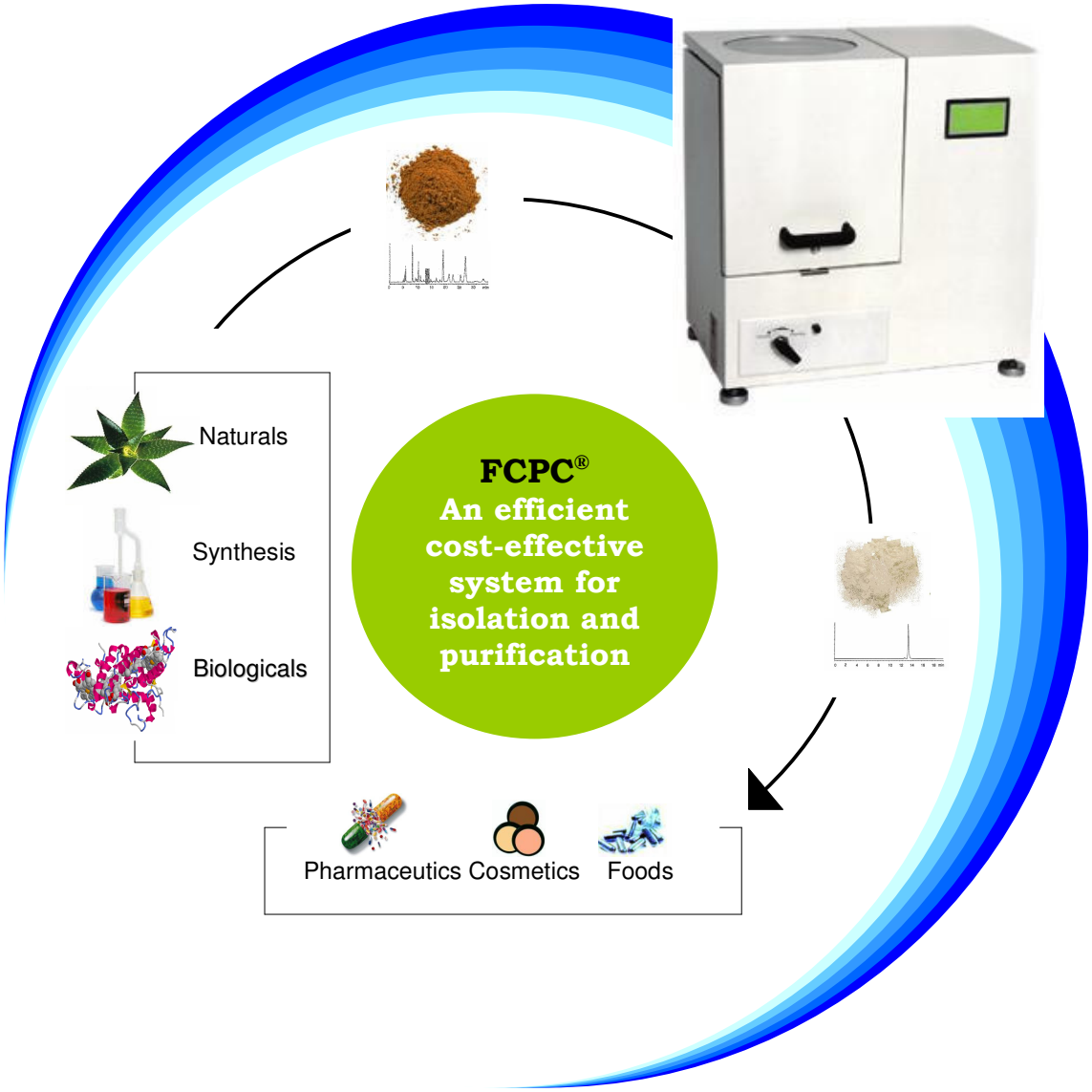
An innovative solution for preparative and industrial chromatography



NO SOLID SUPPORT

- 100% sample recovery
- Low solvent consumption
- No column packing
- Ideal for samples of all polarities

KROMATON

Principle of Centrifugal Partition Chromatography

Fast Centrifugal Partition Chromatography (FCPC®) is an interesting tool for the **isolation** and **purification** of different kinds of extracts or mixtures. This technique is especially useful in the fields of pharmacognosy, pharmaceutical biology, organic chemistry, and fermentation for **preparative fractionation** of synthetic or natural compounds.

It is based on the principle of a liquid-liquid partition between **two immiscible liquid phases** prepared by mixing 2 or more solvents (or solutions). One liquid phase is retained in the FCPC® by centrifugal forces and called “stationary phase”. The second liquid phase is pumped through the stationary phase and called “mobile phase”. Sample compounds are separated according to their **partition coefficient** Kd in both liquid phases (depending on molecule polarity).

Process for the separation of three compounds with different partition coefficient:

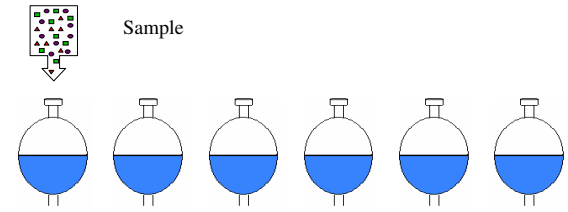
$$K_D = \frac{[A]_{stat}}{[A]_{mob}}$$

$K_D < 1$

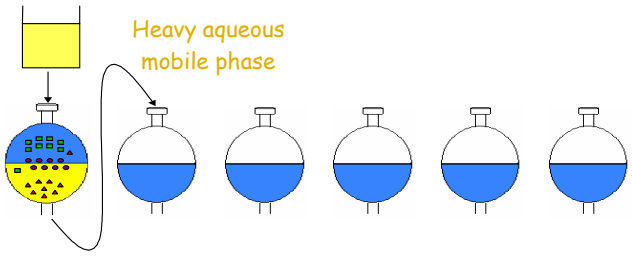
$K_D = 1$

$K_D > 1$

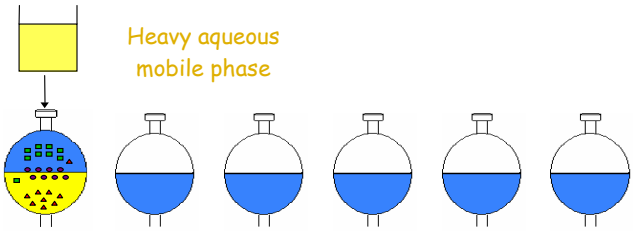
Few funnels are put in series and loaded with half volume of the lightest phase of your biphasic system. During the first step, the sample is added (solubilized in the lightest phase) in the first funnel. In the second step, the heaviest phase (same volume) is added and the funnel shaken. Compounds are shared out according to their affinity for both phases (partition coefficient Kd). In the third step, half of fresh heaviest phase is added in the first funnel and the lower phase is taken and put in the next funnel in the same time (step 3). All steps are repeated until you recover your three compounds at the outlet (step 4).



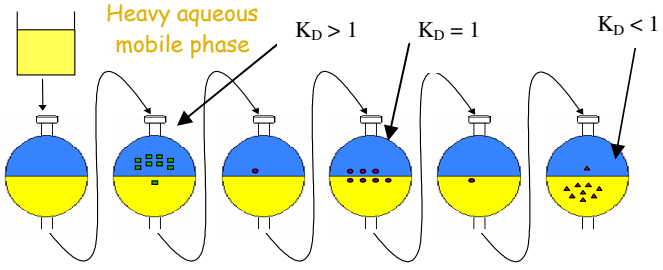
Step 1



Step 3



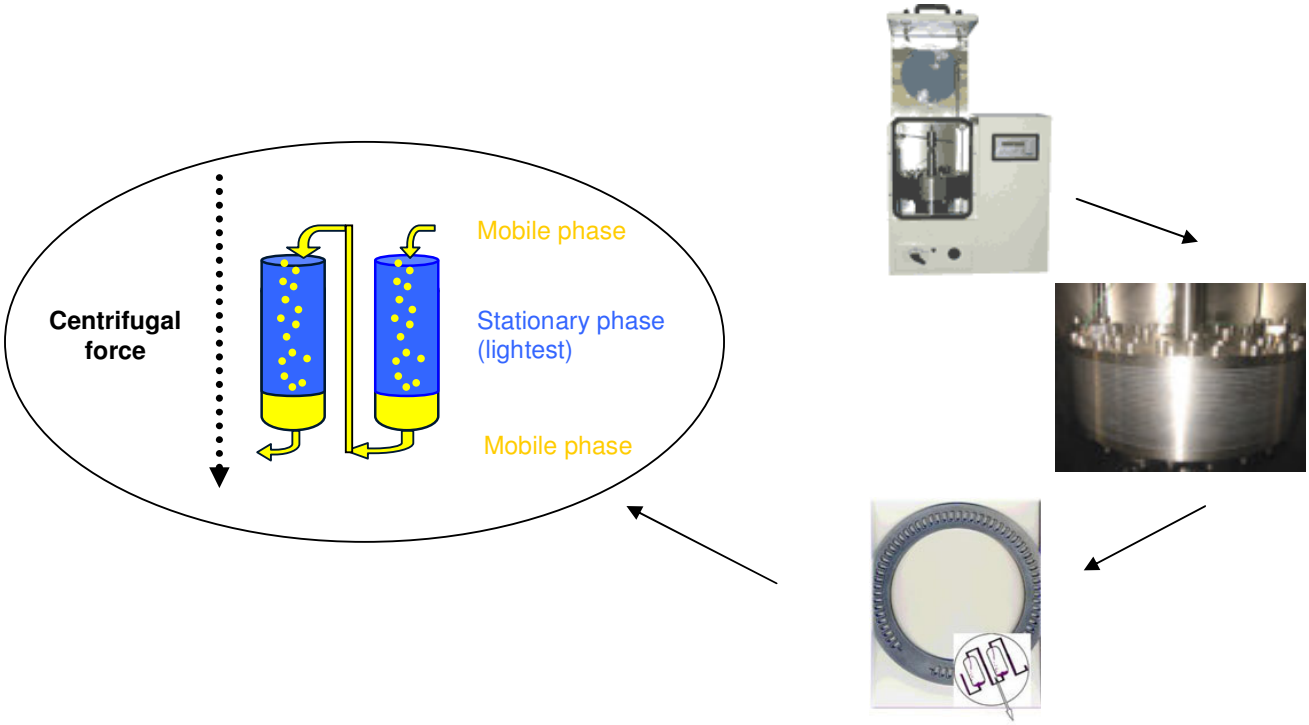
Step 2



Step 4

To sum up, you always inject heaviest phase at the beginning and recover it at this end: this is your mobile phase or eluant. The lightest phase is always kept inside each funnel: this is your stationary phase.

Principle with a CPC machine is the same, the process allows to keep stationary phase with centrifugal force and to elute mobile phase as in classical LC with a pump. More than 1000 mixing chambers (which could be compared with funnel) are connected in series. This allows obtaining enough efficiency to separate very closed structures of molecules.



Compared with preparative HPLC-Chromatography, the FCPC® -Technology is less expensive and easier in handling and maintenance, especially for purification of complex crude extracts from biological matrices.

A versatile technique

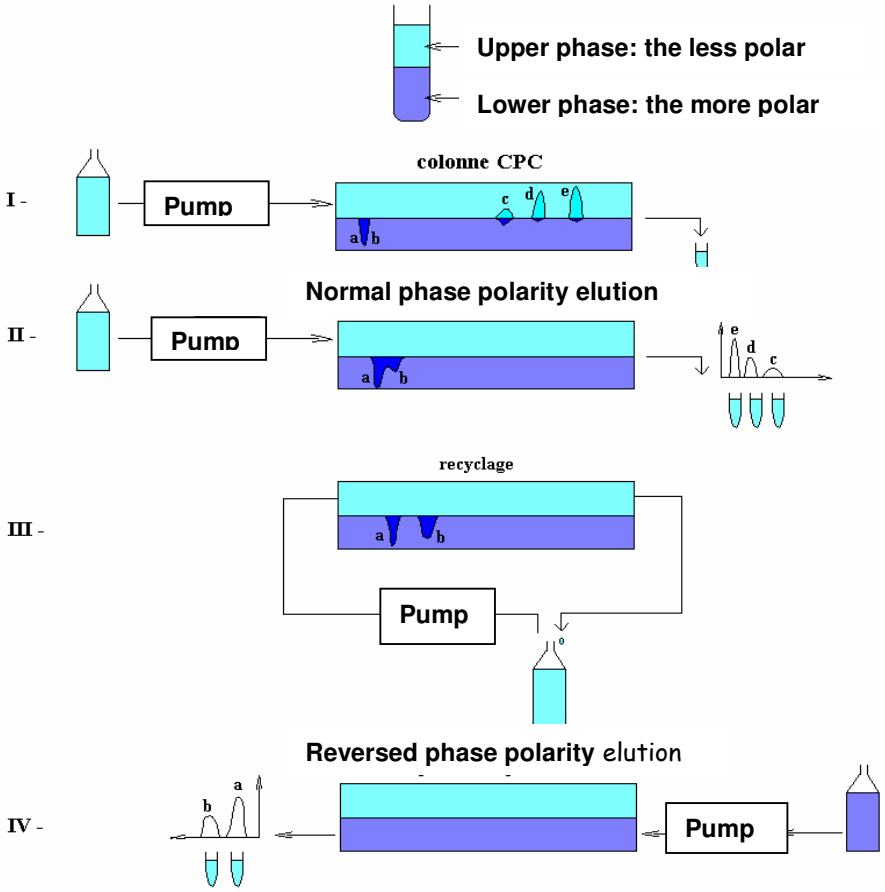
In CPC, one can work in different modes and modulate the selectivity. With both liquid phases, it is possible to choose the stationary phase and the mobile phase. As other chromatographic techniques, **normal mode** is applied when the stationary phase is the more polar and **reversed mode** when the mobile phase is so. We deal with descending mode when the mobile phase is the lower phase and ascending mode in the opposite case.

This allows various development mode possibilities:

- **Elution**: isocratic, gradient and dual mode
- **Displacement**: pH zone refining (devoted to the purification of compounds which electric charge depends on pH value like alkaloids) and ion exchange

In this example, we will use the dual mode, CPC specific mode, for the separation of 5 compounds **a, b, c, d,** and **e**. Their affinity for the mobile phase is different: compounds **c, d** and **e** (the less polar) are isolated by isocratic elution in **normal ascending mode**. Compounds **a** and **b** (the more polar) are strongly retained in the stationary phase. After the elution of the compounds **c, d** and **e** and a slight separation of the compounds **a** and **b**, the inversion stationary phase / mobile phase is done. Thus it is an elution in **reverse descending mode**.

Dual mode allows rapid production of highly purified compounds with reduced solvent consumption (0,8 l of solvents for 5 g of extract) and no loss of compounds.



Highlights of the FCPC®-system

- **Higher degree of purification/fractionation**: thanks to the high number of cells of the column (or rotor), the exchange area of phases is increased and so you can obtain a 99.99% purified product.
- **Fast**: thanks to the original cells design of the rotor, you can work from 3 to 5 times quicker than actual solutions.
- **Cost reduction**: the only running costs are solvent costs (no expensive solid support). Moreover, with FCPC®, your solvent consumption is reduced between 10 to 25% lower than other preparative chromatography techniques. This has also a positive impact on environment.
- **100% recovery**: CPC is a gentle partition technique, your sample is only in contact with solvents or inert materiel (no degradation/decomposition due to the solid support)
- **High selectivity**: you can increase selectivity (>2 or even 3)

- **Unlimited solvent systems :** no pH limitations, ideal for sample of all polarities, from proteins to fatty acids

- **Scaleable**: FCPC® product range allows to work from 50ml to 5 L rotors. Analytical methods developed on an FCPC®50 ml rotor can be directly up-scaled to a 5-liters preparative rotor. Furthermore, with FCPC®, you can use the same bench scale (interchangeable 50ml, 200mL and 1L rotors).

- **High capacity of injection**: with FCPC®, you can inject until 25% of rotor volume. Injection of raw extract without preparation.

- **Several specific development modes**: thanks to the liquid-liquid system, many modes can be chosen, according to the nature of your sample (isocratic, gradient, extrusion, dual mode, pH zone refining).

It is important to point out that this technique, like classical preparative LC, uses the same components including pumps, valve injection, detector and fraction collector. Only the container for the stationary phase is different, because the phase is liquid.

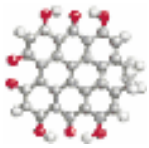
Application fields

General considerations

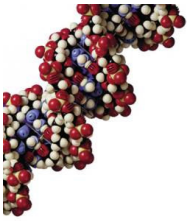
- Analytical to pilot scale separation or purification
- Purification from mg to 150 gr of one compound from complex mixture
- Purification of products with extreme polarities (from non polar fullerenes through polar sulfated polysaccharides)
- Purification of fragile product
- Biphasic aqueous system for polar compound (like proteins)

Compounds already purified by CPC:

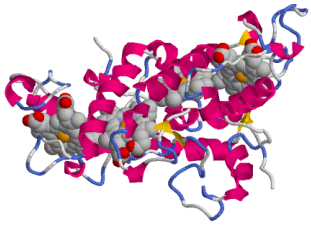
- Natural molecules from plants, soils, marine origin:
  - Alkaloids,
  - Saponins,
  - Flavonoids,
  - Polyphenols as tannins…
  - Sugars,
  - Carotenoids,
  - Fatty acids,
  - …



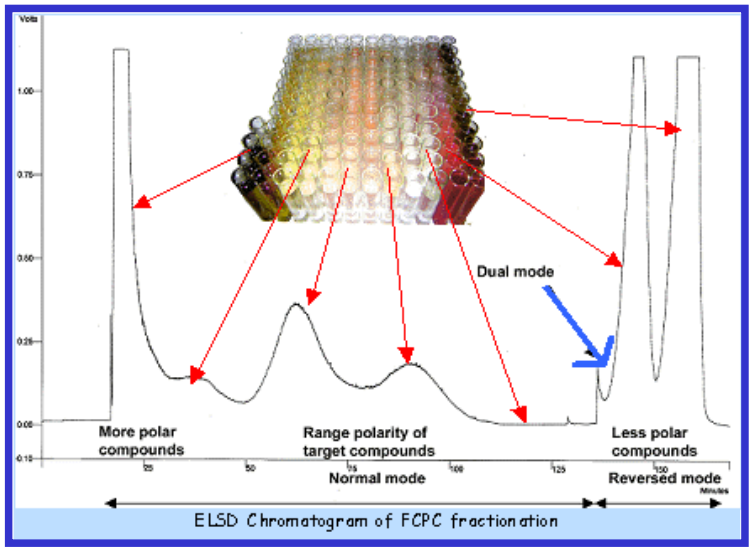
- Biological molecule:
  - Amino acids, peptides and proteins,
  - ADN, ARN,
  - Antibodies,
  - …



- Fermentation product:
  - Antibiotics,
  - Synthetic drug compounds,
  - …
- Pesticides, Weed-killer…



Examples of application

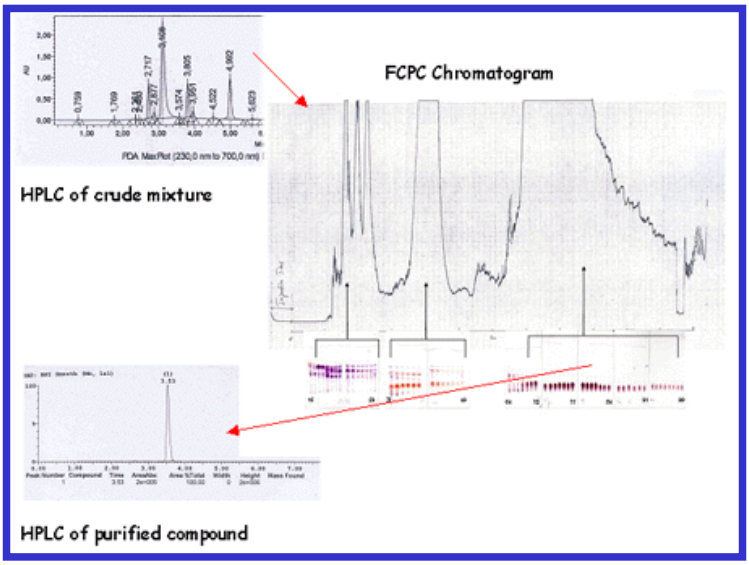


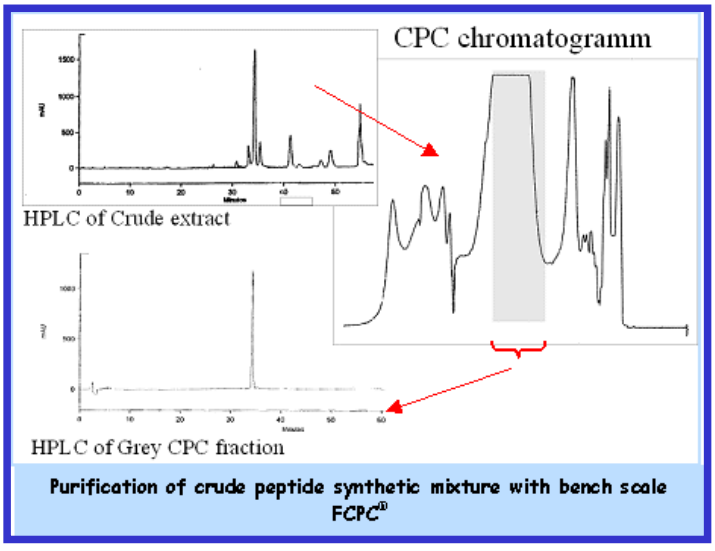
Hypericin purification from St John Wort plant

FCPC	Bench scale FCPC1000
Mass injected	3 gr
Duration	115 min
Solvent consumption	2,3 L
Purified products	Hypericin/Pseudohypericin purity>95%

Synthetic dyes purification

FCPC	Bench scale FCPC1000
Mass injected	8,5 gr
Duration	330 min
Solvent consumption	3,3 L
Purified products	Dyes, 99% purity, 89 % recovery





Synthetic peptide purification

FCPC	Bench scale FCPC200
Mass injected	1,0 gr
Duration	50 min
Solvent consumption	0,6 L
Purified products	Peptide, 98% pure, 70 % recovery