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# CENTRIFUGAL PARTITION CHROMATOGRAPHY: AN OVERVIEW

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**ABSTRACT**

Centrifugal partition chromatography (CPC) is a new and unique method of liquid-liquid chromatography. CPC enables the separation of components with nearly identical partition ratios, and is performed without the aid of a solid support. The method is used for chromatographic reaction in addition to chromatographic separation. Centrifugal partition chromatography is a type of counter current chromatography, which is an automated liquid-liquid extraction process permitting hundreds of automatic successive extractions. A CPC instrument or a CPC column is a series of channels linked in cascade by ducts and aligned in cartridges or disks in a circle around a rotor; setting the rotor in motion submits this assembly to a constant centrifugal field. The originality of CPC is that it uses any biphasic liquid-liquid system as mobile and stationary phases. The United States branch of Sanki contributed greatly to the worldwide acceptance of the technique. Recent works performed in the Netherlands and in France have, by means of visualization of flow-patterns in CPC channels, contributed to a better knowledge of hydrodynamics and mass transfer phenomena. Centrifugal partition chromatography has wide advantages like No column to replace, no silica to recycle, Low solvent consumption, High flow rate for low run time, High performances. Purity > 99%, recovery>90%,No sample losses, No denaturation, no irreversible adsorption of the sample, Huge application fields from petroleum extract to proteins. Hence it is widely employed in the pharmaceutical industry.

**Keywords**: CPC (Centrifugal partition chromatography), Partition Disc, CPC Column, Mono axial rotor, Centrifugal force, Fraction collector.

## INTRODUCTION

Centrifugal Partition Chromatography (CPC) also known as Counter Current Chromatography (CCC) is a preparative, pilot and industrial liquid purification technique that does not require traditional solid supports. The main aims of this technology are 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 [1].

Centrifugal partition chromatography (CPC) is a new and unique method of liquid-liquid chromatography. CPC enables the separation of components with nearly identical partition ratios, and is performed without the aid of a solid support. The method is used for chromatographic reaction in addition to chromatographic separation [2].

CPC is unique because no solid support is used for the stationary phase. Instead, the liquid stationary phase is retained in the column by a combination of centrifugal force, the special tortuous column geometry and the density difference between the two liquid phases. The CPC apparatus consists of coil columns "undergoing one particular mode of planetary motion generated by a synchronous coil planet centrifuge". The column contains one or more cartridges in which channels are engraved, attached to a rotor. The less dense stationary phase remains in the column because of the centrifugal force created by the spinning rotor. Consequently, the mobile phase is able to pass through the stationary phase.

When a mixture of components is introduced into the mobile phase of the CPC column, it distributes according to the individual components' distribution coefficients while passing through the column. The centrifugal force field applied to the coiled columns promotes the retention of the stationary phase against a continuous flow of mobile phase. The mobile phase flow

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enables the two phases interact sufficiently for partition to occur. Chromatographic separation results and separation is sufficiently complete as hundreds to thousands of theoretical plates can be achieved [3].

The degree of separation in centrifugal partition chromatography depends primarily on the partition coefficient of the solute between the two phases. Other important parameters in the separation process include mass transfer coefficients, flow rate, rotational frequency, and the identity of the two phases.

A variety of the two-phase systems are possible using the CPC column. Both organic and aqueous systems are feasible. In fact, using CPC chromatography, aqueous two-phase systems can be used for separation.

Clearly, CPC provides successful chromate graphic separation. This chromatographic technique is also used for chromatographic reaction. The technique causes the equilibrium of a reversible reaction to shift. Reaction takes place in the stationary phase, while products are separated into the mobile phase. Since chromatographic separation of the products occurs simultaneously with production, the reverse reaction is impeded. Therefore, reactions beyond thermodynamic equilibrium are achieved [4].

**CPC/CCC 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 LC instrument in general is the column where the separation occurs. The fundamental difference between LC like flash or HPLC and CPC/CCC 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 generate chemical interaction with mobile phase and molecules to be separate. Centrifugal Partition Chromatography does not require a solid support like silica: two non-miscible phases used, one as the mobile phase or the eluent and the other as the stationary phase maintained by the centrifugal field. The affinity of the solute for each phase can be measured by their partition coefficient that in turn dictates the order of elution for each compound.

**PRINCIPLE**

The centrifugal partitioning chromatograph functions based on the principles of liquid / liquid partitioning chromatography, in which two immiscible liquid phases are mixed together and then separated multiple times. The individual solutes are isolated based on the different partitioning coefficients of each compound in the solvent phase versus the diluents phase.

One of the liquid phases of the two-phase system is used as a stationary liquid phase: it is fed into the column (the rotor) while the latter is spinning at moderate rotational speed. The stationary phase is retained inside the rotor by the centrifugal force generated [5].

The second phase of the two-phase system is used as the mobile phase containing the solutes to be extracted. It is fed under pressure into the rotor and pumped through the stationary phase

Both phases are mixed together. It is at that time that the exchange of molecules between the two phases occurs. The separation of the solutes is achieved as a function of the specific partitioning coefficient (Kd) of each solute between the mobile and stationary phases. The mobile phase then decants at each cell outlet thus entering the next cell. The eluted fractions of the mobile and stationary phases are collected over a period of several minutes to several hours. These fractions, or elutes, will contain the individual purified solutes.

**Operation of the centrifugal chromatograph**

A stationary liquid phase is fed to the rotor while it is spinning at moderate rotational speed. The stationary phase is retained inside the rotor by the centrifugal force generated. The mobile phase, containing the solutes to be extracted, is fed, under pressure, to the rotor and is pumped through the stationary phase. The separation of the solutes is achieved as a function of the specific partitioning coefficient of each solute between the mobile and stationary phases.

The eluted fractions of the mobile and stationary phases are collected over a period of several minutes to several hours. These fractions, or elutes, will contain the individual purified solutes: The fractions can be collected using automatic sample recovery systems.

A four way valve allows a change in the direction of the elution and therefore will work either in ascending mode. When the lightest phase is the mobile phase or in descending mode when the heaviest phase is the mobile phase. By working this way, it is possible to work both in normal and reversed mode without replacing the column [6].

Depending on the solvent system chosen, the CPC can operate in two modes; 1.Descendingmode, and
2. Ascending mode:

**1. DESCENDING MODE**

When operating in the descending mode, the heavier liquid phase is the mobile phase and migrates through the stationary light phase. This is also known as a light phase continuous extraction system.

**2. ASCENDING MODE**

When operating in ascending mode, the lighter liquid phase is the mobile phase and migrates through the stationary heavy phase. This is also known as a heavy phase continuous extraction system. The centrifugal

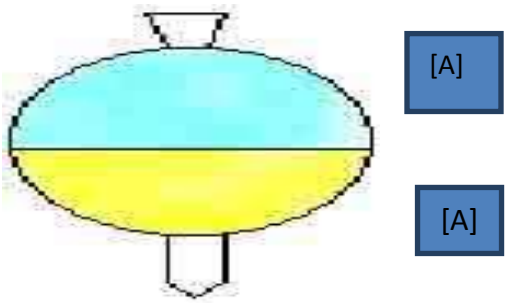
chromatograph is well adapted to non-polar and polar solvent systems.

**Separation of Compounds**

Chromatographic effect in CPC/CCC can be easily explained. Place two non-miscible solvent (for example butanol and water) in a separatory funnel, shake and wait for equilibrium: the upper phase is made of a majority of butanol and lower phase of a majority of water. After dissolution of a molecule A with a concentration [A] in 2 mL of bi physique system made with 1 ml of each phase, shaking and equilibrium (two phases), A is more or less dissolves in both phases. At the equilibrium, for a given temperature, the concentration ratio (respectively [A]upper and [A]lower, [A]=[A]upper+[A]lower) allows us to define

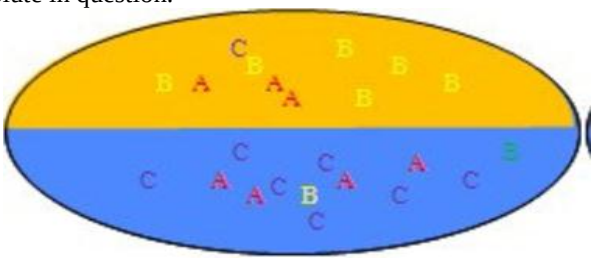
The partition coefficient Kd :Kd=[A]stat/[A]mob

For a given substance "A" the partition coefficient is defined as




αA= [A] upper phase/[A] lower phase

αA is a constant at any given temperature, and unaffected by the presence of other substances or concentration of the solute in question.



KdA=1,KdB<1,KdC>1.

**In this, Upper phase is Butanol,Lower phase is Water.**

From this, it results that for a Kd close to 0, the molecule is completely dissolved in the mobile phase and will not be retained in the system. For a Kd that is too high, the molecule will be too well retained in the stationary phase. So, the best partition coefficient for a good separation is between 0.5 and 5. The Solvent system can then be determined according to the partition coefficients of all molecules that need to be separated [7].

**Conditions**

For a 200 ml rotor, a typical flow rate is between 10 and 15 ml/min, for 1000 ml rotor between 30 and 100 ml/min. The high flow rates allow faster completion of a run, which in-turn, leads to substantial savings of time and costs.

**Working principle**

A bi-phasic solvent system suitable for fractionation of a sample is prepared based on literature data or own experiments. After selecting one of the phases as the stationary, it is pumped into the FCPC® rotor, while it is revolving at 200 rpm. The rotor contains 1,200 separate micro compartments that are connected in series. The stationary phase is kept immobilized due to a special design of the channels combined with the effect of the centrifugal force. The design allows, however, for the mobile phase to pass from one mixing chamber to another.An injected sample passes through these compartments and in each of them, like in a separatory funnel, the components of the sample partition between the two phases. The rotational speed is increased to 700 rpm or higher for the partitioning mode when a mobile phase is pumped into the rotor. When equilibrium is established between the two phases, a sample is injected and the solutes begin partitioning between the phases, much as they do in the separatory funnel. Because the components partition between the two phases differently, the mobile phase will carry out faster the components with the smallest partition coefficients (*p*= [c] stationary phase/[c] mobile phase).

Thus, the component to be eluted first is the one that partitions best into the mobile phase. On exit from the rotor, the eluting mobile phase is directed to an appropriate detector and/or fraction collector [8].

**INSRTUMENTATION**

**1. Hydrostatic CCC columns**

The very first hydrostatic CCC columns used gravity to maintain the liquid stationary phase; they were called droplet CCC (DCCC) columns. They needed very long elution times (days). The DCCC columns are no longer in use today. Modern hydrostatic CCC columns are known and marketed under the name of centrifugal partition chromatographs (CPCs).

Their two main characteristics are:

(1) They have a single axis of rotation generating a constant centrifugal field and

(2) They enclose geometrical volumes, tubes, channels, or locules that repeat themselves through connecting tubes forming a pattern. It can be seen that there is quite a significant volume of connecting ducts which only contain the mobile phase.

**2. Hydrodynamic Columns**

In hydrodynamic CCC the liquid stationary phase is held in a stratified way along the length of a continuous piece of tubing. The mobile phase flows past this stationary phase

and experiences a series of mixing and settling steps as it makes its way from one end of the tubing to the other. The sample is injected with the mobile phase (in either upper or lower phase) and elutes from the other end of the tubing at a time governed by how well it "partitions" between the mobile and stationary phases. If it is only soluble in the mobile phase ($K_d$ =0) it will go through with the mobile phase, but if it is only soluble in the stationary phase (Kd=∞) it will stay in the column. However unlike solid phase chromatography the centrifuge can always be stopped and the stationary phase pumped out and retained substances recovered [9].

The most common (and simplest) form of hydrodynamic CCC column is the multilayer (J-Type) coil planet centrifuge, which consists of a planetary rotor which rotates in synchronised (1 to 1) planetary motion about the main axis of rotation. If you can imagine a gear meshing with and rotating around an identical gear then the rotating gear is the planetary one on which a bobbin is mounted and on which a continuous length of tubing can be wound. The tubing forms a multilayer helix which as it rotates causes the liquid phases to screw up to the head end of the coiled tube. But if the tube is closed then one phase displaces the other liquid phase to the opposite end, just as upper phase is displaced upwards in a test tube by the heavy liquid phase. In hydrodynamic CCC the general rule is that the heavy phase goes to the "Tail" while the lighter phase goes to the "Head". If the mobile phase is the lower phase then it is pumped from Head to Tail and if it is the upper phase it is pumped from Tail to Head [10].

**Hydrostatic CCC columns or CPCs**

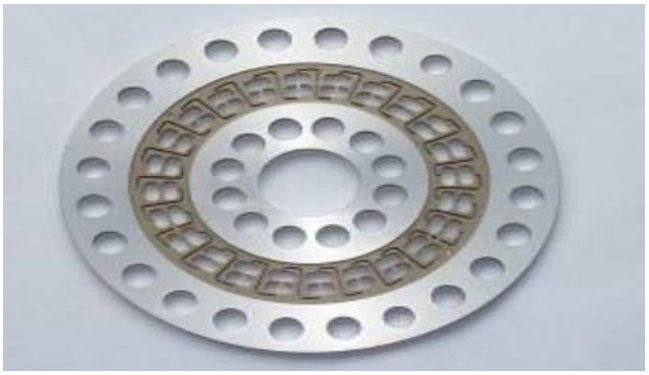
The liquid motion in the two kinds of CCC column is very different. It is relatively easy to understand that the liquid stationary phase will be physically retained in the channels of the hydrostatic columns. If the mobile phase flow rate is stopped, the two liquid phases stay where they are (hence the term hydrostatic).

The way the mobile phase moves through the stationary phase depends on their relative density. In this, the black mobile phase is the lighter liquid (the upper phase in a test tube). It is illustrated entering the channel through the lower end and leaving at the top end—this is called ascending mode where the mobile phase rises through the retained denser stationary phase. The denser or lower liquid phase could be used as the mobile phase. In that case, it would enter in the channel from the top end and leave via the lower end—this is known as descending mode where the mobile denser phase descends through the lighter retained stationary phase. In this, the mobile phase is shown breaking up into droplets. In practice, the mobile phase cascades through the stationary phase, bending in the direction of rotation due to the Corioliseffect, and produces its mixing spray or droplets when the cascade hits the chamber wall or the liquid interface [11].

**MONOAXIAL ROTAR**

- CPC/CCC is made with one axis where one rotor or column is installed.
- It is made up of stainless steel.

**PARTITION DISC**



- The column is made up of stacked disk.
- It consists of specially designed thousand extraction cells.
- Connecting ducts are present from centre to the top of each cell.
- The upper and lower walls consists of inter disc 'Teflon gaskets'.

Note the connecting ducts centered on the bottom and the top of each cell. The upper and lower cell walls consist of the interdisk Teflon gaskets [12].

Where more than a thousand cells are linked together by a thin engraved duct. At each extremity of the column, a rotary seal allows the passage of the liquid from the static to the rotating part. The rotor or column is kept under a homogenous centrifugal field which allows retention of the stationary phase thanks to the specific geometry of the cells [13].

**Fraction collector LS5600**

It collects the different fractions from the mixure of compounds.

**Pump**

AP50 pump : 50 ml/mn, 300 bars Isocratique, binary or quaternary gradient
AP100 pump : 100 ml/mn, 250 bars Isocratique, binary or quaternary gradient
AP250 pump : 250 ml/mn, 230 bars Isocratique, binary or quaternary gradient
AP500 pump : 500 ml/mn, 110 bars Isocratique, binary or quaternary gradient
AP1000 pump : 1L/mn, 80 bars, bars Isocratique, binary or quaternary gradient

**Armen Glider software**

The Armen Glider Software (AGS) 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 fraction collector,With AGS, you develop your complete method from stationary phase loading, equilibration, injection, elution, extrusion or dual mode, put the solvent bottle in the right place and performed your run with one click. Collection of your fractions can be defined according to time, volume, threshold and peak. Methods can also be modified during optimization runs [14].

**Detector**

Armen UV/Vis DAD 600 with prep flow cell.

**Solvent Systems**

**Three criteria**

Numerous two-phase solvent systems with a broad spectrum of polarity or containing extracting reagents can be applied to separate organic, bioorganic, and inorganic substances. Either the aqueous or organic phase of a two-phase liquid system can be used as the mobile phase. In general, solvent systems known from preparative CCC purifications can also be used at the analytical scale. The systems for inorganic separations are very different from those for organic separations, as in most cases the former contain a complexing or extracting reagent (a ligand) [15].

There are three important criteria for choosing a two-phase liquid system.

- First and obviously, it should form two immiscible phases.
- Second, the phase selected to be the stationary phase should be retained by the CCC column.
- Third, the sample should be separated by the two-phase liquid system selected.

**Two immiscible phases**

There are countless solvent mixtures that form two immiscible phases. When the nature of the organic substances to be separated is known, one may find a suitable solvent system by searching the literature for solvent systems that have been successfully applied to similar compounds. In case of organic- aqueous two-phase systems, the organic phase consists of one solvent or of a mixture of different solvents. Various non-aqueous/non-aqueous solvent systems (e.g., heptane/acetonitrile) have been used for separation of non polar compounds and/or compounds that are unstable in aqueous solutions.

Separation of macromolecules and cell particles can be performed with a variety of aqueous/aqueous polymer phase systems. Among the various polymer phase systems available, the following two types are the most versatile for performing CCC. The polyethylene oxide (PEO)/potassium phosphate systems provide convenient means of adjusting the partition coefficients of macromolecules by changing the molecular mass of the selected PEO and/or altering the pH and/or concentration of the phosphate buffer. The PEO 6000/Dextran 500 systems provide a physiological environment, suitable for the separation of mammalian cells by optimizing osmolarity and pH with electrolytes. For pre concentration and separation of inorganic species, a stationary phase containing extracting reagents of different types (cation-exchange, anion-exchange, and neutral) in an organic solvent is usually applied. The mobile-phase components should not interfere with the subsequent analysis. Solutions of inorganic acids and their salts are most often used. The mobile phase may also contain specific complexing agents, which can bind one or several elements under separation [16].

**Applications**

The applications of centrifugal partition chromatography are profuse and diverse. One special feature of the new technique is that partition can be achieved between two distinct aqueous phases. This aqueous two-phase system, ATPS, is created when one or more polymers and a salt are added to water. When the concentrations of polymer and salt become high enough, two-phase result which are both more than fifty percent water by weight. Such a two-phase aqueous system is important because it lends itself to biological applications. Organic systems are typically not mild enough for biochemical compounds. Additionally, the separation of amino acids is not possible in organic systems due to low solubility.[16]

CPC is used for many types of separations. Racemic mixtures can be separated into chi rally pure compounds. Natural products and amino acids are commonly separated on centrifugal partition columns. CPC is also utilized for enzymatic reaction. CPC reactions are advantageous because the reactions proceed beyond thermodynamic equilibrium. Additionally, reaction kinetics can be measured in addition to partitioning and mass transfer.

Since CPC is a new technique, much work remains to be done. Optimization of the columns, study of non-linear systems, and development of new applications are some aspects of the method currently being explored.

From non-polar carotenoids to very polar peptides and proteins, this technique allows one to obtain high purity molecules in very small amounts (mg) or in high quantities(kg) from all kind of matrices:

Here are few examples:

Essential oils,
Chlorophylls,
Lipids,
Antibiotics from fermentation broths,
Alkaloids,
Polyphenols,
Natural products screening.
Synthetic compound
From fulleneris to dyes
Enantiomers
Petroleum extracts
In Bio technology,
Proteins
Monoclonal antibodies

Poly nucleotides
Peptides
CPC Technology has Applications in the Following Industries.
Neutraceutical,
Fine
Pharmaceutical
Biotechnology
Biomedical
Fats and Oils Fermentation

**ADVANTAGES**
The volume of stationary phase available (more than 60 %) and the absence of a 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 low run time
High performances. Purity > 99%, recovery > 90%
No sample losses
No denaturation, no irreversible adsorption of the sample
Huge application fields from petroleum extract to proteins [17].

**Cost efficient**
No column or solid support.
5-10x less solvent and energy.
Direct scaleof from bench to industrial scale.

**Green chemistry**
Low sample volume consumption to recover products.

**Gentle technique**
No denaturation of fragile molecules like proteins and peptides.
No irreversible adsorption or sample loss [18].

**Figure 1. Sequence steps involved in separation**


**Figure 2. Flow diagram of the two operating modes of the centrifugal chromatograph**

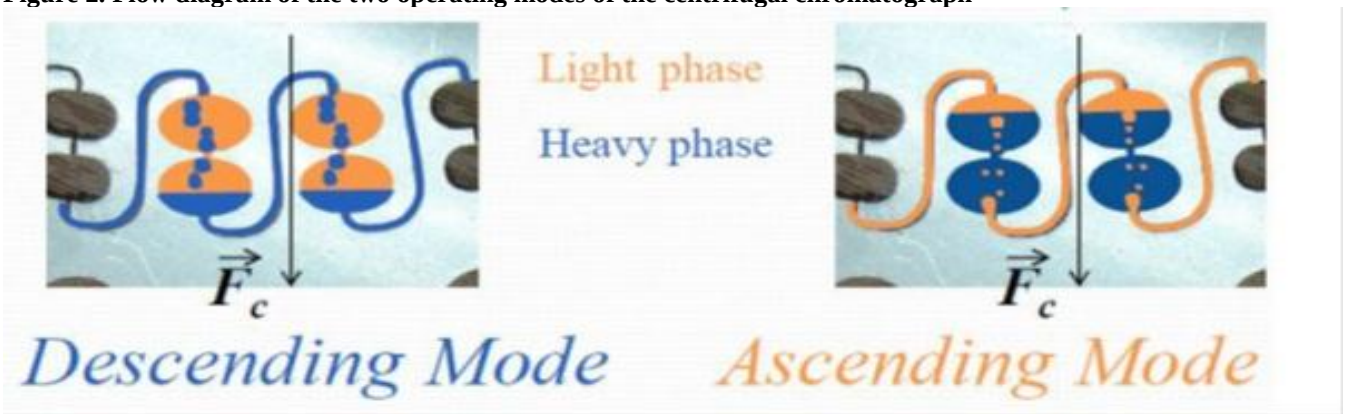


**Figure 3. The Inner Workings of the CPC**

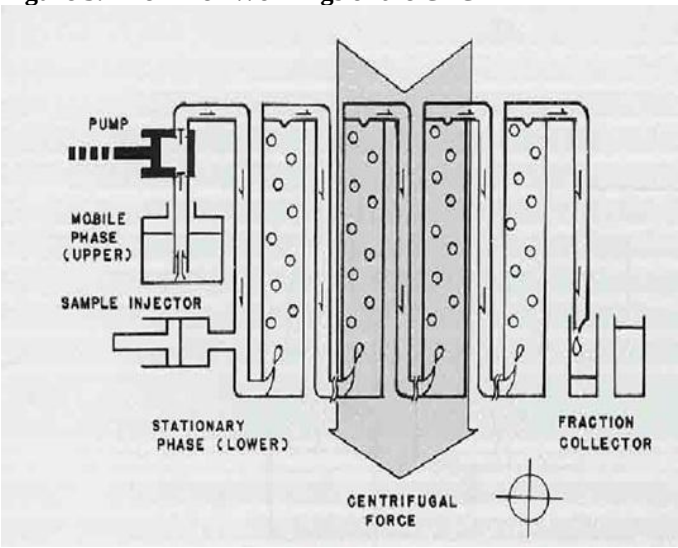



**Figure 4. Separation occurred in CPC column**

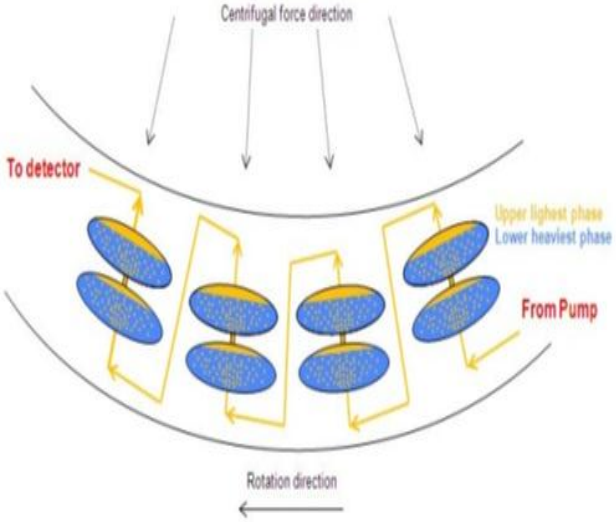



**Figure 5. Liquid motion in CCC columns**

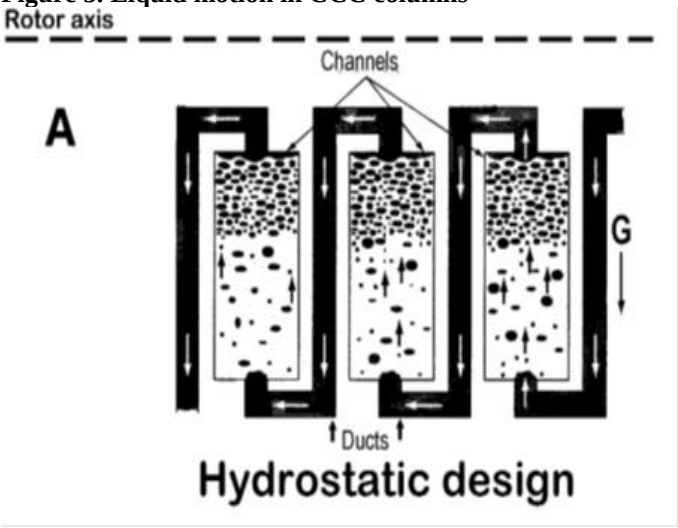



**Figure 6. MonoaxialRotar**

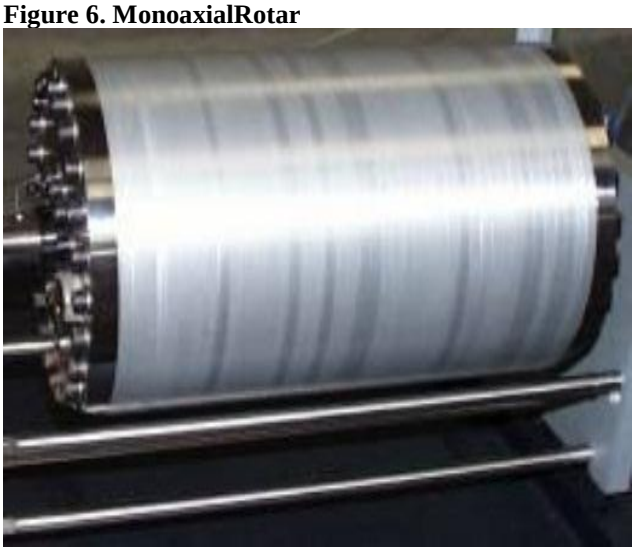


**Figure 7. Partition Disc**

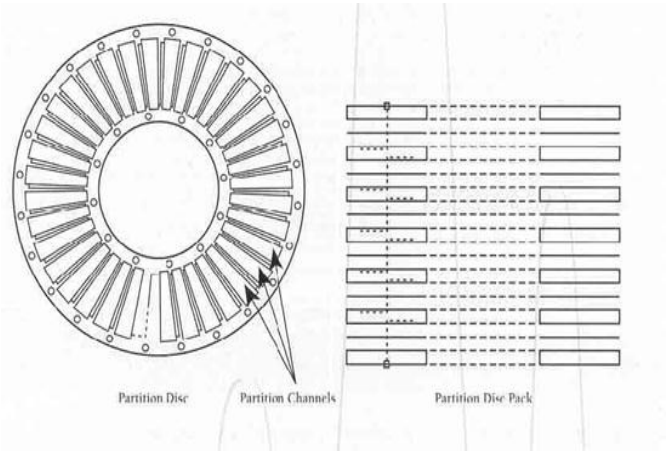



**Figure 8. In Kromaton Technologies FCPC 200 mL rotor (left) and partition disk (right)**

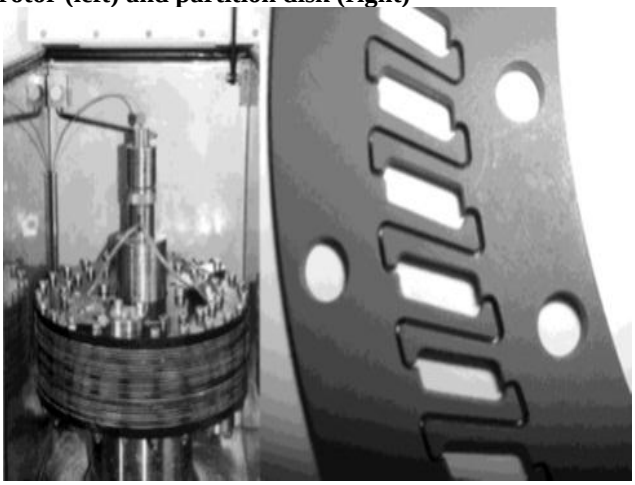


**Figure 9. Motion of liquids in a cell**

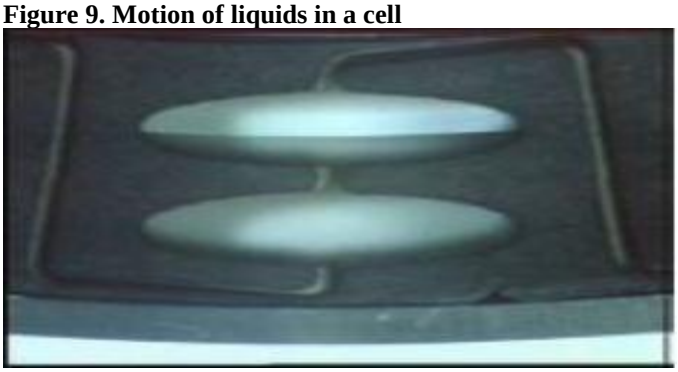
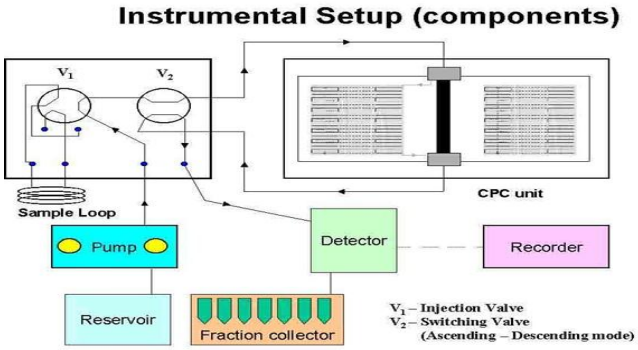


**Figure 10. CPC Instrumental Set up (Components)**




**REFERENCES**

1. García DA. Díez Masa JC. Retention parameter in chromatography (IUPAC Recommendations 2001). *Pure Appl. Chem.*, 73, 2001, 969.
2. Friesen JB, Pauli GF. *J.* Phytoconstituents from Vitex agnus-castus fruits. *Agric. Food Chem*, 56, 2011, 19.
3. Ikehata JI, Shinomiya K, Kobayashi K, Ohshima H, Kitanaka S, Ito Y. Effect of Coriolis force on counter-current chromatographic separation by centrifugal partition chromatography. *J. Chromatogram. A*, 1025, 2011, 169-175
4. Shin Omiya K, Kabasawa Y, Yanagidaira K, Sasaki H, Muto M, Okada T, Ito Y. Protein separation by nonsynchronous coil planet centrifuge with aqueous-aqueous polymer phase systems. *J. Chromatogram. A*, 1005, 2010, 103-112.
5. Zhang TY. Separation and purification of natural products (medicine herbs) by HSCCC. *In Counter current Chromatography, Comprehensive Anal. Chem, Berthod*, XXXVIII, 2009, 201-260.
6. Jin YR, Zhang LX, Zhang LZ, Han SJ. Separation of inorganic compounds: theory, methodology and applications. *In Counter current Chromatography, Comprehensive Anal. Chem, Berthod, A*., XXXVIII, 2008, 261-300.
7. Ettre LS. *Pure Appl. Chem*., 65, 1993, 819.
8. Ito Y. In Counter current Chromatography: The Support-Free Liquid Stationary Phase, A. Berthod (Ed.). *Comprehensive Analytical Chemistry*, 38, 2008, xix–xx.
9. Jin YR, Zhang LX, Zhang LZ, Han SJ. In Counter current Chromatography – the Support free Liquid Stationary Phase, 38, 2007, 261.
10. Marchal L, Foucault A, Patissier G, Rosant JM, Legrand J. Influence of flow patterns on chromatographic efficiency in centrifugal partition chromatography. *J. Chromatogram. A*, 869, 2006, 339-352.
11. Menet JM, Thiebault D. Counter current Chromatography, Chromatographic Science++ Series, Marcel Dekker, New York, 82, 2005.
12. Foucault AP, Chevolot L. Counter-current chromatography: instrumentation, solvent selection and some recent applications to natural product purification. *J. Chromatogram. A*, 808, 2003, 3-22.
13. Ito Y, Conway WD. Chemical Analysis, John Wiley, New York, 132, 2003.
14. Oka H. In High-Speed Countercurrent Chromatography, Ito Y, Conway WD. JohnWiley, Chichester, 73, 2002.
15. Conway WD, Petroski RJ. Modern Counter current Chromatography. ACS Symposium Series No. 593, American Chemical Society, Washington, DC, 2001.
16. Conway WD. Counter current Chromatography, Apparatus, Theory and Applications, VCH, Weinheim, 2000.
17. Mandava NB, ItoY. Counter current Chromatography, Chromatographic Science Series. Marcel Dekker, New York, 44, 2000.
18. Ito Y, Weinstein M, Aoki I, Harada R, Kimura E, Nunogaki K. *Nature*, 212, 1966, 2000.