

Exploring How Centrifugal Partition Chromatography Progresses Green Chemistry Practices

Written by Bence Lovas, Arpad Konczol PhD,
Hajnalka Saranko PhD
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Abstract

Despite its widespread use in the chemical industry, traditional preparative-scale liquid chromatography is considered unsustainable due to its high solvent consumption and solid waste generation. A promising green alternative to this method includes solid support-free techniques like hydrostatic countercurrent chromatography — also called centrifugal partition chromatography (CPC).

In this white paper, we'll discuss CPC's sustainability, demonstrated by its solvent flexibility and recyclability. We'll also investigate how it supports other downstream processes and extracts value from low-value waste compounds.

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The Basics of CPC

In contrast to conventional solid-liquid chromatographic techniques, CPC consists of liquid stationary and mobile phases. The stationary phase is held in place by a strong centrifugal force inside the column, while the mobile phase is pumped through it. The partitioning of the solutes between the two immiscible phases governs purification and affects the time spent within the stationary phase, which determines solute retention.

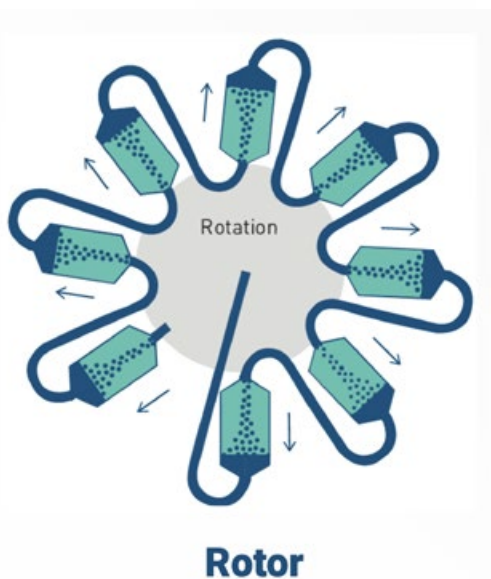
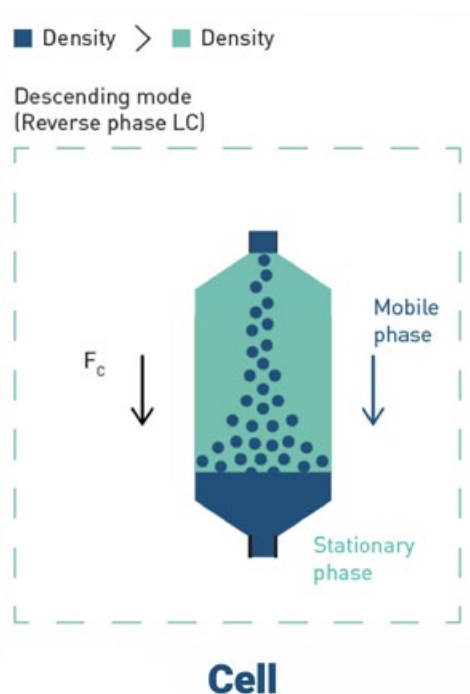


Diagram 1: CPC explained



The iCPC platform is the largest CPC instrument commercially available worldwide and delivers cost-effective continuous or batch industrial-scale purification projects.

An Alternative to Solid-Based Systems

CPC, as a liquid-liquid system, offers significant cost benefits because it eliminates the need to purchase silica gel and reduces the amount of required solvents. The few types of commercially available solid silica-based packing gel limits stationary phase options. And, when the gel, which fills the entire column, is saturated due to adsorption and needs to be changed, the whole column of gel must be replaced which generates a lot of waste.

Being constrained to silica gel stationary phases also limits traditional chromatography's flexibility. When fine-tuning the separation process, chemists usually only alter the mobile phase composition instead of adjusting the stationary phase. CPC takes advantage of a wider range of possible biphasic liquid-liquid systems using the same column.

Substituting halogenated and petroleum-based solvents with green, renewable alternatives reduces the environmental impact of chromatography. Recent regulations have even banned several hazardous solvents, including n-hexane, making the use of green alternatives important.

How To Choose Your Solvents

Determining potential biphasic systems by trial and error is a time-consuming task due to the wide polarity range to cover the most apolar to most polar systems. To overcome this challenge, several regulatory and industry bodies have produced guides on which solvents to use based on properties such as hazardousness, toxicity and recyclability.

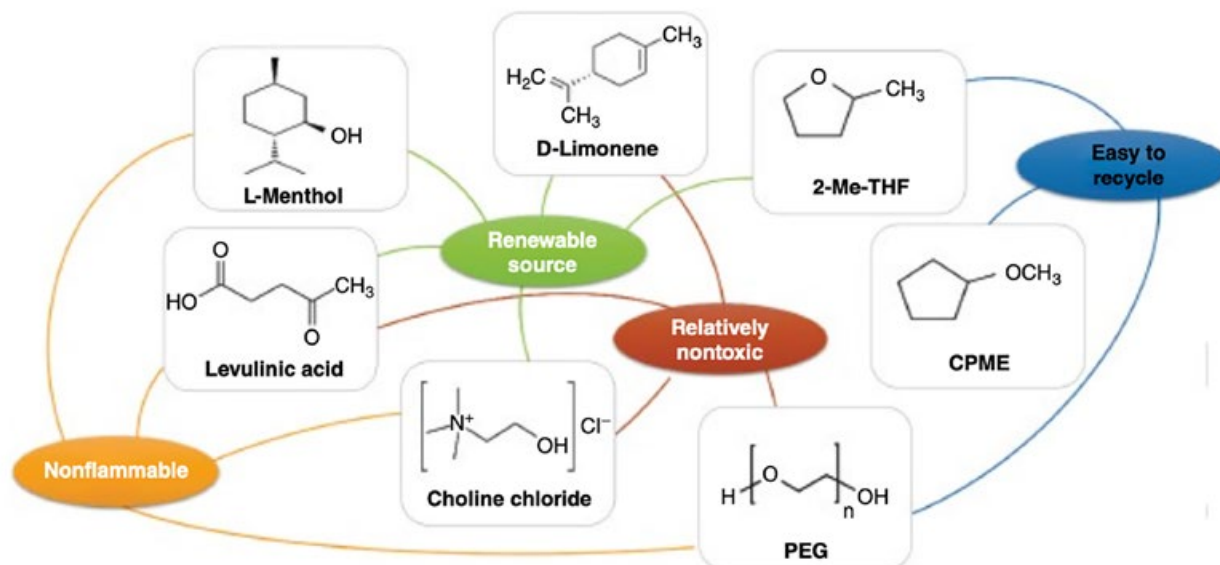


Diagram 2: Solvent characteristics

And, both academic and industrial researchers have composed simple strategies to help you choose the most effective solvent system for a given application.

Ideal solvents for CPC have low viscosity, are UV transparent, cover a wide polarity range and have strong interfacial tension between the aqueous and organic phases. When accounting for additional post-separation fraction processing, solvents with low boiling points work best.

Green solvents include the following:

- **2-Methyl-Tetrahydrofuran (2-Me-THF).** This solvent is a green alternative to THF in synthesis because it is nontoxic, has low miscibility in water and is relatively stable compared to other heterocycles.
- **Limonene.** This apolar solvent has a D-isomer, which is a major component of the essential oils in citrus fruits.
- **Cyclopentyl methyl ether (CPME).** This solvent can directly replace dichloromethane (DCM) in the widely used MeOH-DCM system.
- **Water.** This safe, environmentally friendly solvent is usually the first choice for a green CPC application for aqueous two-phase systems (ATPS).

Another group of potential green compounds are deep eutectic solvents (DES) – more specifically, NaDES, when their components are of natural origin. These solvents are characterized by a large depression of freezing points, usually higher than 150°C. You can acquire DES by simply combining two safe, cheap,

renewable and biodegradable components capable of forming a eutectic mixture. If the challenge of solute recovery and component recycling can be solved, NaDES would be a promising alternative to water-based solvent systems, providing a polarity range that facilitates the separation of less polar and hydrophobic compounds.

Solvent Recycling

Getting the Most Out of Solvent Systems

Sustainable separation processes reuse as much solvent material as possible, and CPC can benefit from solvent recycling through several processes like membrane filtration, ultrafiltration, dialysis and density-based recirculation. Here's a rundown of the research done on each method:

Membrane filtration. It has been demonstrated that combining countercurrent chromatography with organic solvent nanofiltration (OSN) is a cost-effective purification method to isolate an active pharmaceutical ingredient (API) with a molecular weight of about 600 Da. They found that API rejection rates are dependent on the solvents used and even though they achieved more than 99% efficiency with their filter membranes, there is a need to develop membranes with 100% removal efficiency as their total API loss was 2.3% throughout the entire process. It was observed that using OSN to recycle the mobile phase solvent can provide 56% greater mass-intensity and highlights its value in optimizing CCC mass-efficiency.

Dialysis. It has been found that this recycling method shows potential to boost solvent use efficiency. In a CPC purification run with an aqueous two-phase system (ATPS), dialysis was used to isolate separated phenolic compounds and recycle the sodium polyacrylate and polyethylene glycol (PEG)-rich phases of the ATPS. They observed that when compared to CPC runs without dialysis, CPC runs with dialysis can reduce carbon footprint by as much as 36%.

Ultrafiltration. This method provides substantial solvent recovery results. Researchers modeled the continuous separation of PEGylated and non-PEGylated Cytochrome-C (Cyt-c) using CPC. Their results showed that through ultrafiltration, both the product and unreacted protein could be isolated with recovery rates of 88-100% and about 100% purity before recycling the PEG-rich ATPS with a consecutive purification run. Compared to processes without ultrafiltration, processes with ultrafiltration can reduce the carbon footprint by 67%.

Density-based recirculation. A method involving continuous in-line solvent recycling and density-measurement based ternary solvent system readjustment was recently investigated. In this method,



RotaChrom's innovative cell design boosts CPC performance to enable more robust, scalable, cost-effective and efficient separations.

pure solvents are automatically fed into a mixer-settler unit and the solvent system is prepared with the desired ratio based on density measurements. Here, the prepared phases are stored within buffer tanks. These tanks hold the phases until they're ready to be pumped into the CPC during a chromatographic run.

After the run is done, waste solvents are separated from the impurities in the recycler skid and then both the recovered solvents and evaporated solvents from product fractions are fed back into the mixer-settler unit. After readjusting the solvent system based on density measurements, the phases are ready for another CPC cycle. Investigation results provided 14.865 kilograms of crude steroid API with an average purity of 98.74%.

In this experiment, researchers ran 153 cycles and recovered more than 90% of the methyl isobutyl ketone (MIBK) and acetone content for recycling and reuse purposes.

CPC and Downstream Processes

CPC in Bioreactor Applications

Equilibrium-Limited Hydrolysis

It was studied that implementing CPC as a bioreactor is a potential solution for reducing solvent and energy resources. By pairing chemical reactions with the separation process, you can shorten manufacturing times in applications like equilibrium-limited hydrolysis. In this process, lower equilibrium conversions occur at high initial substrate concentrations due to the reverse reaction, making it inefficient to produce large amounts of optically pure amino acids by conventional means.

A CPC reactor can suppress the reverse reaction, but only if the reaction is faster than the reactants' residence time and has no mass transfer resistance. With experimentally determined partition coefficients, stationary phase retention (Sf) values, volumetric mass transfer coefficients and kinetic parameters, you can predict peak shapes, conversion and resolution values as a function of the enzyme and polyethylene glycol (PEG) concentration. Knowing these values allows you to optimize your settings for both hydrolysis conversion as well as product and reactant separation.

When compared to a traditional batch reactor, a CPC bioreactor can double throughput after optimizing CPC and system conditions. It has also been shown that this reaction method maintains performance for up to a 24-hour period before gradually diminishing. And, CPC reactors were found to be more hydrodynamically similar to tubular flow micro reactors since both exhibit diphasic hydrodynamics driven by pulsation.

Multiphase Reaction Systems

CPC also outperforms stirred tank reactors (STR) in converting hydrophobic substrates when using an aqueous organic two-phase system after optimizing buffer type, concentration and system pH. When using an organic solvent, it should be compatible with the enzyme and work as an acceptable biphasic system that facilitates CPC separation. The CPC reactor doubled conversions compared to a STR, showing CPC's capability to handle multiphase reaction systems.

When comparing STR to CPC reactors, CPC provides better mixing conditions and is more efficient when handling lower aqueous phase volumes. However, CPC exhibits earlier limitations than an STR when working with higher enzyme quantities, and the complex fluid

dynamics within CPC constrain scalability. When better rotors and chamber geometries are developed to improve scalability and continuous processes, CPC will grow into an attractive alternative to STR.

Achieving Industrial-Scale Sustainability

Traditional Methods

Coupling CPC with conventional separation and isolation methods was demonstrated with sustainable functionalized taxanoid production. After microbial upcycling low-value feedstocks, CPC can be integrated to economic taxadiene isolation processes to enable consecutive chemo-enzymatic functionalization.

Biphasic, in situ extraction and purification processes like these are traditionally performed by reversed-phase high-performance liquid chromatography (RP-HPLC). Incorporating CPC into a downstream processing strategy is a greener alternative to the traditional method since it combines a rapid two-step extraction procedure with a subsequent CPC purification step.

By introducing CPC into the process, you can avoid the admixing and adsorption issues that can occur during RP-HPLC, providing taxadiene with 95% purity and throughput of roughly 250 milligrams per liter.

In addition, the increased diterpene mass load of taxadiene significantly raised process efficiency, improving recovery from high cell-density fermentation broth. This downstream CPC strategy enables biology-oriented synthesis of ad hoc-designed bioactive taxanoids at an industrial scale.

Pilot-Scale Processes

Another potential application is continuous, pilot-scale recovery of the bioactive components – hydroxytyrosol (HT), oleocanthal, oleacein and the monoaldehydic forms of oleuropein aglycone (MFOA) and ligstroside aglycone (MFLA) – from extra virgin olive oil.

In this example, after using liquid-liquid extractors for fractionation, CPC could enrich the total phenolic fractions during the purification step. Using step-gradient elution extrusion in an n-heptane, ethyl acetate, ethanol and water solvent system, the fractions of oleocanthal, oleacein, MFOA and MFLA can be recovered on the gram-scale with at least 80% purity. For greater purity, preparative HPLC further purifies these bioactive components to greater than 95%.

Because of the large volume of pure compounds recovered, these three industrial-scale techniques make in vivo experiments a possibility for the first time.

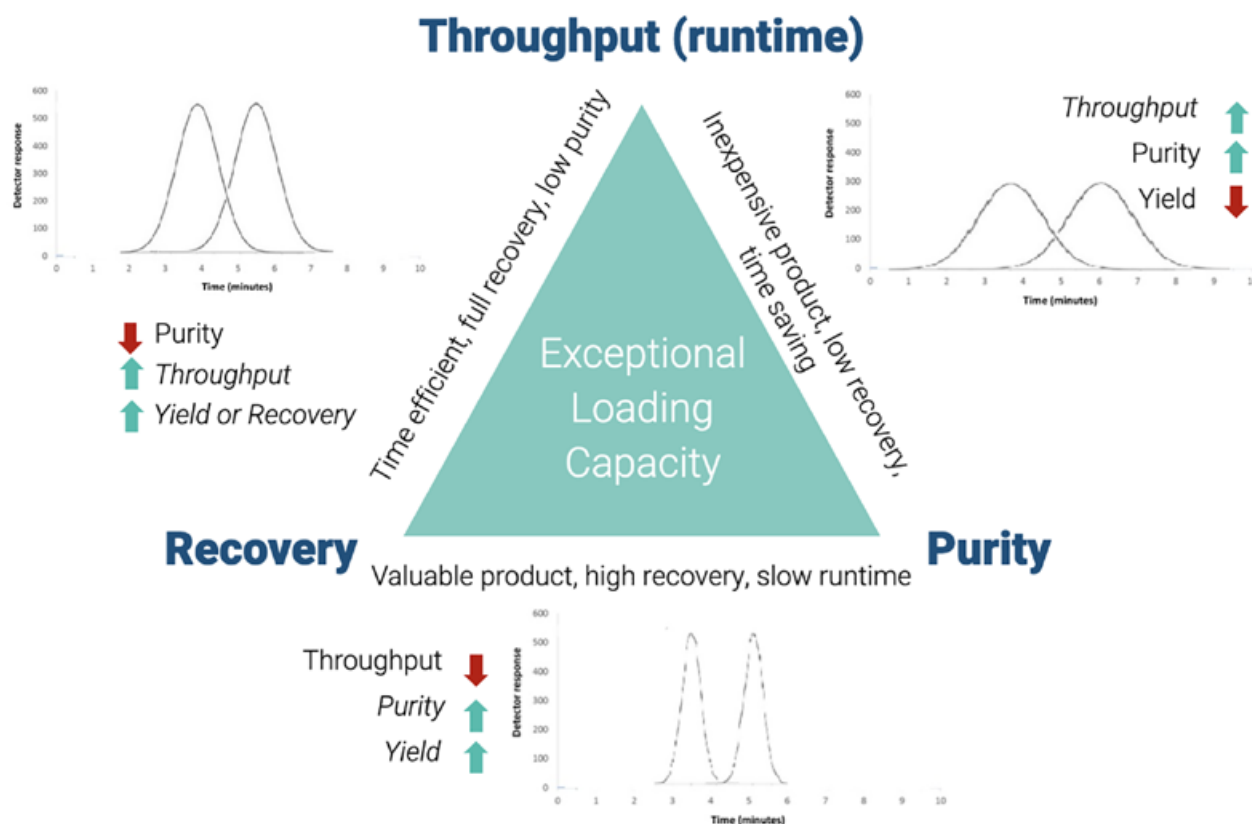


Diagram 3: The versatility of CPC

Multiple Dual-Mode Purification

CPC can support throughput optimization efforts by means of process intensification and switching to continuous manufacturing instead of batch manufacturing. Improving these processes would provide greater automation and quality control, simpler scalability, boosted production throughput and more sustainable manufacturing from start to finish.

Researchers have previously investigated the potential for CPC-based quasi-continuous purification steps to integrate with multi-step, continuous synthesis of key intermediate bioactive carbazoles. A multiple dual-mode (MDM) CPC purification can follow the end of a two-step continuous-flow synthesis of the target molecule.

Using a one-phase intake of the sample solution as the input for the CPC step provides a 59% overall yield, greater than 99% purity and 2.24 g/h/L productivity. Increasing column capacity is a potential method to boost throughput and achieve industrial scale production. The promise of industrial scalability makes CPC ideal for continuous purification in future API and intermediate manufacturing process designs.

Waste Valorization

Each year, millions of tons of agricultural, industrial, municipal and forest waste are generated around the world, and treating this waste is a major undertaking for both industries and governments. Recycling, recovering and reusing these materials with sustainable technologies is a more cost-effective and green alternative than traditional waste treatments.

Centering your waste management strategy around centrifugal partition chromatography (CPC) can reduce raw material usage in manufacturing processes and the environmental impact of waste accumulation. This approach can also help you recover valuable compounds for use in nutraceuticals, cosmetics and pharmaceuticals.

A prime target for waste valorization is hydroxytyrosol (HT), which is found in olive processing wastewater. As one of the main phenols found in olives, HT possesses a natural antioxidative and free radical scavenging activity that make it useful in preventing degenerative diseases. It has been shown that after adsorption resin treatment, a pilot-scale CPC with a cyclohexane/ethyl-acetate/ethanol/water solvent system sorption resin can isolate the phenolic compounds and unlock 1.81 grams per hour per column volume productivity, efficiently recovering HT at a low cost.

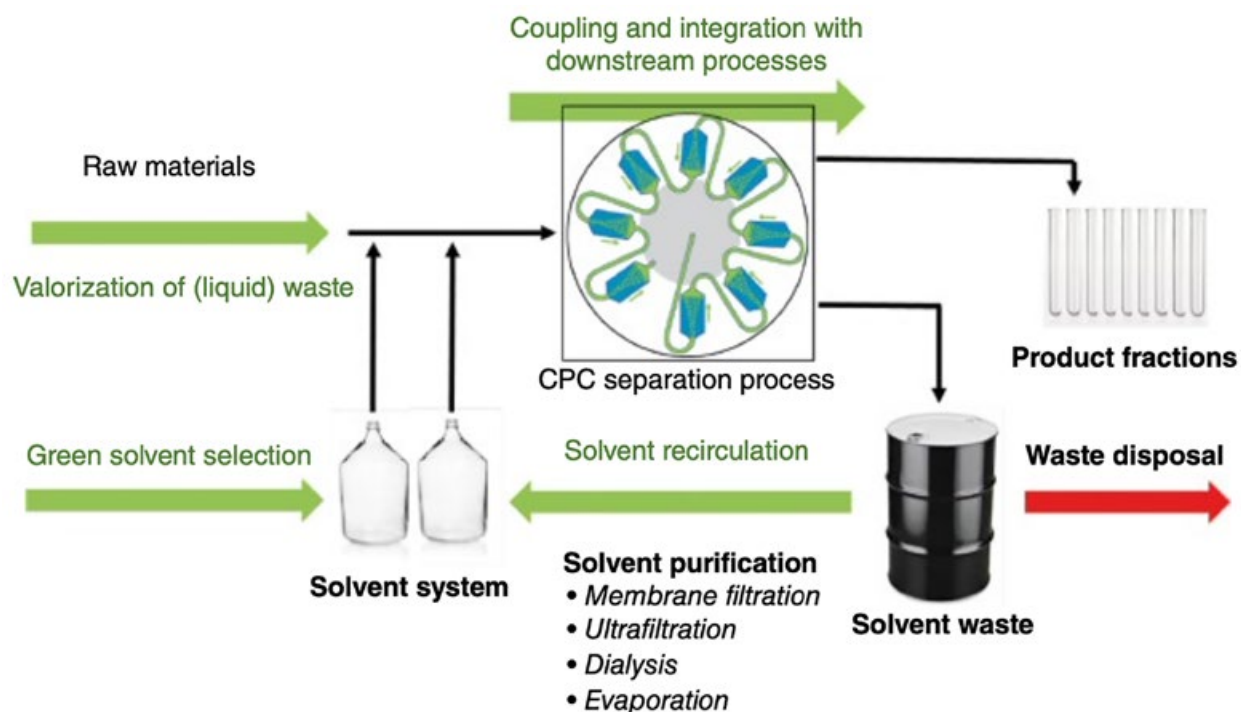


Diagram 4: Basic example of a green, circular CPC process flow

As another example, CPC separation has been implemented to optimize and scale-up monosaccharide fractionation from hydrolyzed sugar beet pulp as an alternative to multiple resin chromatography. Sugar beet pulp mostly consists of cellulose and pectin, making it unfit as a gelling agent, and it is commonly dried and pelleted for use as an inexpensive animal feed. Due to its low cost, prevalence and high carbohydrate content, it can be fermented into bio-ethanol or hydrolyzed into monosaccharides – which can later be fractionated with a CPC after a thermo-chemical pretreatment.

A preparative-scale CPC run with an ethanol/ammonium sulfate solvent system have been implemented to optimize monosaccharide isolation and gain a final throughput of 1.9 grams per liter per hour when

normalized by column volume. In another industrial application, integrating CPC purification into biorefinery processes allowed for purified arabinose to be converted into arabitol or other high-value compounds such as L-gluco-heptulose, a useful chemical in cancer therapy and hypoglycemia treatment.

Conclusion

CPC is a greener, more sustainable alternative to conventional preparatory separation methods due to its versatility and flexibility in available solvents, its wide range of possible isolation, fractionation and purification applications, and its potential as a valuable method of waste treatment.

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hello@rotachrom.com
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**RotaChrom Technologies
PLC (Headquarters)**

Csillag street 2/A, Kecskemet, HU-6000
+36 70 455 2499

**RotaChrom North America
Inc.**

300 Spectrum Center Drive, Irvine, CA 92618, USA
+1 720 220 5669

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