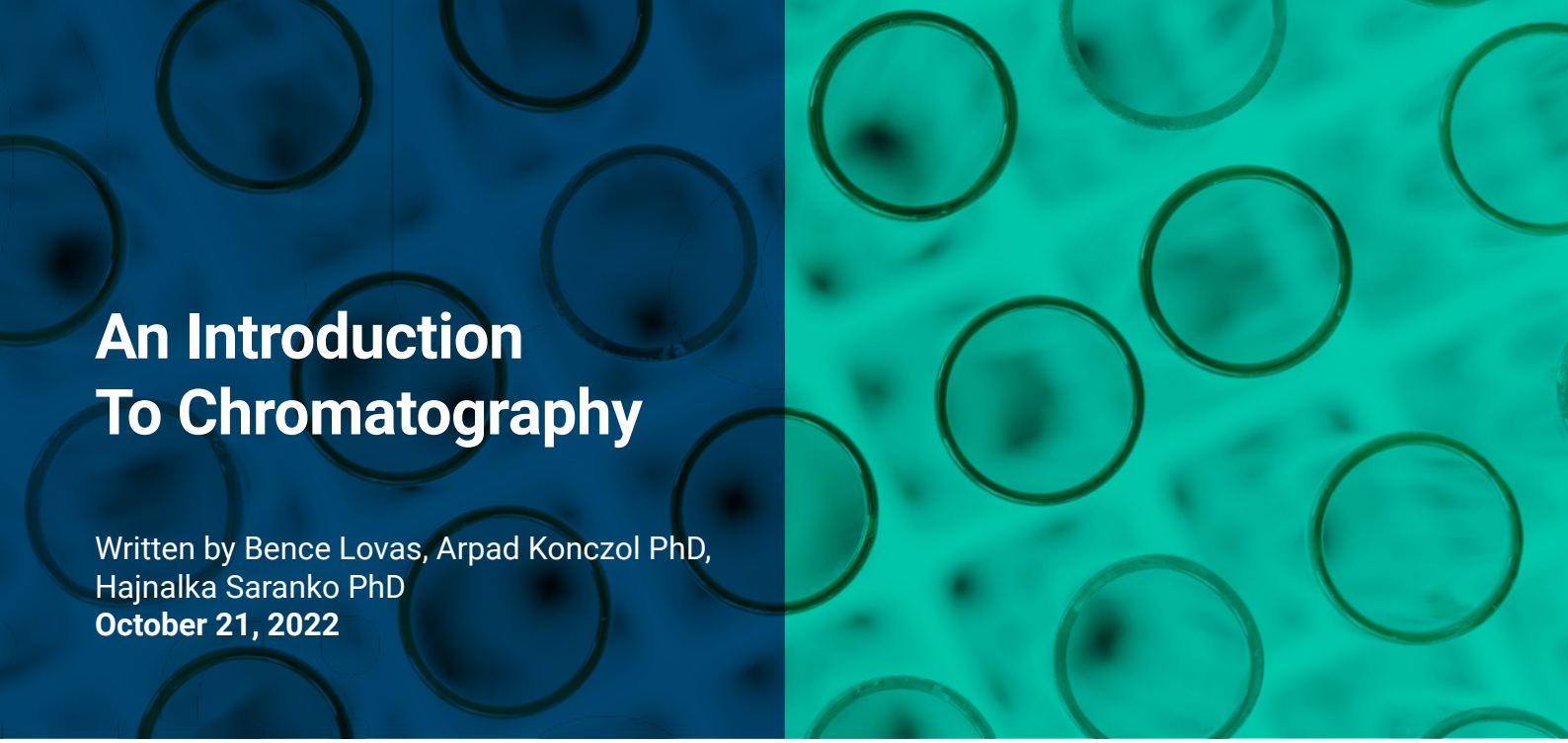

An Introduction To Chromatography

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Abstract

'An Introduction to Chromatography' is RotaChrom's series exploring the basics of chromatography. It analyzies the basics first and slowly progresses towards more complex topics, such as Centrifugal Partition Chromatography.

The goal is to make chromatography a digestible, easy to understand topic, so that anyone, even without a science degree, can get a grasp of the subject.

We divided the bigger topics into five different parts, each dealing with two aspects of the chromatographic process. By the end of the series, you should have a grasp of the workings behind chromatography in general, and have an understanding of the different types of separation techniques in the field.

Contents

Keywords
- Chromatography process steps
- What is chromatography
- Liquid chromatography
- Chromatography

The Downstream Process Chain

The downstream process chain is a series of purification steps, whose goal is to ensure the purity of the product that you receive once you finished all the steps in the chain.
There are several different methods for purification. These range from simple mechanical purification principles to more complex methodologies.
For simpler processes, purifying with only mechanical principles is usually enough. These principles can be:

- Filtration
- Sieving
- Centrifugation
- Dust separation

There are other options available besides the ones mentioned here, but this is just to give a brief overview. Either way, once the "heavy lifting" is completed, it is worth refining your purification chain with more delicate approaches. Let us now take a look where chromatography fits among purification methods.

Introducing chromatography in a downstream process can produce much superior end products, which is the main goal of any purification process. In a typical industrial downstream processing chain, chromatography has a place among separation techniques.

For a successful purification process, it is important to apply the most selective, most efficient method to get rid of contamination, heavy metals, pesticides.

The Basic Chromatographic Process

By definition, chromatography is the physical methodology of separating a component from a mixture by passing it in solution or suspension through a medium in which the components move at different rates.

Or, to phrase it more precisely, chromatography is defined as a physical method of separation in which the components of a mixture to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. So, in short, in the chromatographic process components are divided into two media:

- Stationary phase
- Mobile phase

Why are they called stationary and mobile?
It comes down to another element of the chromatographic process, the driving force. Due to the driving force (which can be gravity or pressure difference, for example) the mobile phase travels through the system and carries sample components with it after the injection of the sample.
Components which interact stronger with the stationary phase will be more retained and move through the system slower than components with weaker interactions. This results in a difference in the time it takes for the components to travel through the system. This also creates a difference in separation.

Chromatography process step variations

The process described so far is, as mentioned before, only the basic description of chromatographic separation. This is just the foundation on which many different chromatographic methods were built. These different methods all capitalize on some aspect (e.g. the driving force used, or the properties of the phases) of the basic chromatographic process and modify or enhance that aspect in some way.

Let's see some examples how this can be done:

- Chromatographic methods based on the driving force of the mobile phase (e.g., gravity, capillary force, electric field)
- Methods developed based on the nature of the phases used (stationary phase: solid/liquid; mobile phase: gas/liquid/supercritical fluid)
- Utilizing the geometry of the chromatographic device (e.g., planar, column)
- Utilizing the interaction of the solutes with the stationary phase (e.g., physicochemical, biospecific)

Many different chromatographic techniques exist, for example:

- High-Pressure Liquid Chromatography (HPLC)
- Supercritical Fluid Chromatography (SFC)
- Flash Chromatography
- Centrifugal Partition Chromatography

Part 2
Liquid-liquid Chromatography

Since we briefly talked about the different types of chromatography, we wanted to take the time to examine Liquid-Liquid Chromatography (LLC) in a bit more detail. This type of chromatography is going to be the basis of centrifugal partition chromatography later on, so it is an essential part of the picture that needs to be understood.

Liquid-liquid Chromatography Basics

LLC is a chromatographic separation technique in which both the mobile and stationary phase is a liquid. Liquid-liquid chromatography is a combination of liquid-liquid separation principles combined with the methodology of chromatography.
In this system separation occurs between two immiscible liquid phases. How separation takes place in such a system is based on the differing partitioning behavior of the solutes between the two phases.

As is the case with traditional chromatographic separation techniques, one of the two phases involved in the process is immobilized, so it becomes the stationary phase. Here, the liquid mobile phase then moves through the column, and the components in the mobile phase will interact with the stationary phase and the components will be separated based on the partition coefficient mentioned beforehand.
High sample loading is made possible as the entire volume of the liquid stationary phase can be accessed by the solutes.

As a plus, the issue of irreversible adsorption is out of the picture, which means that complete sample recovery is possible with liquid-liquid chromatographic separation.

Separation of the injected sample is based on the sample component's varying partition coefficients between the mobile and stationary phases. Partition coefficient (Kd) is the equilibrium constant for the distribution of a compound in a two-phase system. The partition coefficient dictates the amount of time each molecule spends within the mobile and stationary phases and therefore the rate at which each molecule travels through the system.

The versatility of liquid-liquid chromatography is easily demonstrated through the vast range of different applications, which include separation of synthetic compounds, herbicides, pesticides, botanical compounds, vitamins, amino acids, peptides, proteins, and inorganic elements.

The 'Column'

Traditional chromatographic techniques require some form of a column to perform chromatographic separation. In liquid-liquid chromatography, the column is a specially designed housing mounted on the axis of a centrifuge.
For example, in the case of RotaChrom devices, the column is the rotor and the interconnected cells.

Here, the Centrifugal Partition Chromatography cells connected in series, attached to a large rotor, are filled with the liquid stationary phase and immobilized by a strong centrifugal force. The mobile phase moves through the stationary phase in the form of tiny droplets. The large surface area of the droplets assures optimal mass transfer between the phases.

The core advantages of CPC technology over classical, solid support-based preparative liquid chromatography are:

- simplicity of separation mechanism
- easy method development and scale-up
- high loadability and recovery
- no sample loss due to irreversible adsorption
- no need for solid stationary phase and medium solvent consumption

cost effectiveness.

One of RotaChrom's most important discoveries was that our extraction cells can be scaled from 10 mm to 100 mm without impacting performance and increasing flows from mL/mins to multiple L/mins.
Liquid-liquid chromatography is a versatile technique which combines the ideas driving extraction and chromatography. Since it uses a liquid stationary phase, liquid-liquid chromatography offers several advantages over traditional liquid-solid chromatographic separation techniques. These positives include the high sample loading capacity and the possibility of lossless sample recovery. Liquid-liquid chromatography is also capable of being used for many target components and can be optimized for many different operating modes. Despite the benefits, LLC is an underutilized technology, so it is one of our goals at RotaChrom to provide exposure to the technology.

Part 3
The Chromatogram & Fraction Collection

The Chromatogram

In short, the chromatogram is the visual output of the chromatographic system. It is essentially a graph that shows the events of a particular chromatographic process:

- The Y axis represents the detector response.
- The X axis is the time of the separation process.

It is important to understand how to read a chromatogram. There are several questions that it can answer, which provides valuable input about the details of a specific process. Most of the information can be gathered from the exact place, height and width of an individual peak.

The reason why there are many peaks is because, each peak of the Gaussian shapes shows a different type of separated compound. This means you can visualize the individual compounds exiting the system as peaks, even if some of them overlap.
The area under the Gaussian curve is proportional to the amount of the compound. A larger curve means more compound from the mixture was identified in that particular peak. Lastly, the position of the peak is also important. Exactly where you see the peak shows when and in which fraction the elution happens, and the distance and separation of the peaks show selectivity and purity.

As mentioned earlier, the goal of a chromatographic separation is achieving resolution. In a chromatogram, resolution is visualized by peaks that have no overlap between them. Higher resolution (no overlap) means the separation was successful. This results in a purer product. The value of the resolution is always marked with 'R_S' in a chromatogram (Fig. 3).

Overlapping peaks do not have great resolution. An Rs value lower than 1.5 means the peaks in the chromatogram are overlapping. On the other hand, if the R_S value is higher than 1.5, means you achieved 'baseline separation', which is the 'Holy Grail' of chromatography so to say. Baseline separation results in no overlapping peaks at all.

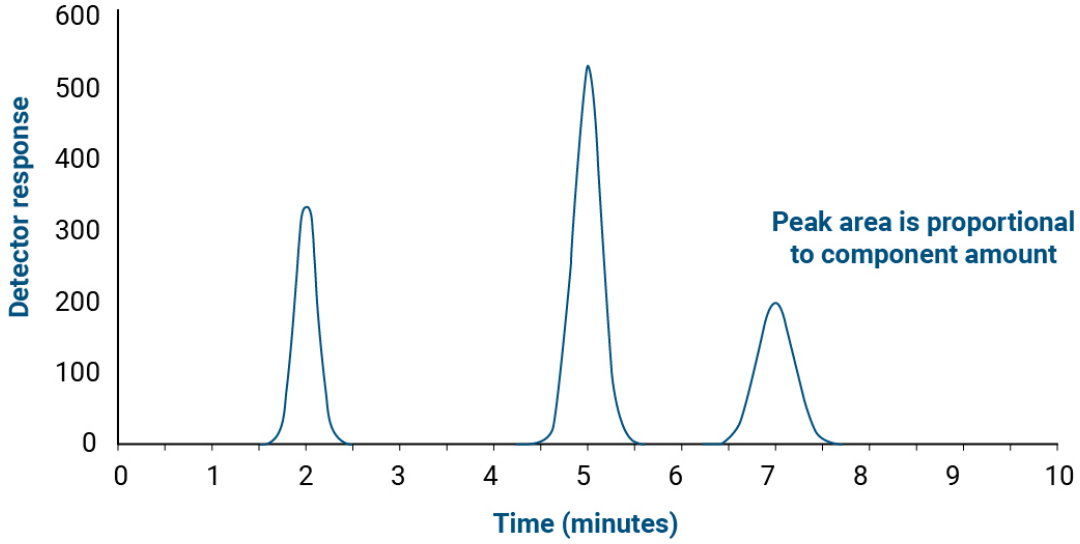


Fig. 1. Example of a chromatogram. Note that three specific compounds are represented by Gaussian shaped peaks at 2, 5 and 7 minutes respectively.

Fraction collection and optimization

The goal of chromatographic isolation is to separate a Compound of Interest (CoI) from its impurities. An impurity can be many different things, including a health threat, a carcinogen, an environmental pollutant, or a legally challenging compound among others. Problems can arise, however, if the impurity that you want to remove comes out around the same time the CoI you want to separate starts to come out. This is an obvious issue, and in such cases, you need to optimize the chromatographic process and take into account that there is going to be some tradeoff between purity and recovery.

This issue only arises if the peaks overlap, but in such cases, you must decide which is more important for your purposes: purity or recovery. The more impurity you remove, the less sample you can recover, and conversely, the more sample you recover, the less pure it will be. Engineers and scientists have been working on methods to balance the purity-recovery gap. The idea is, that if you start the collection at the right time, you can balance between the two extremes, and aim for optimum recovery and purity. This is how you strike a “sweet-spot” between losing some of the material (but not much), and still achieving very high purity.

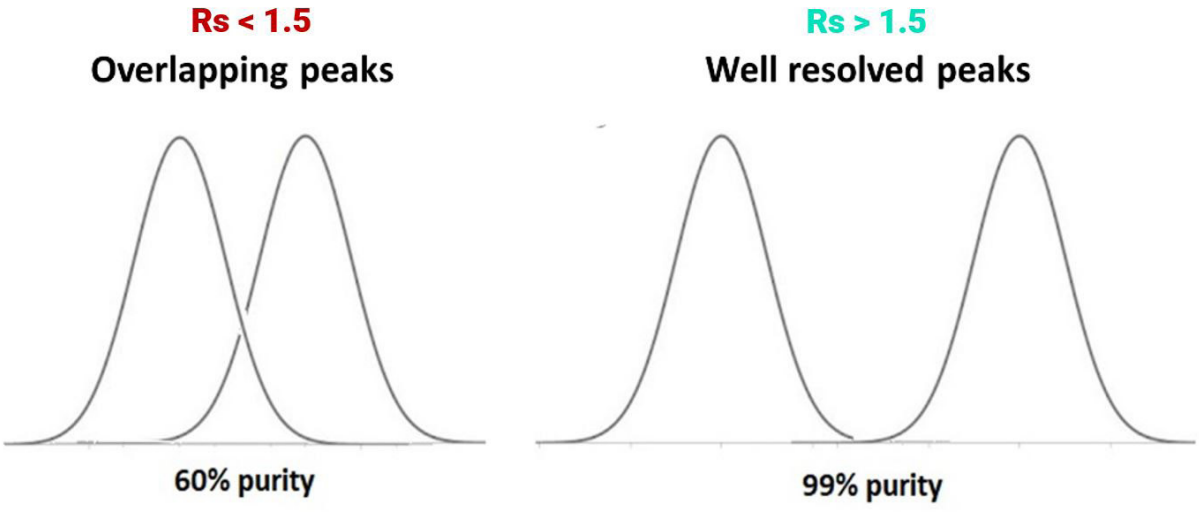


Fig. 2. Examples of overlapping peaks and baseline separation in separate chromatograms.

Part 4
Analytical & Preparative Scale Chromatography

Chromatographic methods can be classified into analytical and preparative ones based on the scale and the goal of the separation process. In the case of analytical methods, the aim of the separation is to achieve qualitative and quantitative information about the sample in question.

On the other hand, the goal of preparative separations is to isolate a specific component of a mixture (called the compound of interest, abbreviated as CoI) or otherwise remediate a single component or group of components (impurity or impurities) from the mixture. These preparative systems are capable of processing hundreds or even thousands of grams of input.

Analytical:
In the case of analytical methods, the aim of the separation is to obtain qualitative and quantitative information about the sample . Analytical is smaller scale. It is mainly for information and research.

Preparative:
Larger scale; it can process substantial amounts.
The goal is to isolate a specific compound from a mixture (i.e., the CoI) or alternatively remove a single compound or group of compounds (i.e., impurity or impurities) from the mixture.
Preparative systems can process hundreds or even thousands of grams of input material and work in multi-liter per minute flow rate range.

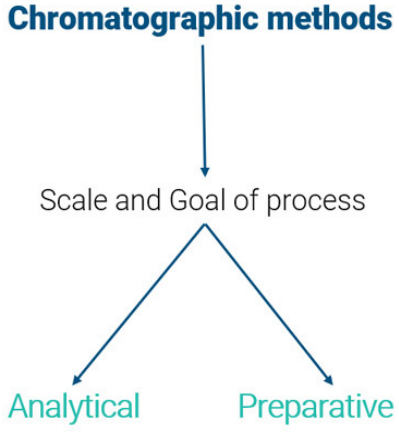
Industrial-scale purification

As a next step of preparative chromatography, it is important we touch on industrial-scale chromatography. To explain the concept, we would like to talk about the iCPC, RotaChrom Technology's Industrial-Scale Centrifugal Partition Chromatography device.

One of the main benefits of industrial-scale purification is its scalability. This means, indusctrial-scale devices can be scaled up from g/day to several kg/day.

As an example, RotaChrom's iCPC is capable of:
- up to 1200 g/cycle of load capacity
- Several Kg/day throughput of purified material

This means the iCPC has the capacity to process hundreds of kilograms of crude input material per month, much more than what is expected from traditional chromatographic solutions.



	Analytical	Preparative
Goal	Information	Production, Separation, Isolation
Limitation	No, every technique applicable	Making sure CoI is intact
Parameters of optimization	Resolution Peak capacity Speed Selectivity	Modelling Scalability Purity Yield Throughput Economy/Productivity Safety Sustainability

Fig. 4. Differences between analytical and preparative chromatography.

Basics of CPC

Centrifugal Partition Chromatography (CPC) is a liquid-liquid preparative chromatographic technique, in which separation occurs between two immiscible liquid solvent systems: acting as both the stationary and mobile phases. Separation of the injected sample is based on the sample component's varying partition coefficients between the mobile and stationary phases. Partition coefficient (Kd) is the equilibrium constant for the distribution of a compound in a two-phase system.

In CPC, a pattern of cells (columns) connected in series by ducts attached to a large rotor is filled with liquid stationary phase, which is immobilized inside the rotor by a strong centrifugal force. The other phase of the two-phase system is the mobile phase containing the sample to be purified. It is fed under pressure into the rotor and pumped through the stationary phase in the form of tiny droplets. The core advantages of CPC technology over classical, solid support-based preparative liquid chromatography are: (i) simplicity of separation mechanism – easy scale-up; (ii) high loadability and recovery – no sample loss due to irreversible adsorption; (iii) no need for solid stationary phase and medium solvent consumption – cost effectiveness.

RotaChrom's CPC solutions

RotaChrom has developed a revolutionary preparative purification instrument called Industrial-Scale Centrifugal Partition Chromatography (iCPC) device. This novel system does not utilize any solid stationary phase (such as silica gel) to achieve precision molecular separation and is demonstrably superior to conventional liquid chromatographic techniques in terms of yield and purity. In addition, the iCPC platform has radically reduced the costs and number of steps associated with downstream method development for a diverse array of purification challenges.

In this system separation occurs between two immiscible liquid phases. The CPC cells, attached to a large rotor, are filled with the liquid stationary phase and immobilized by a strong centrifugal force. The mobile phase moves through the stationary phase in the form of tiny droplets. The large surface area of the droplets assures optimal mass transfer between the phases.

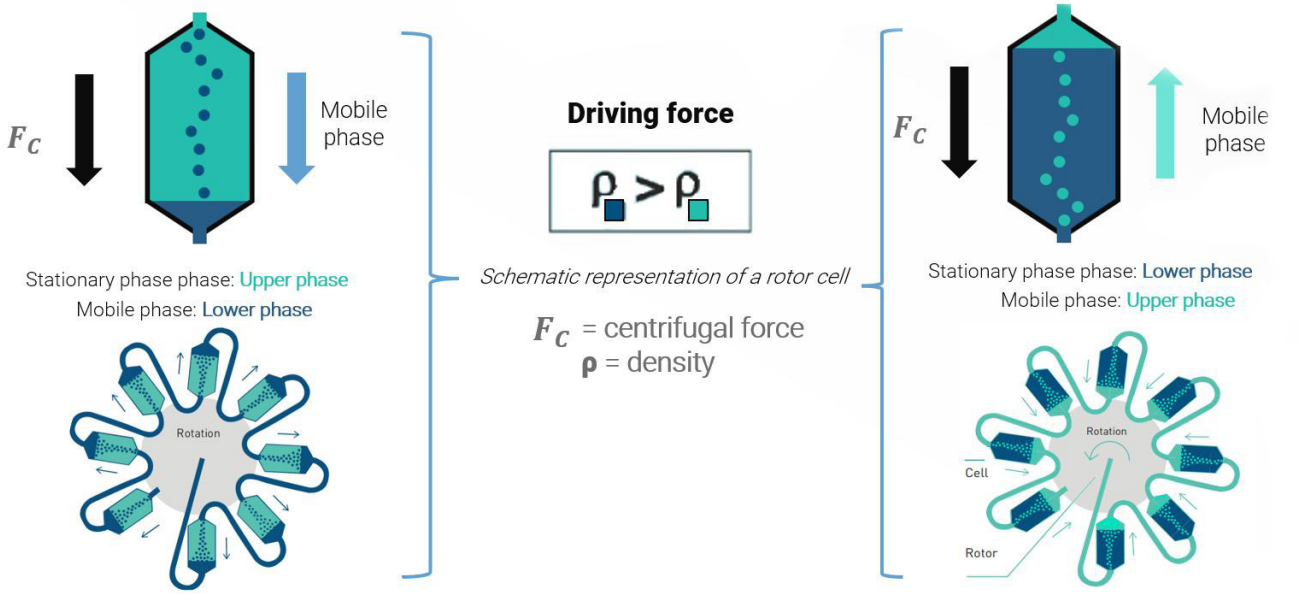


Fig. 5. Visual representation of ascending and descending modes.

Like conventional liquid chromatography, separation of the injected sample is based on the sample component's varying partition coefficients between the mobile and stationary phases.

The partition coefficient dictates the amount of time each molecule spends within the mobile and stationary phases and therefore the rate at which each molecule travels through the system. At the end of the purification process automated fraction collectors retain all selected fractions based on the program settings.

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