Aalto University
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Bioprocess Technology II

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PARTITION CHROMATOGRAPHY

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1. Abstract

Partition chromatography has been one of the most significant classes of separation methods since its development by Martin and Synge in the 1940s. They were awarded by Nobel Prize in Chemistry in 1952, which highlights the importance of their discovery. All partition chromatography techniques apply the same principle: there are two phases, one stationary and one mobile, and the sample is partitioned between these two phases, based on their greater affinity to either one. Mobile phase can be liquid or gaseous. Partition chromatography has been used in industrial scale since 1960s. Nowadays it is the main workhorse in downstream operations of bioprocessing whenever high levels of purity are needed, such as in the production of recombinant proteins. New research avenues include e.g. the development of centrifugal partition chromatography, in which centrifugal forces are applied to make the other phase stationary.

2. History of partition chromatography

The theory and the practice of partition chromatography was invented by Archer Martin and Richard Laurence Millington Synge during 1940s. They were both students at Cambridge University. After graduation, Martin worked on separating carotenes by distribution between two solvents using separating funnels. As he was interested in fractional distillation since his high school days, during his work at the university he built a laboratory machine that can carry out highly efficient countercurrent extraction. At the same time, Synge was working on removing N-acetly amino acids and peptides from carbohydrate moiety by using exhaustive extraction with chloroform. During his research, Synge first measured the partition coefficients of acetyl amino acids between chloroform and water phase. As the partition coefficients had distinct differences, he wanted to carry out separation by liquid-liquid extraction. Since Martin had been working on extraction, Synge was suggested to contact Martin. (Ettre, 2001)

In 1940, Martin came up with the idea of packing a glass tube with a mixture of wool and cotton, to have chloroform above and the water below the packing. The idea was that the fibers would separate two flows and amino acids would distribute differentially between the two solvent flows. However, it did not work as the theory has suggested. Martin realized that the problem was coming from trying to create equilibria between two liquids moving continuously in opposite directions. Then he tried to keep one of the liquids stationary, which then paved the way for the birth of partition chromatography. Martin and Synge decided that water would be the stationary phase and the chloroform would be the moving phase. They impregnated silica gel with water and packed the column with it, the acetylamino acid mixture was added from top and chloroform was poured down the column. They added methyl orange as indicator to water to see amino acids passing through the column as red bands. (Ettre, 2001)

An important problem to tackle was using the column for analysis of dicarboxylic acids. However, silica gels did not work for that purpose, they had to find another material. Martin and Synge tried paper chromatograms by impregnating the paper with water, the stationary phase and adding butanol as the mobile phase. A drop of the solution of two amino acids were placed in the center of the paper. Addition of Butanol moved the paper up by capillary action, moving the amino acids at different speeds. As they developed the method further, the setup became simpler with the use of paper strips placed in a closed container where air was saturated with water and the tops of the strips were dipped into mobile phase. However, even though they have tried different solvent they couldn't separate the complex mixtures of common amino acids. Therefore, they have tried the what now we call two-dimensional chromatography by turning the paper strips 90 degrees and used a different solvent for further separation of initially formed spots in the first method they tried. The use of paper chromatography advanced quite rapidly due to simplicity of the method and commercial availability of the filter papers used. (Ettre, 2001)

3. Theory and mechanisms

Partition chromatography refers to any form of chromatography, where a mixture of components is separated and distributed between two phases (Cammack, 2006). One of the phases is called the stationary phase, while the sample to be analysed is contained and dissolved in a fluid called the mobile phase. In column chromatographic analysis, components of the sample (solutes) travel through the chromatographic column at different speeds and partition between the two phases based on their different affinities towards the stationary phase; this is the very basis of all partition chromatography. (McNair & Miller, 1998) Due to their applicable nature in analytical laboratory operations, this paper focuses solely on column chromatography techniques, where the stationary phase is contained inside a tube. All column chromatographic techniques can be used for both qualitative and quantitative analysis.

Different chromatography processes are divided into two main classes according to the physical state of the mobile phase: in liquid chromatography (LC), the moving phase is a liquid, whereas in gas chromatography (GC), the mobile phase is a gas. Furthermore, chromatographic techniques can be further divided according to the state of the stationary phase, e.g. in gas-liquid chromatography (GLC), the mobile phase is a liquid. A classification hierarchy of column chromatographic methods is shown in Figure 1. (McNair & Miller, 1998)

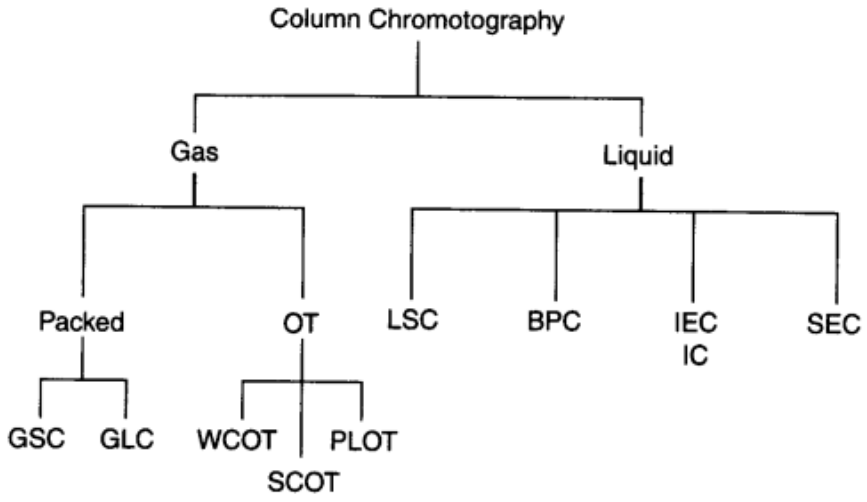

Figure 1. Classification of different chromatographic methods. (McNair & Miller, 1998)

3.1 Gas chromatography

GC is an analytical technique, where the mobile phase of the column is a gas. The basic operating principle of gas chromatography is that the organic component mixture that is analysed is first dissolved in a solvent (e.g. methanol or diethyl ether), and subsequently vaporized and passed through a column containing the stationary phase. After injection and passing through the column, the sample reaches a detector that records the time and intensity of the given solute, making it possible to conduct quantitative analyses. A schematic representation of gas chromatography operation is presented in Figures X and Y. (McNair & Miller, 1998)

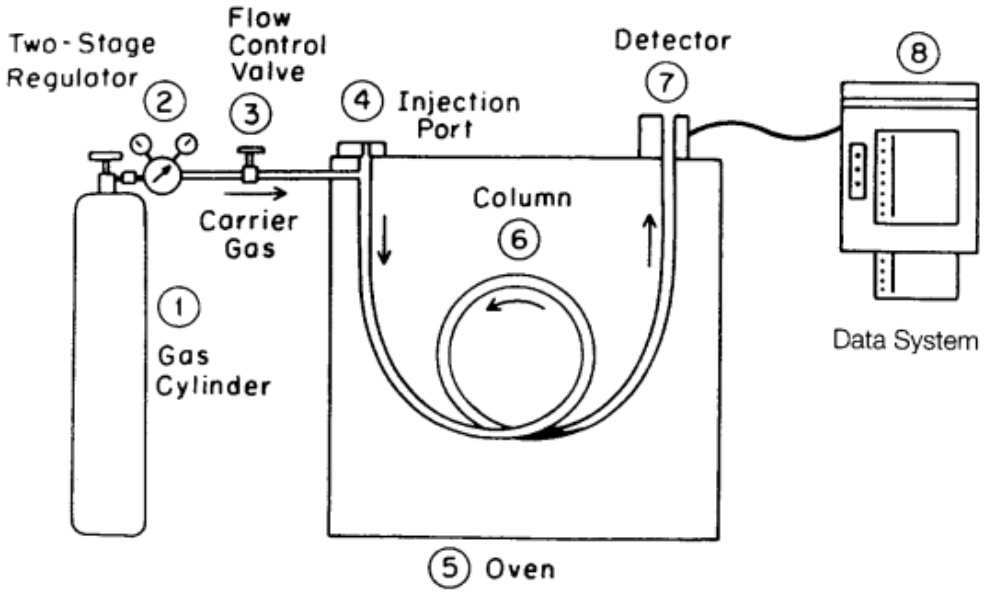

Figure 2. Schematic of a gas chromatographic analyser. (McNair & Miller, 1998)

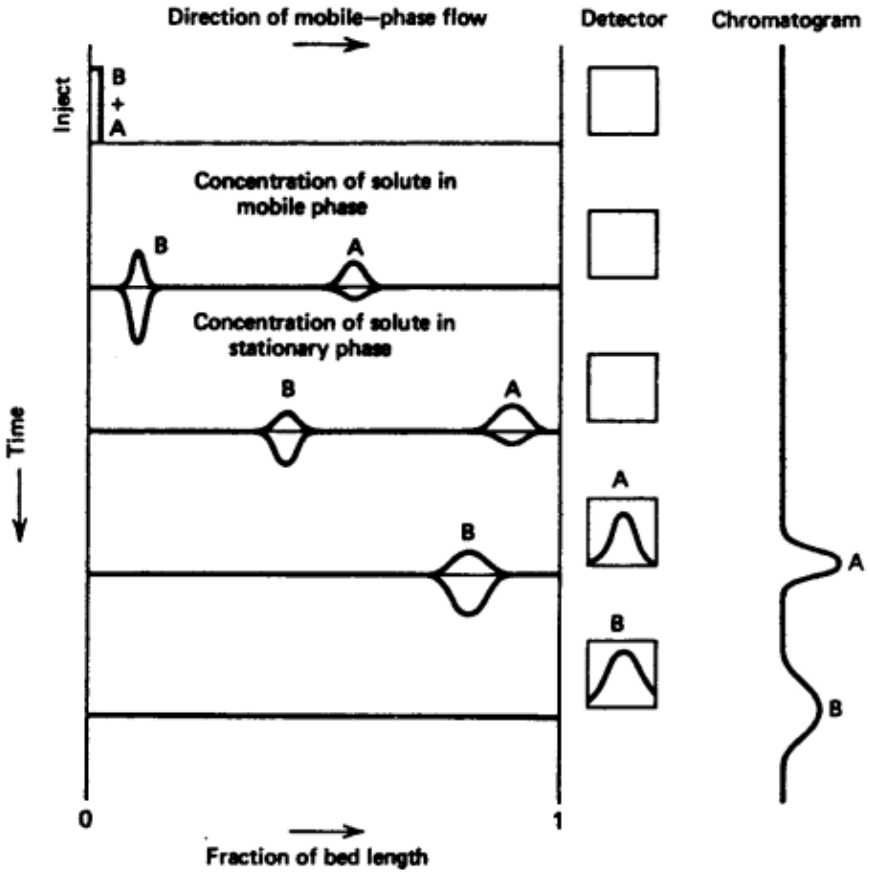

Figure 3. Representation of a chromatographic process. (McNair & Miller, 1998)

The mobile phase is a chemically inert carrier gas, which carries the solute molecules through and out of the column; the carrier gases most often employed are nitrogen (N_2) and helium (H_2). Additionally, the column containing the stationary phase can be either open-tubular (OT) or a packed column: in packed columns, the tubing is packed with tiny, spherical supports. Often used column materials include glass and stainless steel, and a common support for the stationary phase that is nowadays utilized in a laboratory setting is silica. The difference between packed and open-tubular columns is summarized in Figure X. (McNair & Miller, 1998; Hubschmann, 2001)

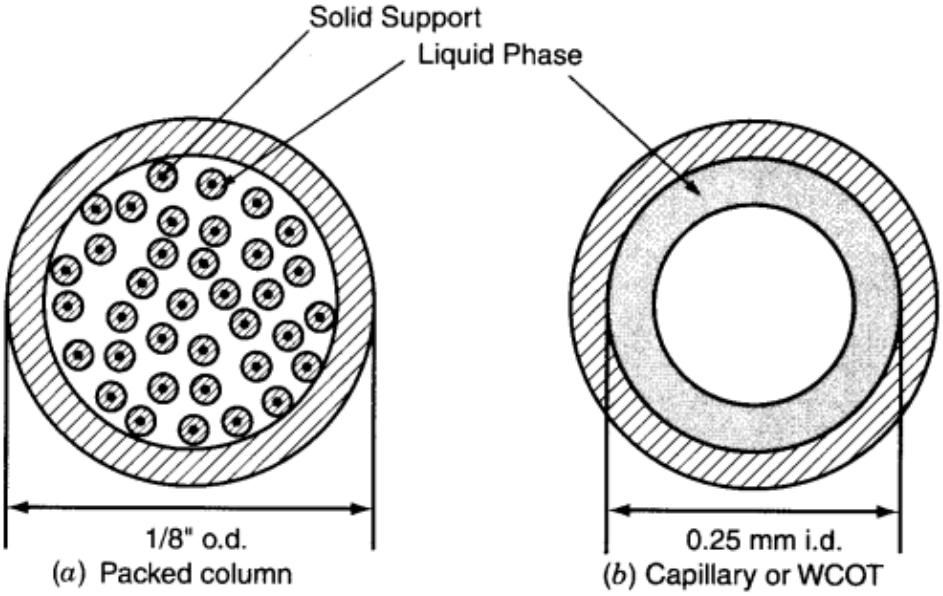

Figure 4. Schematic representation of a packed column and open tubular column.

The liquid phase can be either a solid adsorbent (GSC) or liquid on a chemically inert support (GLC): out of these two techniques, GLC is by far the most utilized. A very important distinction between the two is that in GLC, the solutes interact with the thin layer of liquid phase that is adhered and adsorbed onto the surface of the stationary phase: substances with greater affinity towards the mobile phase exit the column and reach the detector first. (McNair & Miller, 1998)

Factors that influence component separation and measurement are the vapor pressure of the solvent, polarity of analysed components, column temperature and length, injected material amount and carrier gas flow rate: by increasing column length, a better degree of separation is achieved, but the analysis takes a longer amount of time. However, the advantages of the technique are numerous: the analyses typically take only minutes, it is sensitive up to ppm and ppb levels, high resolutions are achievable, it is inexpensive and non-destructive, and the amount of sample needed is very low. However, GC is limited to samples that are volatile, are not thermally labile, and the technique usually requires a supportive technique that can reliably identify the peaks: such an instrument could be e.g. mass-spectrometer (MS). (McNair & Miller, 1998)

3.2 Liquid chromatography

Contrary to GC, liquid chromatography (LC) employs a liquid mobile phase. However, it can be used to separate a wider variety of components than GC since only a marginal number of components are volatile enough to be analysed utilizing GC. The most employed type of liquid chromatography utilized nowadays is high performance liquid chromatography (HPLC), which uses higher operational pressures in the range of 50 to 350 bar, compared to a normal LC which relies on gravity alone. Similar to GC in operation, the sample is injected into a column and the mobile phase (carrier/solvent) flows past the stationary phase: the stationary phase is an immobilized layer of liquid adhered to a support surface. Separation occurs depending on the relative solubility of solutes in coated liquid surface, i.e. different components have different partition coefficients. (Bakalyar, 1981)

4. Partition chromatography in bioprocess technology

Chromatographic techniques are widely used in downstream operations of bioprocessing. All techniques are based on a similar mechanism that solutes have different rates of migration in the column. The simplest type of chromatography is adsorption chromatography that relies on adsorption of the solute by Van der Waals forces. However, adsorption chromatography is not very selective and thus more refined techniques are needed. In bioprocessing, adsorption chromatography is most applicable in water treatment. (Clarke, 2013).

Partition chromatography has been used since 1960s to separate products of bioprocessing and nowadays it downstream operations of bioprocesses are dominated by large-scale partition chromatography. Basically, same principles are used in large scale as in lab-scale with similar elements: pump, injector, column and separators. Early support materials in lab-scale chromatography were different kinds of gels but they were mostly unsuitable for process scale. The expansion of chromatographic techniques was enabled in the 1960s by the development of carbohydrate-based supports. Separation chromatography operations are an important determinant also in the total cost and feasibility of the recombinant protein production. Process

chromatograpy has been noted as the biggest single cost center in downstream operations of bioprocessing. (Curling, 2007).

Especially biotechnologically produced medicines such as recombinant proteins require separation methods that can reach high levels of purity. Liquid chromatography can be used to obtain recombinant protein separation with over 95 % purity. In industrial applications, several liquid chromatography steps in series are used. (Geng and Wang, 2008).

One of early examples is the separation of insulin, reported by Porter (1953). Insulin is one of the most traditional biotechnologically produced compounds and is used as a medicament for diabetes patients. In this study, several aqueous phases containing organic solvents and salts were used. Porter reported two major challenges, which need to be considered regarding the choice of phase mixture: protein denaturation caused by organic solvents may not occur and the partition coefficient should be high enough, at least 10:1 – 40:1. Insulin recovery obtained by partition chromatography technique was close to 100 %.

Recently, there has been a lot of studies on the use of centrifugal partition chromatography (CPC). In this technique, the other liquid phase is kept stationary by centrifugal forces and supporting solid material is not needed. One example is the separation of active laccases by CPC, reported by Schwienheer et al. (2015). Aqueous two-phase system (ATPS) was used in the experiment; in ATPS, both liquid phases are mainly composed of water. CPC in combination with ATPS was found to be a gentle separation method that retained the activity of laccase enzymes. CPC seems to be a feasible separation method for bioactive compounds, but the activity loss of the end product should be checked in each individual case. The shear forces did not lower enzyme activity in this case.

The CPC has been applied also to low-value products such as fractionation of monosaccharides from sugar beet pulp, reported by Ward et al. (2017). In this study, the CPC method was found to remove impurities and fractionate beet pulp to rhamnose, arabinose and D-galacturonic acid.

5. Conclusions

Among all separation methods, chromatographic techniques are the most precise and separation chromatography is even more precise compared to e.g. adsorption chromatography. Nowadays, chromatographic methods that are based on the partition of compounds between mobile and stationary phases are the foremost analytical separation method, not only in research but also in industrial processing of such compounds as biopharmaceuticals. Most probably chemistry and biochemistry as disciplines would have never developed to their current state without the discovery of partition chromatography. Moreover, partition chromatography has anabled the industrial production of compounds, which require high degree of purity such as recombinant proteins and other biopharmaceuticals.

6. References

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