# Chemical Microengineering. II. Partition Chromatography

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## Introduction

This is the second in a series about a Virginia-Tech senior-elective course, entitled "chemical microengineering", that includes the study of (a) the continuity-of-species equation for "small chemical systems"; (b) clever ideas and interesting gimmicks that are used within small chemical systems; and (c) interesting molecules. The introductory article is available as a downloadable file at URL **http://www.che.utexas.edu/cache/newsletters/fall2000_contents.html** (see file fall2000_chemmicro.pdf in the Fall 2000 issue, No. 51).

## What is Partition Chromatography?

***Partition chromatography*** is defined as a differential migration separation technique that employs the distribution of a migrating component between two or more different states whose relative velocities are not all equal to zero.

## Example No. 1: Gas-Liquid Partition Chromatography

As an example, consider gas-liquid partition chromatography, where a pulse containing a mixture of voltatile components is injected at the inlet of a long, hollow, stainless-steel or glass capillary column coated on the inside with a thin film of a non-volatile liquid. The column is long, perhaps 10 meters or more. A continuous, steady flow of helium gas passes through the column. After injection, the components later appear, in sequence, as a series of separated, broadened, Gaussian pulses at the exit of the column (Figure 1). Why is the injected mixture separated into its components by this apparatus? Because each component has a unique value of its solubility in the non-volatile liquid. A component that is completely insoluble in the liquid phase passes quickly through the capillary column with a residence time equal to the residence time of the flowing helium gas. A component that is highly soluble in the liquid phase takes considerably longer to elute from the column. Components with solubilities that are intermediate between "insoluble" and "highly soluble" elute at intermediate times.

## What is the Gimmick Associated with Ideal Partition Chromatography?

The basic "gimmick" behind ideal partition chromatography is the existence of two phases -- one stationary and one mobile -- between which the components of a mixture rapidly equilibrate. In the ideal case, the equilibration of each component is ideal, in the sense that there exist no mass-transfer limitations to equilibration and the quantity of each component is sufficiently small such that linear partition coefficients apply.

Ideal partition chromatography can be considered to be, in effect, an example of two-dimensional thermodynamics. The three vector directions in Example No. 1 include a single, axial coordinate direction (the length of the capillary tubing; see Figure 1) and two lateral coordinate directions (one being the radial direction) over which thermodynamic equilibrium exists at every point ***z*** and at all times ***t*** within the chromatographic column.

## Typical Chromatographic Apparatus: A Tubular Flow System

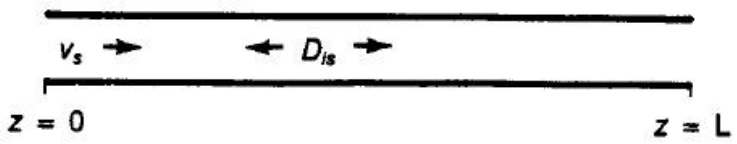


*Figure 1. Non-steady-state diffusion and convection in a tubular flow system. A mixture of components is injected rapidly into the column at z=0 and t =0 and elutes later in time as a series of separated Gaussian peaks at the exit, z = L .*

## What is the Theoretical Basis for Partition Chromatography?

The teaching of the basic principle behind partition chromatography has not been a part of chemical engineering curriculum for decades, with the significant exception of Howard Saltzman when he was a faculty member at the University of Rochester. For example, the author needed to understand a basic derivation for partition chromatography while at the Monsanto Corporation several years after he received his Ph.D. degree in 1965. He found it difficult to find a satisfactory answer in textbooks (most of which focused on either the discrete Craig countercurrent apparatus or on HETP), most of which were oriented towards chemists.

At the time, the author discovered the key gimmick to chromatography in an unusual place, namely, the classic textbook, "The Mathematics of Diffusion", by J. Crank [1]. Crank's Chapter VIII treats simultaneous diffusion and chemical reaction.

Section 8.2 in Crank's book [1] describes instantaneous reeaction, in which "In the simplest case, the concentration, S , of immobilized substance is directly proportional to the concentration C of a substance free to diffuse," i.e,

$$S = RC \tag{1}$$

The result is given by Crank as:

$$\frac{\partial C}{\partial t} = \frac{D}{R+1}\frac{\partial^2 C}{\partial x^2} \tag{2}$$

Crank stated [1]: "Clearly the effect of the instantaneous reaction is to slow down the diffusion process. Thus, if R + 1 = 100, the overall process of diffusion with reaction is slower than the simple diffusion process by a hundredfold. In fact, if the linear relationship (1) holds, solutions of the diffusion-with-reaction problem for given initial and boundary conditions are the same as for the corresponding problem in simple diffusion, except that the modified diffusion coefficient D/(R + 1) is to be used. This is true irrespectively of whether the diffusion-with-reaction occurs in a plane sheet, cylinder, or sphere, or any other geometric shape, and whether diffusion occurs in one dimension or more." [1]

In the author's opinion, Equation (1) describes the partitioning of a component between immobilized (S) and freely diffusing (C) states. This so-called "instantaneous reaction" appears to be a reversible equilibrium rather than an irreversible reaction of C to S. Equations (1) and (2) provided the key clue to the author as to why a partition chromatographic separation yields an individual elution peak for each component.

## Partition Chromatography as a Linear Multistate System

The author has rederived Equation (2) based upon the following linear partition coefficient between states 1 and 2 for an eluting component **i**,

$$k_{i2} = \frac{c_{i2}}{c_{i1}} \tag{3}$$

which has units of concentration/concentration. The resulting conservation-of-species equation -- involving diffusion, convection, and irreversible first-order reaction along the axial coordinate direction z -- is,

$$\frac{\partial c_{is}}{\partial t} - D_{ieff} \frac{\partial^2 c_{is}}{\partial z^2} + v_{ieff} \frac{\partial c_{is}}{\partial z} + k_{ieff} c_{is} = 0 \tag{4}$$

In the absence of axial convection and first-order reaction, Equation (4) simplifies to,

$$\frac{\partial c_{is}}{\partial t} - D_{ieff} \frac{\partial^2 c_{is}}{\partial z^2} = 0 \tag{5}$$

which is identical to Equation (2).

## What is a State?

Partition chromatography basically is the superposition of two-dimensional thermodynamics -- a mobile phase and a stationary phase -- upon non-steady-state diffusion, reactopm. and convection in a third dimension. For simplicity, the two-dimensional equilibrium and third-dimension dynamics can be characterized by a pair of states for each eluting component in a chromatographic column (see Figure 2).

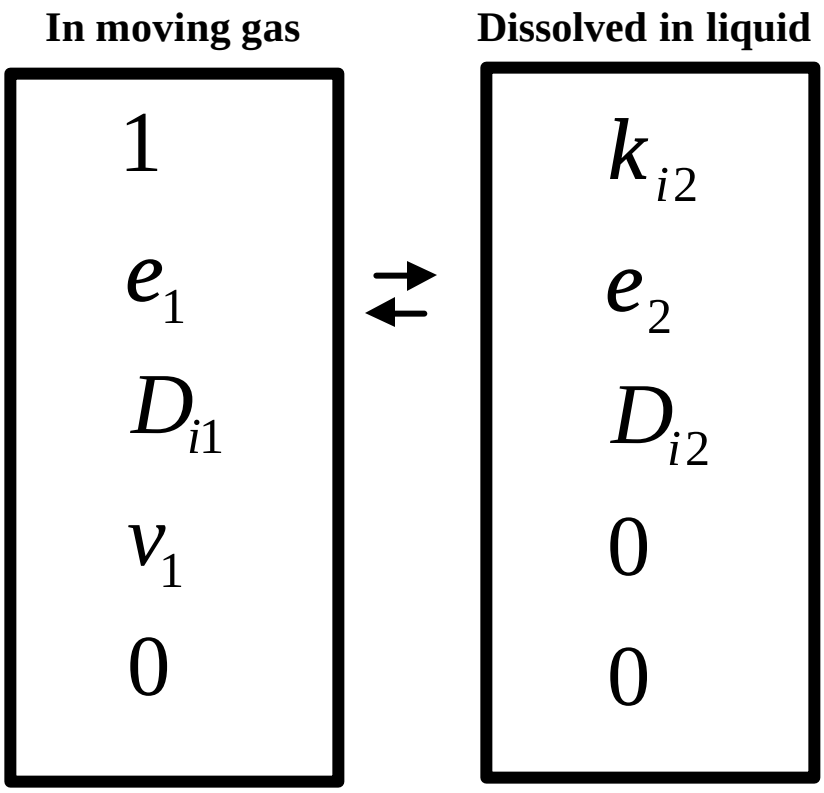


*Figure 2. Box representation of the two states (for each eluting component* ***i****) in gas-liquid partition chromatography (in a tubular column system).*

In Figure 2, the subscript **i** represents an eluting component; the subscript numbers ***1*** and ***2*** represent the mobile and stationary phases, respectively; D is the diffusion coefficient in a phase; and v is the convective velocity of phase ***1***. At the bottom of each state box, the value 0 indicates that no irreversible first-order reaction is occurring in each phase.

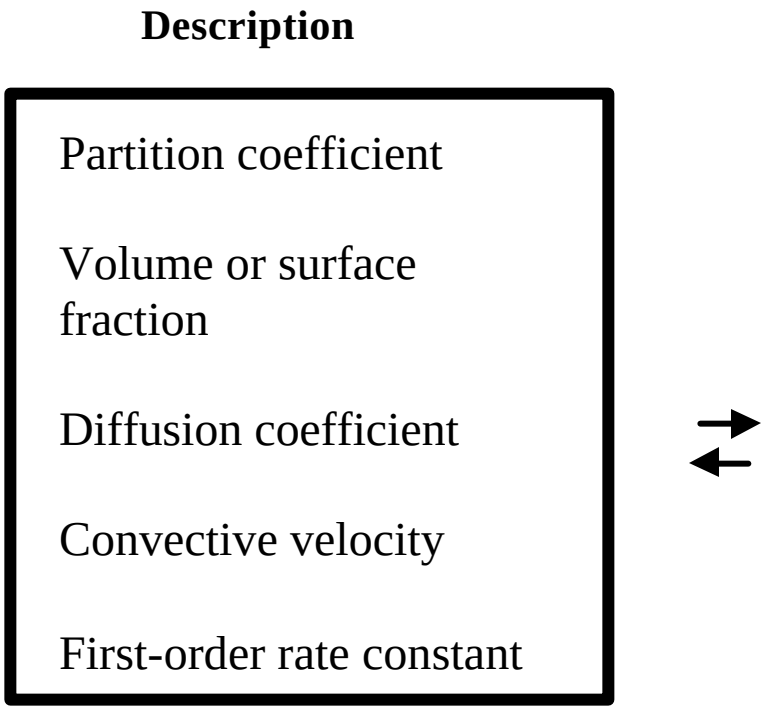


*Figure 3. General box representation of a state in a linear multistate chemical system.*

Figure 3 provides a general representation of a state box in any two-dimensional thermodynamic system in which reaction and mass transfer processes occur in the remaining coordinate direction. The quantity, Vieff , is calculated as follows from Figure (2),

$$v_{ieff} = \frac{e_1 v_1}{e_1 + k_{i2} e_2} \tag{6}$$

If we multiply the numerator and denominator of Equation (3) by the ratio of the volumes of phases 2 and 1, respectively, we obtain the distribution coefficient,

$$K_{i2} = \frac{c_{i2} V_2}{c_{i1} V_1} = k_{i2} \frac{e_2}{e_1} \tag{7}$$

which has units of moles/moles. Therefore, Equation (6) becomes,

$$v_{ieff} = \frac{v_1}{1 + K_{i2}} \tag{8}$$

Equation (8) is the well-known equation for the migration velocity of a component in a partition chromatographic system.

## Example No. 2: Oxidation-Reduction Chromatographty

Lederer and Lederer have mentioned Cassidy's unsuccessful attempts to separate components with the aid of "electron exchange resins" [5]. The flaw in Cassidy's experiments was that both the oxidized and reduced forms of the migrating components were completely soluble in the liquid phase. To counteract this problem, strong adsorption sites -- such as those found in ion exchange resins -- should be provided in order to make this type of chromatography practical for components that are stable to oxidation-reduction cycles. Figure (4) depicts the four states associated with a practical oxidation-reduction chromatography system.

## Example No. 3: Carrier Electrochromatography, Single-Phase Chromatography

How do you separate hexasaccharides in an electrophoresis machine? Sugar molecules are uncharged, so they do not migrate in an electric field. The clever resolution is to perform the electrophoretic separation in an aqueous borate medium [6]. Sugar molecules engage in reversible complexing with borate ions to produce sugar-borate ionic complexes,

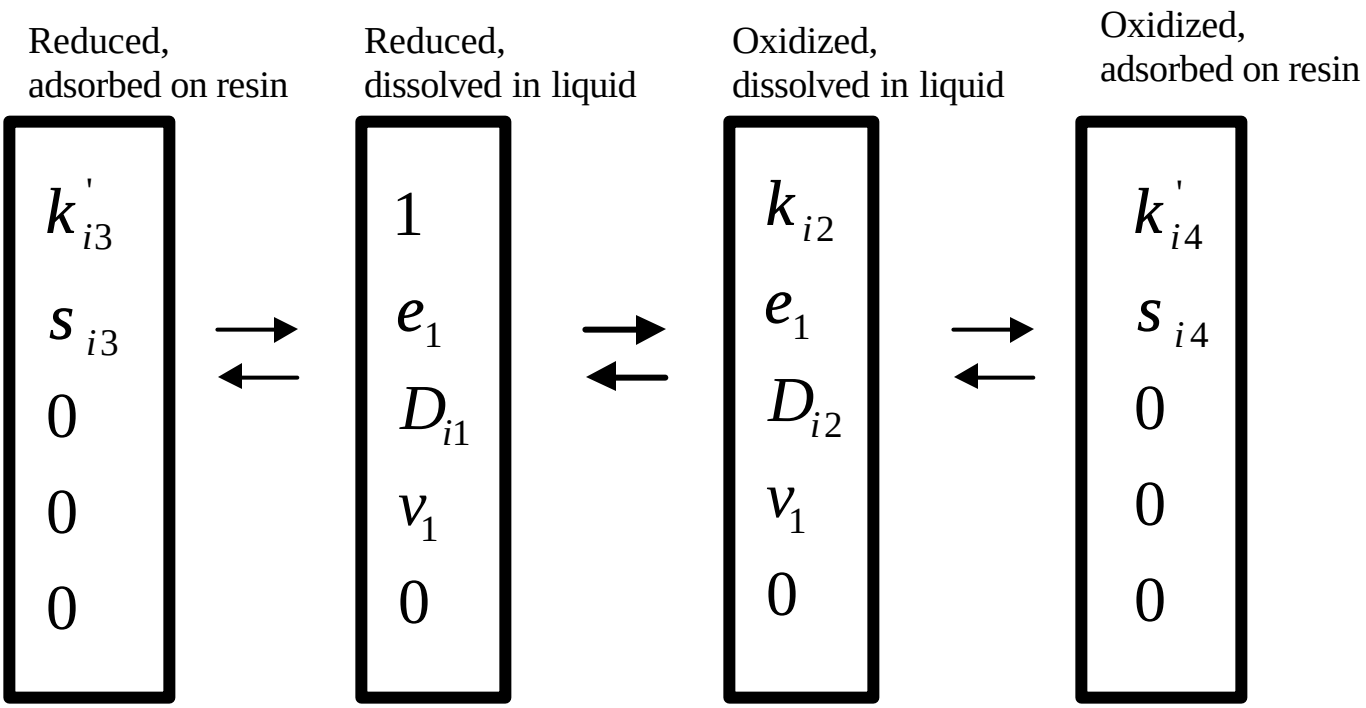


*Figure 4. Four-state box representation for oxidation-reduction chromatography.*

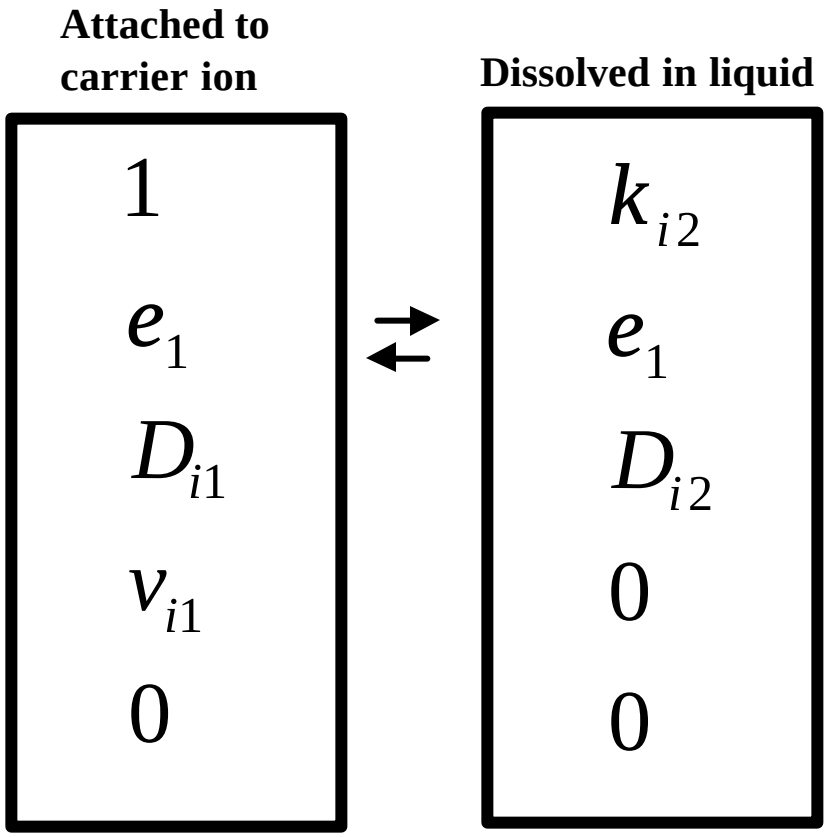


*Figure 5. Two-state box representationm for carrier electrochromatography.*

| | | | | |
|---|---|---|---|---|
| Dissolved | | | Keq | sugar-borate |
| Sugar | + | borate | -------> | ionic |
| Molecule | | ion | <------- | complex |

If the equilibrium constant of complex formation, Keq , is very large, the sugar molecules are completely complexed and migrate in the electrophoresis apparatus at a rate determined by the mobility of the sugar-borate complex (not by the mobility of the free borate ion). If Keq is extremely small, the sugar molecules remain uncomplexed and do not migrate in the apparatus. Between these two extremes are intermediate migration velocities that depend upon the chemical nature of individual hexasaccharide molecules. With sucha reversible complexing reaction, this becomes a single-phase chromatographic system that we call "carrier electrochromatography".

## Examples of Partition Chromatographic Techniques

*Table I. Standard chromatographic techniques*

| Standard Chromatographic Technique | Mobile Phase | Stationary Phase |
|---|---|---|
| gas-liquid chromatography | gas | liquid contained in porous solid |
| gas-solid chromatography | gas | polymeric solid particles |
| liquid-liquid chromatography | liquid | organic liquid contained in porous solid |
| solid-liquid chromatography | liquid | polymeric solid particles |
| molecular-sieve chromatography | gas | molecular sieve particles |
| ion-exchange chromatography | liquid | porous ion-exchange particles |
| ion-exclusion chromatography | liquid | porous ion-exchange particles |
| gas-solid adsorption chromatography | gas | porous solid particles |
| HPLC | liquid | porous solid particles |
| liquid-solid adsorption chromatography | liquid | porous solid particles |
| reversed-phase chromatography | organic liquid | water contained in porous solid |
| gel-permeation chromatography | liquid | cross-linked solid particles |

Table I summarizes standard chromatographic techniques with the names by which they were known during the early 1970s. The only exception is HPLC, which initially was called high-pressure liquid chromatography and is now known as high-performance liquid chromatography.

*Table II. Non-standard chromatographic techniques*

| Non-Standard Chromatographic Technique |
|---|
| aerosol chromatography |
| dust chromatography |
| solid-liquid fiber chromatography |
| solid-liquid membrane chromatography |
| gas-solid membrane chromatography |
| gas-solid fiber chromatography |
| liquid-liquid membrane chromatography |
| flotation chromatography |
| oxidation-reduction chromatography |
| electrode chromatography |
| carrier chromatography |
| inverse carrier chromatography |
| carrier magnetochromatography |
| solid-phase carrier electrochromatography |

Table II lists unusual, or non-standard, chromatographic techniques that were initially proposed in the author's book [2] and then communicated in the literature [3,4]. The carrier chromatogrpahic techniques are conducted in an electrophoresis apparatus that, in principle, requires only a single phase. The mobile state consists of ions complexed to eluting components, whereas the stationary state is the water itself. Electrophoresis is not a chromatographic technique because it does not have both mobile and stationary states for an eluting component. The component migrates because of its charge-to-mass ratio in an electric field. Accordingly, electropheresis is a single-state separation technique.

*Table III. Categories of chromatographic techniques*

| Categories of Chromatographic Technique | Special Characteristics |
|---|---|
| Two-phase techniques | none |
| High-performance techniques | apparatus consists of column under high pressure |
| Column techniques | apparatus consists of a cylindrical column |
| Paper techniques | stationary phase is paper |
| Thin-layer techniques | stationary phase is thin layer of porous solids |
| Capillary techniques | apparatus consists of a capillary column |
| Micro-column techniques | column is very small |
| Single-phase techniques | mobile state consists of dissolved mobile ions |
| Reverse phase techniques | water is stationary phase |
| Superimposed chemical equilibria | system contains dissolved complexing agents |
| High free-energy techniques | non-aqueous liquid with high pKa equilibrium |

Table III summarizes different categories of chromatographic techniques. The techniques differ in the type of apparatus -- glass column, capillary column, stainless-steel, high-pressure column, paper, or glass plate; whether the technique employs one or two phases; and the possible existence of superimposed chemical equilibria within a liquid phase.

## Summary

***Partition chromatography*** is defined as a differential migration separation technique that employs the distribution of a migrating component between two or more different ***states*** whose relative velocities are not all equal to zero. The ***state*** can be a dissolved component within a phase, an adsorbed component at an interface, or a reversibly complexed (to an ion or molecule) component within the liquid phase. The apparatus can consist of a vertical glass tube; a high-pressure stainless-steel column; a long glass or stainless-steel capillary tube; paper; an electrophoresis apparatus; or a plate with a thin-layer coating of porous solid.

## Nomenclature

**Arabic letters**

| | |
|---|---|
| c | Concentration of eluting component, defined by Equation (3) |
| C | J. Crank concentration of diffusing component, defined by Equation (1) |
| D | Diffusion coefficient, defined in Equations (2), (4), and (5) |
| k | First-order rate constant, defined by Equation (4) |
| K | Distribution coefficient, defined by Equation (7); units of moles/moles |
| K | Equilibrium constant |
| R | J. Crank ratio, defined by Equation (1) |
| S | J. Crank concentration of stationary component, defined by Equation (1) |
| t | time |
| v | velocity, defined in Equation (4) |
| x | x axis |
| z | z axis |

**Greek letters**

| | |
|---|---|
| ε | Volume fraction, defined in Figure (3) |
| κ | Partition coefficient, a ratio of concentrations, defined by Equation (3); units of concentration/concentration |
| σ | Surface-to-volume ratio, defined in Figure (3) |

**Subscripts**

| | |
|---|---|
| ***eff*** | Effective value index, defined in Equations (4) and (5) |
| eq | Equilibrium value |
| ***i*** | Eluting component index |
| ***s*** | Environment index |
| ***i1*** | Component ***i*** in phase or environment **1** |
| ***i2*** | Component ***i*** in phase or environment **2** |
| ***i3*** | Component ***i*** in phase or environment **3** |
| ***i4*** | Component ***i*** in phase or environment **4** |

**Superscripts**

| | |
|---|---|
| ' | Indicates a surface state |

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6. R. Consden and W. M. Stanier, Nature ***169*** , p. 783, 1952.