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# THEORY AND USE OF CENTRIFUGAL PARTITION CHROMATOGRAPHY

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## INTRODUCTION

Centrifugal Partition Chromatography (CPC) is a variant of countercurrent chromatography (CCC). As in CCC, two immiscible liquids are used. The first liquid is the stationary phase, the second is the mobile phase. The liquid stationary phase is held in channels engraved in several polychlorotrifluoroethylene (PCTFE) plates. One hundred channels are engraved on each PCTFE plate. Four PCTFE plates are assembled together in a cartridge. Up to 12 cartridges (4800 channels) can be loaded in the rotor of a centrifuge. The centrifugal field, generated by the spinning rotor, holds the stationary phase sufficiently that a mobile phase can be pumped through in (Figure 1). The system is analyzed in detail.

## RESULTS AND DISCUSSION

### *1-Factors Affecting Pressure Drop*

The total pressure drop in CPC originates from hydrostatic and hydrodynamic sources (1). The pressure difference  $\Delta P$  (in Newtons per square meter,  $N/m^2$ ) between the top and bottom of a channel filled with two immiscible liquids in a centrifugal field is given by:

$$\Delta P = n \Delta \rho \omega^2 R h \quad (1)$$

where  $\Delta \rho$  is the difference in liquid densities ( $\text{kg/m}^3$ ),  $h$  is the height of the stationary phase in one channel,  $\omega^2 R$  is the centrifugal field generated by a rotor of radius  $R$  and spin rate  $\omega$  and  $n$  is the total number of channels.

In addition to the hydrostatics term represented by equation 1, a hydrodynamic term must be present in the pressure drop equation. This can be derived from Poiseuille's law for an open tube:

$$\Delta P = 8\eta L F / (\pi r^4) \quad (2)$$

where  $\Delta P$  is the driving pressure ( $\text{N/m}^2$ ),  $\eta$  is the liquid mobile phase viscosity ( $\text{kg/m} \times \text{s}$ ),  $L$  and  $r$  are the tube length and radius, respectively (m), and  $F$  is the flow rate ( $\text{cm}^3/\text{s}$ ). The ratio  $8 L / \pi r^4$  corresponds to the geometrical characteristics of the tube and is referred to as the coefficient  $\gamma$  ( $\text{m}^{-3}$ ).

During a 2-phase separation experiment the total pressure drop is given by:

$$P = n [\Delta \rho \omega^2 R h + \eta \gamma F] \quad (3)$$

Even though the crooked shape of the channels (Figure 1) is certainly not an open tube of constant radius, the  $\gamma$  value of eq. 3 corresponds to an open tube of geometrical characteristics equivalent to a channel and its duct (1). Equation 3 links the experimental driving pressure to the physico-chemical properties of the liquids used and to the spin and flow rate (1). Of these factors, the linear increase of the driving pressure with the density difference between the two liquid phases was reported by Murayama et al. (2).

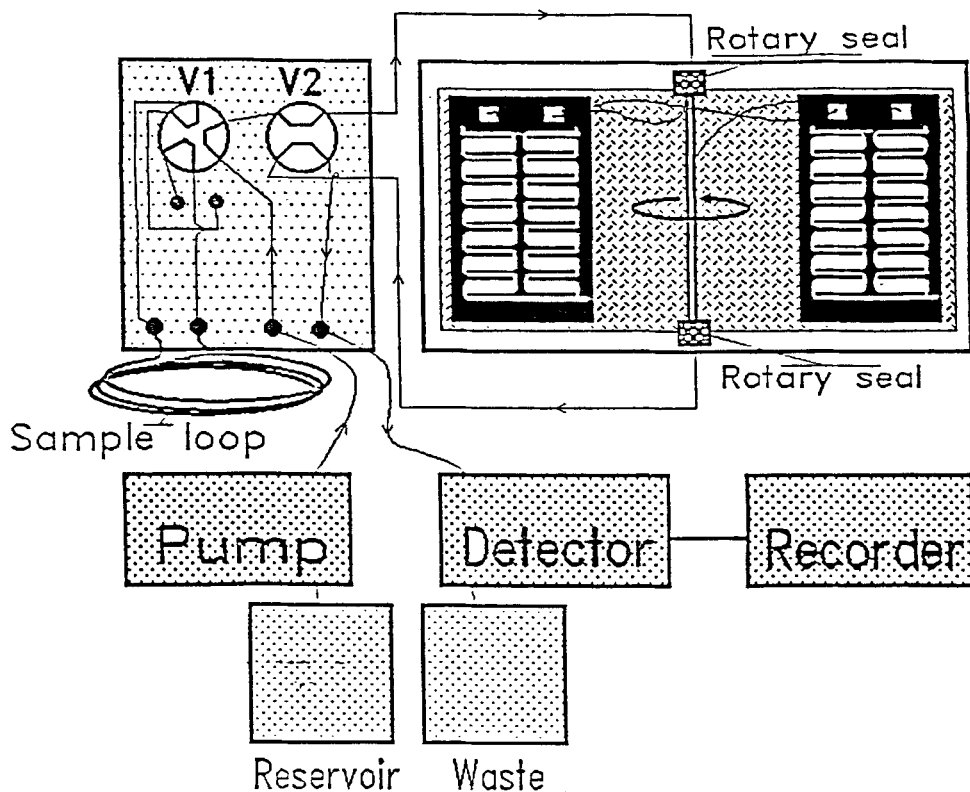


Figure 1. The general CPC setup in the descending mode.  $V_1$  = injection valve;  $V_2$  = switching valve (ascending or descending mode). The sample loop volume is 3 mL. Concerning the channel design, an actual cartridge contains 400 channels in four PCTFE plates, with 25 channels on each side of each PCTFE plate. Only 14 channels are represented here for clarity. At rest, each channel and connecting duct is 12.4 mm long and 1.1 mm deep. The width of the channel was 2.4 mm while that of the duct was 0.9 mm. The dividing wall between the channel and connecting duct was 1.1 mm wide. The rounded corners of ducts and channels have radii or curvature of approximately 0.45 mm and 1.0 mm respectively. (Taken from Reference 1).

The relative importance of the hydrostatic and hydrodynamic terms of eq. 3 depends greatly on the experimental conditions. If the density difference between the two immiscible liquids is high and the viscosity is low (system hexane-water, for example), the hydrostatic term will be the principal factor acting on the pressure drop. The flow rate will have a slight effect. For example, with the system hexane water ( $\Delta\rho = 0.339 \text{ g/cm}^3$  and  $\eta_{\text{hexane}} = 0.29 \text{ cP}$ ), with 3 mL/min and 500 rpm as the flow and spin rates, respectively, the hydrodynamic term contributes to the total pressure drop for only 3.6%. On the other hand, when the viscosity of the mobile phase is high (octanol, for example) and the density difference is low, the hydrodynamic term of eq. 3 becomes more important. With the system octanol-water ( $\Delta\rho = 0.137 \text{ g/cm}^3$  and  $\eta_{\text{octanol}} = 5 \text{ cP}$ ), 3 mL/min and 500 rpm, the hydrodynamic term corresponds to 61% of the total pressure drop.

The spin rate remains the most important parameter. It increases the pressure drop quadratically. For the octanol-water system, a 60% spin rate increase (from 500 rpm to 800 rpm) multiplies by 2.5 the pressure drop due to the hydrostatic term. In such conditions (octanol-water system, 3 mL/min and 800 rpm), the hydrodynamic term of eq. 3 contributes only 38% to the total pressure drop.

### *2-Physico-Chemical Factors that Affect CPC*

Some important physico-chemical solvent parameters that affect centrifugal partition chromatography (CPC) were evaluated. They are (i) the mutual solubility of the solvent(s) in each phase which was analyzed using phase diagrams and tie-lines, the results of which can be used to evaluate the behavior and stability of the biphasic CPC system; (ii) solution density which affects the pressure drop; (iii) interfacial tension which affects droplet formation and (iv) viscosity which affects both pressure drop and chromatographic efficiency (3). The solubility properties can be

characterized using ternary mass diagrams such as that for water-hexane-methanol in Figure 2. The black areas correspond to the monophasic mixtures which cannot be used in CPC. The area containing the tie-lines delineates the biphasic region that can be used in CPC. The tie-lines allow one to calculate the quantity and composition of the two phases obtained when three liquids are mixed. To do this, the lever rule must be used (4). Consider, for example, the solution represented by point B in Figure 2 which corresponds to a mixture of hexane (60 g), methanol (20 g) and water (20 g). It is possible to calculate the quantity and composition of the two phases obtained with the Point B mixture as follows. According to the lever rule:

$$\text{distance AB} \times \text{mass of A} = \text{distance BC} \times \text{mass of C} \quad (4)$$

and

$$\text{mass of A} + \text{mass of C} = \text{mass of B} \quad (5)$$

The compositions of Points A and C are: methanol 49%-water 51% w/w (traces of hexane) and hexane 99%-methanol 0.9%-water 0.1% w/w, respectively. The length AB is equal to 0.61 x AC. The length BC is equal to 0.39 x AC.

From Eqs. 4 and 5, one obtains:

$$\frac{0.61 \times AC}{0.39 \times AC} = \frac{\text{mass of C}}{\text{mass of A}} = \frac{100 - \text{mass of A}}{\text{mass of A}} = 1.56 \quad (6)$$

from which the mass of A is calculated to be 39 g and the mass of C, 61 g. The 100 g mixture, corresponding to Point B, separates into two phases: 39 g of aqueous phase with the A composition and 61 g of organic phase with the C composition.

In CPC, the useful quantity is the volume of each phase. The solution density must be used to convert the mass to a volume. Table I lists several

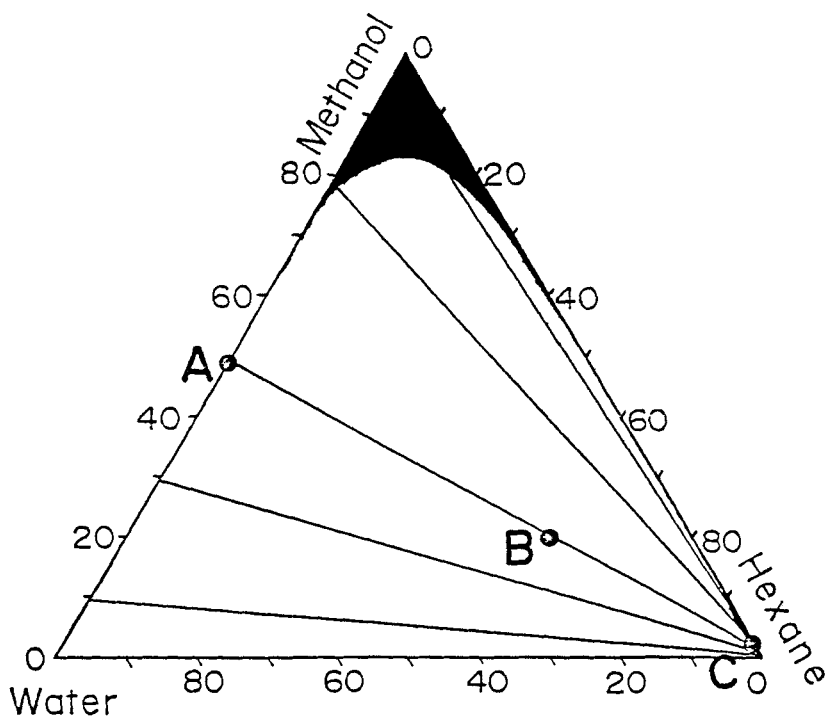


Figure 2. Ternary mass diagram for the hexane-methanol-water system. The white area containing tie-lines represents the solvent compositions at which 2 phases exist (biphasic). The dark area indicates a monophasic (homogeneous) solution. See the Results and Discussion section for an explanation of points A, B and C. (Taken from Reference 3).

Table I. Volume characteristics and density of selected mixtures

hexane	Mixture (mL)		Aqueous Lower Phase		Organic Upper Phase	
	methanol	water	volume	density	volume	density
25.0	25.0	0.0	42.0	0.720	8.0	0.666
25.0	24.75	0.25	35.9	0.726	14.1	0.664
25.0	23.75	1.25	28.4	0.773	21.6	0.664
25.0	22.5	2.5	26.3	0.796	23.7	0.653
25.0	20.0	5.0	25.2	0.828	24.8	0.652
-----						
pentanol	methanol	water	volume	density	volume	density
25.0	0.0	25.0	23.7	1.000	26.3	0.831
25.0	1.25	23.75	22.7	0.978	27.3	0.834
25.0	2.5	22.5	21.8	0.967	28.2	0.846
25.0	5.0	20.0	20.4	0.960	29.6	0.850
25.0	10.0	15.0	12.5	0.950	37.5	0.865

Temperature 20°C; if 25 mL of water was shaken with any 25 mL octanol-hexane mixture, both aqueous and organic phases had about the same volume (25 mL).

different phase volumes obtained after mixing different amounts of various solvents. As expected, by looking at the mass diagram of Figure 2, the mixture of 25 mL of hexane with 25 mL of methanol gives only 8 mL of hexane upper phase and 42 mL of methanol rich lower phase.

Temperature changes also induce numerous physico-chemical changes in liquids (5). Vapor pressure, density, refractive index, viscosity and



surface tension are such temperature dependent parameters. The mutual solubilities of the stationary and mobile phase liquids are also temperature dependent. For example, the ternary diagram of the water-methanol-hexane system changes somewhat between the temperatures of 20° and 40° C (5). It is shown that the flow behavior change, as determined by an efficiency study, is temperature dependent because the viscosities of the liquids are modified by the temperature (see Table II). A solute's partition coefficient is also dependent upon temperature. Using Van't Hoff plots, it is possible to determine the enthalpy of transfer of the solute from the mobile phase to the stationary phase. Plotting the  $\ln K$  values versus the inverse of the corresponding absolute temperature (Van't Hoff plot) produced a straight line (regression coefficient 0.998) with a slope of 1500 K. With the Van't Hoff equation:

$$d(\ln K) / dT = \Delta H_o / (RT^2) \quad (7)$$

one can estimate the variation of enthalpy,  $\Delta H_o$ , corresponding to the transfer of hydroquinone from octanol to water, to be -12.5 kJ/mol or -3 kcal/mol. It should be noted that in this approach it is assumed that the composition of the two phases does not change significantly over the temperature range used.

There were two additional temperature effects that can severely affect detection. They occur when operating the system at temperatures  $\geq 30^\circ\text{C}$  (5). The first effect occurred when using an octanol-water system at  $35^\circ\text{C}$ . The solution emanating from the heated centrifuge became turbid as it reached the relatively cooler detector. The octanol mobile phase was saturated with water at  $35^\circ\text{C}$  but became supersaturated at room temperature ( $21^\circ\text{C}$ ) thereby producing aqueous microdroplets that produced extensive

Table II - Evolution of Efficiency and Retention Volume with Temperature<sup>a</sup>

Temperature °C	Pentachlorophenol		Hydroquinone		K <sup>b</sup>
	v <sub>r</sub> mL	N plates	v <sub>r</sub> mL	N plates	
15	59.4	390	77.0	90	0.269
20	64.2	350	81.6	100	0.286
25	68.1	450	85.8	110	0.311
30	60.6	790	82.2	125	0.336
35	64.8	1190	86.4	180	0.359

<sup>a</sup>Flow rate 3 mL/min, mobile phase-octanol, Stationary phase -water.

<sup>b</sup>Because water was the stationary phase, K is the water-octanol partition coefficient. The classical octanol-water partition coefficient is the reverse ( $K_{\text{oct}} = 1/K$ ).

detector noise. This problem is eliminated by heating the tubing between the rotor chamber and the detector to 35°C. The second temperature effect was observed when using low boiling point mobile phases (e.g., hexane) at low flow rates. The heat generated at the end rotary was sufficient to induce local vaporization that produces numerous observed bubbles that interfered with detection. This problem was eliminated by cooling the seal with a small fan during operation, thereby eliminating vaporization.

### 3-Partition Coefficient Determination

Octanol-water partition coefficients ( $K_{\text{oct}}$ ) are one of the accepted physico-chemical parameters for predicting the biological effects of organic

chemicals. It is demonstrated that CPC can be used to determine directly the  $K_{\text{Oct}}$  values from about 0.003 to 340. The mobile phase must be water and the stationary phase must be octanol for accurate determination of the larger  $K_{\text{Oct}}$  values. To reduce retention times and volumes, the stationary phase volume can be decreased. This can be done using an original mode: the "underload" mode. In this procedure one pumps the mobile phase in the opposite direction as required by the manual (i.e., pump in the ascending mode if you are supposed to pump in the descending mode (6). Technically this is supposed to push the entire stationary phase out. However, if the centrifuge is rotating, it does not, and a small amount of stationary phase is trapped (see Figure 3) (6). To extend the  $K_{\text{Oct}}$  range which can be determined by CPC, the system hexane (60%) octanol (40%)-water was used. A single linear relationship between  $\log K$  in this system and  $\log K_{\text{Oct}}$  was established allowing to determine the  $K_{\text{Oct}}$  values up to 1000 ( $\log K_{\text{Oct}} = 3$ ). Under standard conditions it is theoretically demonstrated that CPC can be used to determine partition coefficients over a five order of magnitude range, from 0.003 to 340, by adjusting the stationary phase volume (6,7).

#### *4-Efficiency*

The theory of nonequilibrium band broadening and factors giving rise to back pressure in CCC and centrifugal CCC have not been considered adequately. Plots of efficiency versus flow rate show behavior roughly opposite that of modern liquid chromatography (LC) or gas chromatography (gc) (8). Indeed, van Deemter-type plots have maxima rather than minima (Figure 4). Consequently, the best efficiency is obtained at very high or very low flow rates. A model to explain this behavior is given below (9).

#### Model

A model can be formulated to account for the observed flow efficiency behavior. A single channel or cell in the CPC apparatus has a cross sectional

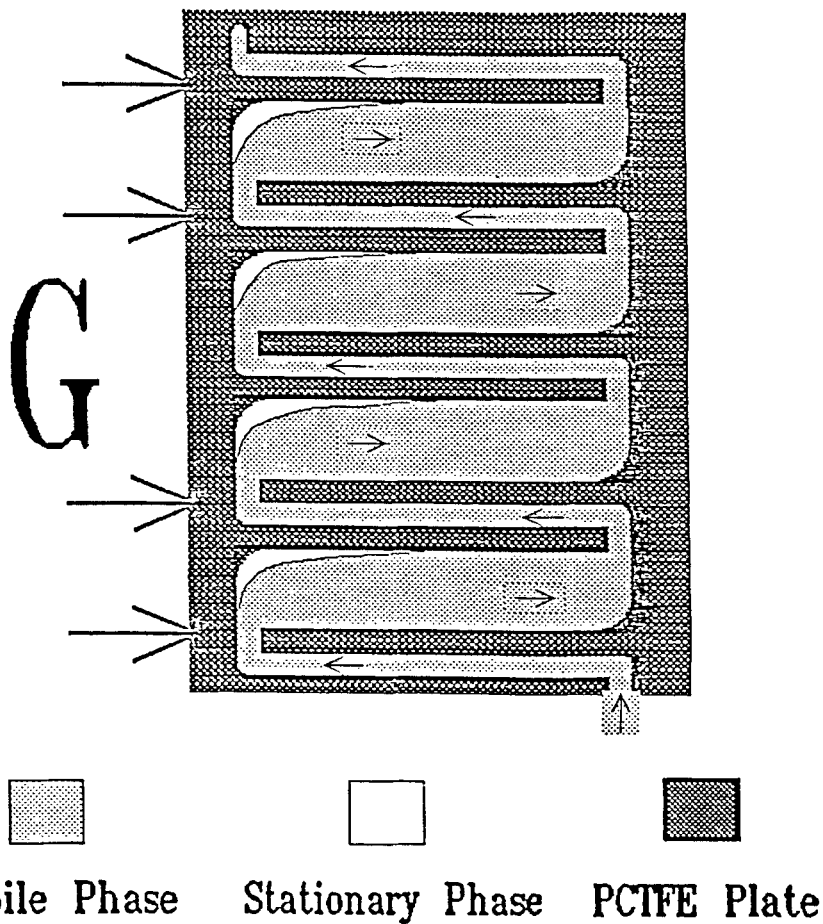


Figure 3. The "underload" mode. Oversimplified view of the possible way octanol is entrapped in the channels. The arrows indicate the flow direction. (Taken from Reference 6, CPC V).

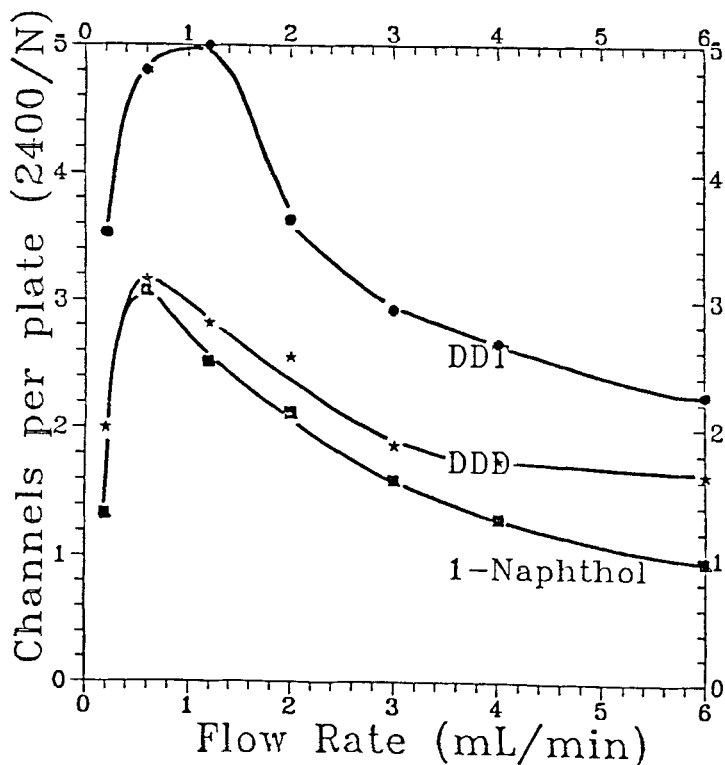


Figure 4. A van Deemter-type plot where the number of channels required to obtain one theoretical plate is plotted versus the flow rate. The solutes are: DDT (●), DDD(\*) and 1-naphthol (■). The runs were made at 20°C and 1100 rpm. (Taken from Reference 8).

area ( $A_c$ ) and a total volume ( $V_t$ ).  $V_t$  is divided into the stationary phase volume ( $V_s$ ) and the mobile phase volume ( $V_m$ ). The extraction process is divided conceptually into three stages. Initially, the cell contains only the less dense stationary phase. The mobile phase is introduced at the top of the stationary phase as  $N_D$  droplets of volume  $V_D$  and surface area  $A_D$ . These drops fall through the stationary phase with velocity ( $v$ ), exchanging solute

across a surface area ( $A_1$ ) for a period of time ( $t_1$ ). Normally, this time is very short.

The droplets do not immediately coalesce to form the bulk stationary phase, but exist for a period of time as droplets in an emulsified band between the two bulk phases. Solute exchange continues during this period, still across the surface area ( $A_2 = N_D A_D$ ). The average time of residence in this settling condition ( $t_2$ ), is assumed to be proportional to the flow rate raised to some power, since agitation by flow stirs the boundary and broadens the emulsified band. This time is expected to be inversely related to the velocity of the falling droplets, since those factors which increase the velocity (difference in density, centrifugal force, fluidity, etc.) tend to decrease the time of settling and the thickness of the band. Surface tension should also have an inverse effect on this time.

The remainder of the residence time ( $t_3$ ) is spent in a fairly quiescent state with stirred but non-turbulent exchange across the cross-sectional area ( $A_3 = A_c$ ). The total residence time ( $t = t_1 + t_2 + t_3$ ) is equal to the volume of the mobile phase divided by the flow rate ( $F$ ,  $t = V_m/F$ ). After this time, the mobile phase is removed and the process is repeated in the next cell.

**Kinetic Considerations:** Long residence times and high surface areas facilitate the attainment of equilibrium and maximum efficiency of the exchange process. The time of falling is independent of flow rate *per se*, and this stage of the process is not expected to be of major importance. At very slow flow rates, residence times approach infinity allowing attainment of equilibrium across the relatively small cross-sectional area ( $A_c$ ). Increasing the flow rate decreases the total time of residence, and thus decreases the efficiency of the exchange if kinetic factors are important. However, as the flow rate increases, there is an increase in the amount of emulsified region as well as a decrease in the size of the emulsified droplets. This results in an

increase surface area, thereby increasing efficiency of the process. This increasing flow rate, the process becomes increasingly efficient, but eventually the broadening emulsified region will approach the exit port and "flooding" will occur. Equations have been derived based on this model and evaluated using experimental results. These are discussed in detail in another work (9). However, both theory and experiment tend to support the aforementioned model.

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