A Study of Inhomogeneity Effects on Packed High Performance Liquid Chromatography Columns

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A Study of Inhomogeneity Effects on Packed High Performance Liquid Chromatography Columns

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Abstract

The packing quality of a High Performance Liquid Chromatography column has significant impact on the resulting separations. In practice, problems in a packing occur through normal usage of a column. Resulting chromatograms from inhomogeneities exhibit varying problems such as extreme tailing and double peaks. Early detection of such problems is critical to fixing problems before their effect harms efficiency, but a fully developed theory of how inhomogeneities affect separations does not exist. The purpose of this work was to experimentally characterize HPLC column inhomogeneities and to begin preliminary simulations of HPLC columns with the computer simulation package STAR-CD. To do this, columns were first packed with a standard packing recommended by the manufacturer of the column. Later, two types of inhomogeneity were individually packed into columns: cavities and balls. The resulting chromatograms were analyzed and compared. The results show that as was expected, introducing inhomogeneities reduced separation quality. A simulation was created to model a one-dimensional column and a chromatogram was produced from this simulated column. From the simulated column, one can see that producing chromatograms entirely from a simulation produces an accurate, adjustable, and quick method for studying the column. Recommended further research includes more experiments involving inhomogeneities and simulations incorporating inhomogeneities. A further outlook includes research into pattern recognition with the goal of being able to analyze a series of chromatograms produced by the same column and notice small changes that indicate a specific inhomogeneity is forming.
1. Background Theory

Chromatographic processes have long been used in science and industry to complete useful separations. Such processes include thin layer chromatography as well as gas and liquid chromatography. Today, the field of high performance liquid chromatography (HPLC) is divided into two main fields: preparative and analytical. The most prominent difference between the two is the intention of the process: whether to analytically collect information, or to preparatively collect unaltered samples. [Schmidt-Traub05]

Fundamentally, liquid chromatographic apparatus consist of a solid stationary phase and a liquid mobile phase in some sort of a packed column (with inlet and outlet on opposite ends). After a sample is injected into the flowing mobile phase into the column, the sample particles adsorb onto (and into the pore system of) the stationary phase. As shown in Figure 1, different samples will undergo different equilibrium processes; and thus, samples that spend more time in the mobile phase than adsorbed onto the stationary phase will elute faster than those samples having a higher affinity toward stationary phase adsorption. The adsorption process is governed by thermodynamic behavior and mass transfer and fluid flow kinetics. The thermodynamic behavior can be described by adsorption isotherms. For a given sample and system temperature, this graph shows the relationship between the sample’s loading of solid adsorbent and the concentration of the sample in the fluid phase. An example isotherm can be seen in Figure 2. This type of isotherm

![Figure 1](image1.png)

Figure 1 [Schmidt-Traub05]

![Figure 2](image2.png)

Figure 2 [Schmidt-Traub05]
is called Langmuir and is the norm for the processes discussed here. Thus, samples with different isotherms will exhibit unique equilibriums and separate differently.

When a detector of some kind is connected to the outlet of a column, the concentration detected is converted to a signal that can be recorded electronically. When this recorded signal is graphed against time, a standard chromatogram is produced, as shown in Figure 3. The three peaks in Figure 3 are produced from 3 different samples. The area of a peak on a chromatogram is proportional to the concentration of the injected sample. The chromatogram is the main source of

![Chromatogram Diagram](image)

**Figure 3 [Schmidt-Traub05]**

information regarding the experimental chromatographic process. Thus, to characterize a system, one must first characterize resultant chromatograms.

Many parameters can be gathered from a chromatogram; these include overall retention time \( t_R \), net retention time \( t_{R,\text{net}} \), total dead time \( t_{\text{total}} \), dead time of the column \( t_0 \), and dead time of plant without column \( t_{\text{plant}} \). The total dead time represents the time required by a non-adsorbed sample to be detected, and the net retention time is the effective time that a sample is adsorbed onto the stationary phase. The retention time of a sample can vary drastically depending on mobile phase flow rate, so a value called the retention factor \( k' \) is used. The retention factor is the ratio of the time a sample is adsorbed to the stationary phase to the time a sample is in the mobile phase; it can also be described in mole ratios:

\[
k' = \frac{t_R - t_0}{t_0} = \frac{t_{R,\text{net}}}{t_0} = \frac{n_{\text{stat}}}{n_{\text{mob}}} \tag{1}
\]

In addition to this, the selectivity, \( \alpha \), between two components is as follows, where the selectivity of component \( j \) is the longer retained component:

\[
\alpha = \frac{k'_j}{k'_i} \tag{2}
\]
Many different volume parameters are important for the characterization of a chromatogram. The total volume of the packed column \( V_c \) is divided into the interstitial volume of the fluid mobile phase \( V_{\text{int}} \) and the volume of the stationary phase \( V_{\text{ads}} \), such that:

\[
V_c = \frac{\pi \cdot d_c^2 \cdot L_c}{4} = V_{\text{ads}} + V_{\text{int}}
\]  

(3)

Accordingly, the solid phase volume is composed of the volume of strictly the solid material \( V_{\text{solid}} \) and the volume of pore system \( V_{\text{pore}} \). A variety of porosities are based on these volume parameters, namely void fraction \( \varepsilon \), porosity of the solid phase \( \varepsilon_p \), and total porosity \( \varepsilon_t \); with the volumetric flow rate denoted as \( \dot{V} \), these porosities are defined as such:

\[
\varepsilon = \frac{V_{\text{int}}}{V_c}
\]  

(4)

\[
\varepsilon_p = \frac{V_{\text{pore}}}{V_{\text{ads}}}
\]  

(5)

\[
\varepsilon_t = \frac{V_{\text{int}} + V_{\text{pore}}}{V_c} = \frac{t_0 \cdot \dot{V}}{V_c}
\]  

(6)

These porosities are not independent of each other. They are related by the following equation:

\[
\varepsilon_t = \varepsilon + (1-\varepsilon) \cdot \varepsilon_p
\]  

(7)

The interstitial velocity, or the average linear velocity of the fluid through the column, can also be specified:

\[
u_{\text{int}} = \frac{\dot{V}}{\varepsilon \cdot t_0 \cdot \pi \cdot d_c^2 / 4}
\]  

(8)

Another velocity can also be defined - the superficial velocity \( u \), or the theoretical velocity through an empty column:

\[
u = \frac{4 \cdot \dot{V}}{d_c^2}
\]  

(9)

Not mentioned before is the fact that an ideal chromatogram would exhibit the same peak as that of the injection but many things alter this shape. Mentioned earlier, one of these things is the thermodynamic behavior of the system described by adsorption isotherms. Another effect is the resistance of mass transfer in the column. All effects that are not related to adsorption processes or mass transfer resistance are lumped into the term axial dispersion. Two major effects that belong to axial dispersion are eddy diffusion and the laminar interstitial velocity profile. Eddy diffusion is simply a statistical effect that describes how a particle can take an infinite number of paths through the column, thus changing the residence time for each individual particle. The laminar velocity profile between the particles simple means that as a sample is traveling through two adsorbtent particles, the velocity it travels will be fastest in the middle of the particles and slower as it approaches either particle. An example of axial dispersions can be seen in Figure 4. Here, a square injection is shown at the inlet and the maximum concentration slowly becomes less and the peak broadens as it passes through the column.
Figure 4 [Schmidt-Traub05]
Because peaks are not always symmetrical and sometimes can exhibit strange geometry, it becomes necessary to further characterize the column using the parameters already defined. A term called the theoretical plate number or column efficiency ($N$) is essential in determining the quality of the chromatographic column. The height of the equivalent theoretical plate (HETP) is also useful in characterization; where $\sigma_t^2$ is the variance of the chromatogram peak (with respect to time), HETP is defined as:

$$\text{HETP} = \frac{L_c}{N} = \frac{\sigma_t^2}{t_R} \cdot |L_c|$$

(10)

For unsymmetrical peaks, the method of moments is needed to find the retention time. The following integrals (and their finite sum equivalents) are used to calculate $N$ and HETP, with $\mu_t$ equivalent to the retention time $t_R$ in (8):

$$\mu_t = \frac{\int_0^\infty t \cdot c(t) \, dt}{\int_0^\infty c(t) \, dt} \approx \frac{\sum_{j=1}^{n_p} t_j c_j \cdot \Delta t}{\sum_{j=1}^{n_p} c_j \cdot \Delta t}$$

(11)

$$\sigma_t^2 = \frac{\int_0^\infty (t - \mu_t)^2 \cdot c(t) \, dt}{\int_0^\infty c(t) \, dt} \approx \frac{\sum_{j=1}^{n_p} (t_j - \mu_t)^2 c_j \cdot \Delta t}{\sum_{j=1}^{n_p} c_j \cdot \Delta t}$$

(12)

With HETP and $N$ defined, axial dispersion can now be calculated [Seidel-Morgenstern95]:

$$D_{ax} = \frac{u_{int} \cdot \text{HETP}}{2}$$

(13)

With all of the above information, a chromatographic column and subsequent chromatograms can be characterized.
2. Models

Modeling of chromatographic columns becomes necessary to effectively understand the inherent processes. Assumptions made are: packed bed is homogeneous and composed of spherical particles; fluid density and viscosity are constant; radial distributions are negligible; the process is isothermal; the eluent is inert; no convection inside the particles; and size-exclusion effects are neglected. [Schmidt-Traub05]

To most accurately model a chromatographic process, a model would need to account for the effects of convection, dispersion, mass transfer of the eluent, pore diffusion, diffusion along surface of adsorbent, and adsorption kinetics [Schmidt-Traub05]; however only a very comprehensive model such as the general rate model uses all of these effects. Instead, simplification is used to produce models that have varying usages. One such simple model – the simplest, in fact – known as the ideal equilibrium model takes into account only convection and adsorption equilibrium and ignores the effects of axial dispersion, mass transfer, and kinetics.

\[
\frac{\partial c}{\partial t} + \frac{1 - \varepsilon_t}{\varepsilon_t} \frac{\partial q}{\partial t} + u_{int} \cdot \frac{\partial c}{\partial x} = 0
\]  \hspace{1cm} (14)

Models that account for only one band-broadening effect are a step above the ideal equilibrium model. The equilibrium dispersive model is an important member of this group and the primary focus of this discussion. Because all peak broadening effects are lumped into a single coefficient, the term accounting for axial dispersion (as well as mass transfer resistance) is best labeled as the apparent dispersion coefficient \(D_{app}\). With this as the only addition, the ideal model of chromatography becomes the equilibrium dispersive model:

\[
\frac{\partial c}{\partial t} + \frac{1 - \varepsilon_t}{\varepsilon_t} \frac{\partial q}{\partial t} + u_{int} \frac{\partial c}{\partial x} = \frac{\varepsilon_t}{\varepsilon_t} D_{app} \frac{\partial^2 c}{\partial x^2}
\]  \hspace{1cm} (15)

Other, more complex models offer greater accuracy; however, they are beyond the scope of this paper, as the computer simulation package used only deals with dispersion effects modeled by the equilibrium dispersive model.
3. Simulations

Simulating chromatographic processes using models like the ones described has become more feasible with the advent of more powerful computing systems. Today, accurate predictions of separations can be performed with little time or resources required. Particularly of interest for this paper, a computational fluid dynamics package called STAR-CD will be discussed.

STAR-CD simulations are composed of several components. The geometry and setting up of the problem is done with a graphical user interface (GUI) known as PRO-STAR. With this program, meshes of cells can be created along with boundary conditions. Physical data are also selected for molecules being used in the system. The solving of the system is done with a program called STAR. It is run by simply passing the setup from PRO-STAR to STAR and telling STAR how long to run or how many iterations to perform (depending on whether solving for a transient or steady-state solution). These three main steps – cell mesh building, physical data input, solving of system – obviously must be completed in order for the program to operate correctly.

STAR-CD does have some limitations; mainly, a user must define coding to allow for monitoring of the output cells to mimic a detector in an experimental setup. With recorded concentration and time data, a simulated chromatogram can be produced and thus studied in the same ways described in the previous section.

The number of cells used to create a model in STAR-CD is determined by the user; furthermore, a greater number of cells will result in a more defined model compared to a model with fewer cells. However, required computation goes up with higher cell count as well. This aspect of modeling has been researched and in a summary by [Su05], it is described how to calculate the minimum number of cells needed to model a real-world column accurately. An example of this increasing accuracy with increasing cell number is shown in Figure 5, where [Su05] demonstrates that as number of cells increases, the pressure drop calculated converges on an expected value.

![Figure 5](image)

[Su05] further determined that by setting the Peclet number equal to approximately 1, an optimal cell size could be determined:

$$p_{eul} = \frac{u \cdot \delta x}{\varepsilon D_{ax}}$$  \hspace{1cm} (16)

The quantity of interest is $\delta x$, which represents the width of each cell in the direction of fluid flow. Dividing the height of the column by $\delta x$ yields the number of cells needed.
4. Inhomogeneities

As mentioned previously, many things can affect a separation. Specifically, the packing of a column has significant impact on the resulting separations. Of interest to this work are inhomogeneities in a column’s packing. In practice, these problems in a packing occur through normal usage of a column. For instance, a common inhomogeneity is a cavity formed at the inlet of a column. This happens primarily because the pressure entering the column is sufficient to further compress the solid packing (settling also has an impact). The resulting chromatograms from a cavity exhibit broad peaks with sometimes extreme tailing. Channels can also form in a bad column packing often causing double peaks from a single sample due to the large variation in the time it takes to complete each path. These effects can be seen in Figure 6, where the first chromatogram pictured is a normal, well-packed column.

In industry and research, inhomogeneities greatly affect a column’s ability to separate samples, and thus they reduce the usefulness of using such a separation means. Early detection of such problems is critical to fixing problems before they hurt efficiency too much, but a fully developed theory of how inhomogeneities affect separations does not exist. The purpose of this work was to experimentally characterize HPLC column inhomogeneities and to begin preliminary simulations of HPLC columns. With a firm experimental basis, simulations are given credibility and thus can be used to quickly make changes in a column that would be inconvenient in physical experiments.
5. Experimental Procedure
In an effort to experimental characterize real world column inhomogeneities, a series of experiments were completed. Silica beads of particle diameter 40 to 70 µm were used as the packing material in a column produced by Merck. The fluid pump that was used was set by adjusting the stroke length in the range 0 to 15 mm. The spectrophotometer detector (wavelength 256 nm) was connected via a voltmeter to a computer running FlexPro-Control 5.0 to record output voltage as well as the in and out column temperatures and pressures. A picture of the experimental setup can be found below in Figure 7. In Figure 7, the column and injection loop are in the far left. In the middle are the detector and valve controller; and on the left is the outlet measurement valve and the lab computer. In Figure 8, a flow diagram of the experimental setup can be found.

To begin with, normal column packings were used. Normal, in this case, means that Merck’s recommendations for packing the column in the column operation manual were used. That is, the silica was poured into the empty column, filling it to within a few centimeters of the opening. The fluid pump was then started at a low flow rate (5 mm setting equivalent to a flow rate of 2.4 ml/s); this flow pushed the silica packing up through the column, so the pump was intermittently turned off to allow the packing to settle again. When the fluid reached the top of the silica packing, the pump was turned off and the top outlet frit was put into place and secured. Between 40 and 50 bar of pressure was then applied onto the column with a hydraulic pump. The frit holder was tightened and the height of the column was measured and recorded.

For running experiments, isopropanol was used as the fluid phase. For the single component sample, diethyl phthalate of varying concentrations in isopropanol (10, 20, 30, 40, and 50 g/L)
was used. The flow rate was measured by closing the recycle feed and opening the measurement valve during pump operation. The measurement valve was set up to drain into a graduated cylinder. With a stopwatch, the time necessary for 400 mL to accumulate (200 mL for 5 mm and 7 mm settings) was measured. After this, with the recycle valve open and the measurement valve closed. The detector readings were zeroed by pressing the “Zero” button. A sample was injected into the injected loop and the valve was opened to the column feed-through simultaneously as a new FlexPro run was started. The output was voltage of the detector was monitored and when it began to rise (the sample began elution), the recycle valve was closed and the waste valve was opened. The output drainage was allowed to flow into the waste container until elution had completed and the detector readings returned to their zero state. Only samples of concentration 20 g/L and 40 g/L were used for each pump setting; however, for the first few initial experiments, calibration tests were run using all concentrations at a pump setting of 7 mm.

After a suitable number of such normal packing experiments, packing of the column with inhomogeneities was begun. The first deliberate faults used were custom manufactured balls. The balls were produced out of aluminum oxide foam to have a specific porosity. For their use, the packing process went as normal except with a few additional steps. The ball was degassed with isopropanol in an ultrasonic water bath until bubbles no longer appeared (roughly 10 minutes). The ball was placed into the column after half of the silica had been poured in. Its location was adjusted to be directly in the center, radially and vertically, and its height from the top of the column was measured. After the rest of the silica packing was poured into the column, the packing procedure as outlined above was followed. Calibrations were not performed, but the same pump flow settings and concentrations were tested.
Accordingly, another form of inhomogeneity, a cavity in the packing, was also tested. For the experiments, the steps were also similar to a normal packing. The silica was poured into the column up to a desired height from the top. The height difference from the top of the silica to the top of the column denoted the cavity height. Using the process described previously, fluid was allowed to reach the top of the packing and the top outlet frit was installed. Here, the only difference is that the packing was not pressurized. The pump was turned on for 10 minutes to give ample time for the packing to stabilize to the fluid pressure. After this, the experiments were completed as above with the exception that a 12 mm pump setting was not tested to prevent damaged to the inlet frit. Measurements were recorded with their cavity height. Cavities of 4.5 cm, 4 cm, 2 cm, and 1 cm were each tested.

6. Experimental Results
Figures 9, 10, and 11 show example chromatograms with concentrations of 20 g/L and 40 g/L from the three column packing types. Each was measured with the same flow rate (a setting of 10 mm on the pump).
HETP, porosity, pressure drop, and axial dispersion were calculated for each sample run. The results are summarized below for each type of column packed. All values are for only a sample with concentration 20 g/L.
Figure 12. HETP

Figure 13. Porosity
Figure 14. Pressure Drop

Figure 15. Axial Dispersion
7. Simulation Procedure
Preliminary simulations to model a normal column began with a simple one-dimensional case. Using STAR-CD tutorials and the work of [Su05], an initial procedure was created. The description that follows is very general and only includes major steps performed as ample documentation for STAR-CD already exists that describes how to complete each of the steps. First, a cell mesh was created with only one cell in the X and Y directions and 100 cells in the Z direction. This effectively made the mesh one-dimensional. The scale used for the mesh created a simulated column of height .33 m and diameter .1 m. Next, boundaries were created. The inlet and outlet were assigned to the first and last cells, respectively. All cell exterior cell faces not lying in the XY plane were added to the symmetry plane. Next, boundary conditions such as flow velocity (the equivalent of a 7 mm flow rate setting from the experiments above was used) were applied. Physical properties of isopropanol were also input as the only fluid eluent. A steady-state solution was then solved to find the velocity and pressure profiles that would be used in the transient solution. After completion of the steady-state solution, a boundary condition with a molar concentration of .4 diethyl phthalate was imposed. User coding provided from [Su05] was implemented to allow for monitoring of the output concentration at the outlet. This mimicked a detector at the outlet of a real column. The length of the simulation was set to 1 second. The solution from the previous steady-state run was used as a restart file. This effectively modeled an injection into the column. After the 1 second simulation, the inlet boundary condition’s concentration was changed back to 0. The simulation was then run for 1300 seconds to allow for complete elution of the injected sample. At the end of this time, the output of the user coding was opened and recorded into a Microsoft Excel spreadsheet.

8. Simulation Results
The chromatogram produced from the simulation above is shown below in Figure 16.
9. Conclusions and Recommendations

Cleary, inhomogeneities in a column’s packing greatly affect its separation properties. As can be seen, HETP and axial dispersion both increase when an inhomogeneity is introduced. Furthermore, the more pronounced the inhomogeneity, the greater the affect on separation. From the simulated column, one can see that producing chromatograms entirely from a simulation produces an accurate, adjustable, and quick method for studying variables related to the column. One recommendation for further research is to complete more experiments on inhomogeneities. This should include further testing of the cavity and ball, as well as other untested inhomogeneities. With these data, simulations that incorporate inhomogeneities can be completed and validated.

A further outlook includes research into pattern recognition with the goal of being able to analyze a series of chromatograms produced by the same column and detect small changes that indicate a specific inhomogeneity is forming.
10. References

