

# How Viscous Fingering Can Spoil Your Separation

## And You May Not Even Suspect It

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**T**echniques of both analytical and preparative liquid chromatography have advanced substantially in recent years. Nowadays, samples are often analyzed or purified in systems that incorporate one or more flow-stream changes from one mobile phase to another. It happens, for example, in column switching, in multidimensional separations, and in simulated moving bed (SMB) chromatography, where a feedstock stream enters continuously into a mobile-phase stream.

When flow-stream switching is necessary, care must be paid to the compatibility of the different flow solutions. Obviously, the streams must be miscible, but it is less obvious that their viscosities should be similar. An important consideration is the solvent in which a sample is dissolved before injection. Usually that solvent is the

same as the mobile phase, but sometimes a solute must be dissolved in a stronger solvent with a different viscosity. In some instances a mismatch between mobile phase and solute plug, or mobile phases in two-dimensional (2D) separations, or feedstock and mobile phase in SMB, leads to viscosity differences large enough to cause a phenomenon known as *viscous fingering* (1–3). Occurrence of viscous fingering (VF) can have a catastrophic effect on separation performance, leading to separation failure (4).

In short, *viscous fingering* is a flow instability phenomenon that occurs at the interface of two fluids of differing viscosities. When a high-viscosity fluid is displaced by a lower viscosity fluid, their interface is unstable. After a time (depending on the viscosity contrast), the lower viscosity fluid penetrates the other in a pattern resembling a set of fingers. Those “fingers” multiply and develop further into a complex network (1–10). VF can be detrimental to chromatographic separations when a low-viscosity mobile phase fingers into a high-viscosity solute plug (2), or conversely, when a low-viscosity sample plug fingers into a higher viscosity mobile phase (1). Similar adverse effects take place when a mobile-phase stream is replaced with one of different viscosity in the complex schemes now developed for 2D chromatography (3).

Analysis of polymers and isolation of proteins using size-exclusion chromatography (SEC) present ideal



In an extreme case of VF, the band width expands to the column length

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environments for development of VF. Solutions of high molecular weight polymers and proteins are more viscous than their mobile phases and do not dilute appreciably during rapid elution (4). When proteins are isolated at the preparative level, sample solutions have a high viscosity for exactly the same reasons as polymer solutions. In a worst-case scenario, a set of multiple peaks instead of a

**PRODUCT FOCUS:** BIOPHARMACEUTICALS

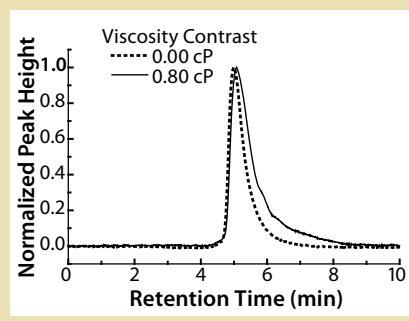
**PROCESS FOCUS:** DOWNSTREAM PROCESSING

**WHO SHOULD READ:** PROCESS DEVELOPMENT AND MANUFACTURING, SEPARATION SCIENTISTS

**KEYWORDS:** VISCIOUS FINGERING, MOBILE PHASE, STATIONARY PHASE, ANALYTICAL AND PREPARATIVE LIQUID CHROMATOGRAPHY, MULTIDIMENSIONAL CHROMATOGRAPHY, VISCOSITY

**LEVEL:** INTERMEDIATE

**Figure 1:** Chromatograms illustrating the relative change in band shape for solute plugs, where viscosity of the solute plug matches exactly the viscosity of the mobile phase (dotted line); and viscosity of the solute plug is 0.80 cP (centipoise) higher than the viscosity of the mobile phase.



single peak (8) may be observed. At the least, VF will alter band profiles and may cause excessive band broadening during separation or purification of solutions of polymers and proteins (11, 12).

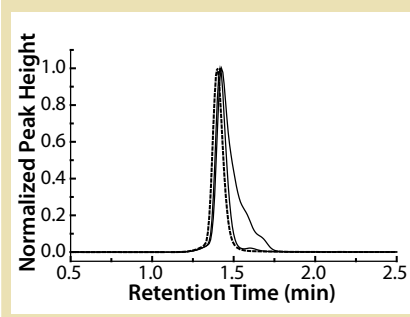
According to Moore, VF will generally be observed in SEC when the product of sample volume, the intrinsic viscosity of the sample, and sample concentration exceed 0.05–0.10 mL (11). Other authors claim (without robust evidence) that a significant loss of efficiency and random peak deformation may occur when the viscosity difference between the solute plug and the mobile phase is greater than 10% (4). In previous work we have shown that the onset of viscous fingering depends on whether the sample plug has a higher or lower viscosity than the mobile phase (1, 2): VF is more visually apparent if the sample plug viscosity is lower. Even if that viscosity contrast is insufficient to cause spectacular VF, small differences between viscosities are sufficient to generate changes in band shape, which can affect separation performance and quantification.

We believe that the VF phenomenon has largely been under- or misreported. In this article we present a number of VF scenarios in the hope that separation scientists will begin to understand its significance and perhaps its widespread nature.

## EXPERIMENTAL

**Chemicals and Reagents:** High-performance liquid chromatography (HPLC)–grade dichloromethane and

**Figure 2:** Chromatograms illustrating the relative change in band shape for solute plugs, where viscosity of the solute plug matches exactly the viscosity of the mobile phase (dotted line); and viscosity of the solute plug is 0.32 cP higher than the viscosity of the mobile phase (solid line). The small peak trailing the band is a result of the viscous fingering phenomenon.



toluene used for the VF experiments were purchased from LabScan (Lomb Scientific, Sydney, NSW, Australia; [www.lomb.com.au](http://www.lomb.com.au)). The solute-plug and mobile-phase viscosities were modified with the addition of cyclohexanol (Ajax Chemicals Sydney, NSW, Australia; [www.ajaxfinechem.com](http://www.ajaxfinechem.com)). Viscous fingering was visualized using Oil Red O dye purchased from Sigma-Aldrich (St. Louis, MO; [www.sigmaaldrich.com](http://www.sigmaaldrich.com)). Nucleosil 10- $\mu$ m ODS (2) stationary phase used to pack the glass columns was purchased from Alltech Associates (Deerfield, IL; [www.alltechweb.com/US/Home.asp](http://www.alltechweb.com/US/Home.asp)) and used as received.

Under the selected experimental conditions, the dye is not retained, so the injected band behaves as a tracer compound. Reagent-grade carbon tetrachloride used for visualizing the wall effect was purchased from Sigma-Aldrich ([www.sigmaaldrich.com/Area\\_of\\_Interest/Biochemicals.html](http://www.sigmaaldrich.com/Area_of_Interest/Biochemicals.html)). Care must be exercised when handling carbon tetrachloride because of its toxic and carcinogenic effects. All work was performed in a well-ventilated hood, and protective precautions were taken as prescribed by the material safety data sheet (MSDS). Iodine (99.9%) was obtained from General Chemical Division (New York, NY).

**Equipment:** The chromatographic system consisted of a Waters model 6000 HPLC pump (Milford, MA; [www.waters.com](http://www.waters.com)) used to deliver the

**Photo 1:** Showing the clarity of packed columns as evidenced by the frit, when the refractive indices of the mobile phase and stationary phase match exactly. Stationary phase: Nucleosil C18, particle size 10  $\mu$ m. Mobile phase: dichloromethane/toluene/cyclohexanol (14%/17.2%/68.8%).



mobile phase at the prescribed flow rates as noted in the figure captions. Sample injection was achieved using a Valco VICI EHMA ([www.vici.com](http://www.vici.com)) six-port, two-position switching valve with a 200- $\mu$ L injection loop.

The glass column was housed in a rectangular reservoir filled with dichloromethane to remove the cylindrical lens effect caused by the column curvature. The reservoir has four glass windows, two lit with fluorescent lights (15 watt) and a third used for "on-column" visualization, recorded using a Pentax ZXM SLR 35-mm camera fitted with a Tamaron 90-mm macro lens. For all experiments, a shutter speed of 125 and aperture of 8 were used. The film was Kodak professional PORTRA 160VC. It was developed by a commercial photographic processor (Paxton, Parramatta, NSW, Australia). The remaining window could be looked through to observe the solute plug as it passed along the column, and video was sometimes recorded. A GBC LC 1200 UV–vis detector ([www.gbcs.com](http://www.gbcs.com)) set at 575 nm was used for conventional postcolumn detection. Postcolumn detection responses were collected on a Lawsons lab A/D converter set at 2 Hz ([www.lawsonlabs.com](http://www.lawsonlabs.com)).

Chromatographic columns used in this study were packed in a 17 mm i.d. borosilicate (Pyrex) glass tube (Upchurch Scientific, [www.upchurch.com](http://www.upchurch.com)) with end fittings made from Delrin plastic by the University of Tennessee workshop (USA). The fittings include a fixed length outlet fitting and adjustable inlet fitting, which allow for the axial compression of the stationary-phase bed.

**Photo 2 (below, left):** Illustrating a normal band profile where the viscosities of the injection plug and mobile phase (0.38 cP) are exactly the same.



**Photo 3 (at right):** Illustrating a 10- $\mu$ L injection of an iodine solution in carbon tetrachloride injected against the wall of a chromatography column. Viscosities of the mobile phase (carbon tetrachloride) and solute plug were matched exactly. (Left) Initial injection, time = 0; (right) time = 3.00 min. Note the two wall effects. The first one leads to a very high rate of migration of the band in the region in the immediate vicinity of the column wall, the second to an increasing rate of migration of the band with increasing distance from the wall.



### Column Packing Procedure:

Chromatographic columns were slurry packed in a downward configuration, and the empty column was filled with dichloromethane as a displacement solvent. A slurry of packing material and methanol was pushed into the column and consolidated by a steady stream of methanol at 15 mL/min. The bed was then subjected to axial compression by mechanically applying a piston stress equivalent to a pressure of 48 kg/cm<sup>2</sup>, with both ends of the column open to allow a free flow of solvent within the bed.

The compression piston was then removed, and the adjustable inlet fitting was tightened into place. Sample injection in the vicinity of the wall was achieved using a modification of the central point method previously described (13). A needle was inserted in the inlet frit and then bent at an angle so that sample could be loaded at the desired radial location. This head fitting was then inserted into the bed, with minimal disturbance to the column inlet.

**Image Analysis:** Image analysis has previously been discussed in detail (1, 2, 9, 13) and is beyond the scope of this article.

**Measuring the Solute-Plug-Solvent-Mixture Viscosity:** The viscosities of solute plug and solvent mixtures were measured at various

compositions, using an Ubbelohde glass capillary viscometer type OC. In each case, the mixture was allowed to equilibrate in a 25 °C water bath for at least one hour before measuring its viscosity. All measurements were made in triplicate, and the viscometer was calibrated using toluene.

### RESULTS AND DISCUSSION

Chromatographers undertake separations using systems that rarely, if ever, allow us to look inside a chromatographic column. Therefore, almost everything we know about a separation is gauged from a postcolumn detection record. When separations are performing well, there is no concern. When for apparently inexplicable reasons separations fail, deducing the nature of the problem from postcolumn detection records can often be difficult.

For example, in Figure 1 the poor band profile in the chromatogram could be caused by a column that has exceeded its useful lifespan, or maybe by an incorrectly fitted piece of HPLC tubing that leaves a void in the system. The solution to solving this problem may therefore very well be to replace the column or check each ferrule. But if that is done, at least in this case, the same result would occur when the separation is repeated. Thus the problem is not solved, and failure comes at the cost

of a new column. Whether that column is commercially purchased or made in-house, the cost is often too high for such action to be repeated without consideration of other possible reasons for the problem.

The postcolumn chromatographic record shown in Figure 2 looks entirely ordinary. If such a chromatogram were to be recorded during analysis of a raw material, the small peak trailing the larger one would perhaps be reported as an impurity. It is difficult to believe that the small band is actually the result of the VF phenomenon and, in fact, contains exactly the same component as the main band. Imagine that the main band was the limiting impurity in a preparative-scale isolation. The small trailing peak might then coelute with the target compound and contaminate the product. Without knowledge of viscous fingering, how would any analyst be reasonably able to suspect the problem?

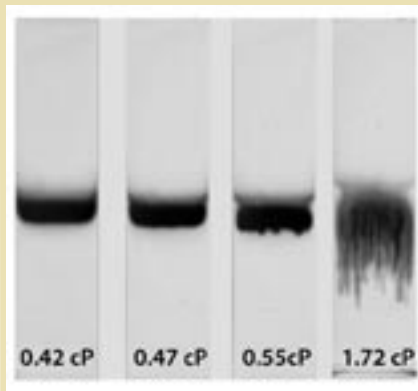
Being able to see inside a chromatographic column can allow these complex phenomena to be understood through visual, real-time interpretation of a separation process. The ability to see inside a column necessitates that both column container and stationary phase be transparent. One simple way to see through a column container is to use a glass tube. Seeing through the usually opaque stationary phase is often more difficult, but if the stationary and mobile phases have exactly the same refractive indices, then the stationary phase becomes entirely transparent. The migration of the band of a colored solute injected onto the column can then be visually monitored, and a series of photographs can be recorded and analyzed to give quantitative information about the migration of the solute band.

In the present study, mobile phases consisting of mixtures of dichloromethane and toluene were used for matching the refractive index of the mobile phase to that of the C18 stationary phase. Under such conditions, the bed is transparent as shown by the photograph of the frit at

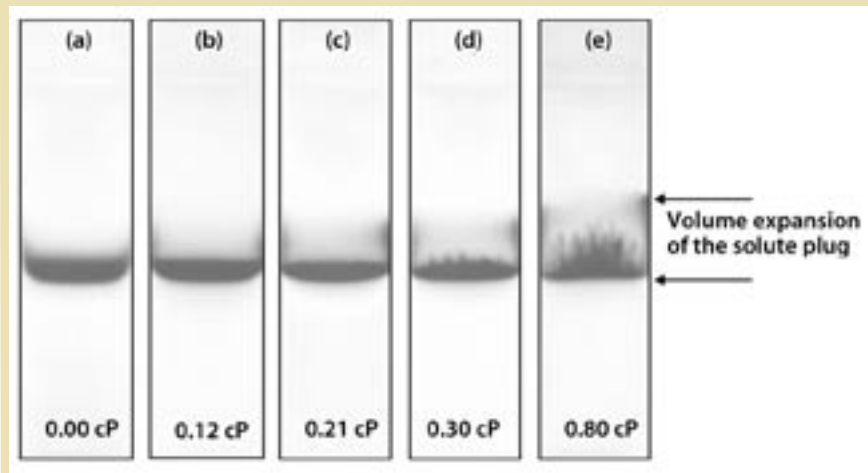
the column outlet in Photo 1.

Injection of a dye allows visualization of the solute plug (Photo 2). The dye sample migrated along the bed in an idealized way associated with pressure-driven flow. However, even this idealized plug flow comes with complications. Because we can see inside the column, we see nonideal flow behavior such as the wall effect, as is illustrated in Photo 3. This example shows two significant regions of flow. In the immediate vicinity of the wall, the solute plug migrates with its highest velocity because the particles cannot pack closely against the rigid, uniform wall; hence, the void fraction in this region is greatest. Away from the wall, the central region of the column's solute velocity is less densely packed than the region near the wall;

**Photo 4:** Illustrating viscous fingering when the viscosity of a solute injection plug is less than the mobile phase. Solute injection plug viscosity was 0.42 cP; the mobile phase viscosity is noted below each photograph.



**Photo 5:** Illustrating the change in the band profile as a function of the viscosity contrast between the mobile phase and the solute injection plug. The mobile phase viscosity was constant at 0.4 cP. The solute plug becomes progressively more viscous as indicated by the stated viscosity contrast on each photograph.



thus, the apparent idealized pressure-driven plug flow is not what is seen inside a chromatography column.

Photos 2 and 3 were recorded in systems in which viscosities of the mobile phase and the solute plug were matched exactly. A viscosity contrast, however, makes the pressure-driven flow much more complex. The series of images shown in Photo 4 illustrate how the shapes of the plugs change as the mobile-phase viscosity increases relative to that of the injection plug. First, more and more spectacular VF effects develop. Then as the viscosity contrast increases, the solute in the plug is pushed away from the wall by the more viscous mobile phase, which elutes faster in the region of the column with the highest permeability; that is, in the immediate vicinity of the wall. The plug develops nodes on its leading edge, and when the viscosity contrast is sufficiently high, those "fingers" propagate. The severity of the fingering effect increases with increasing viscosity contrast. The postcolumn response of a UV detector is shown in Figure 3 for the sample illustrated in Photo 4.

If viscosity of an injection plug is greater than that of the mobile phase, fingers develop from the rear instead of propagating forward. Photo 5 illustrates this effect. In our series of photographs, the leading edge of the plug flattens, but the peak volume increases as the trailing section develops in exactly the reverse process

to the forward fingering situation. Mobile phase fingers cannot pierce through the solute band, however, because the same mobile phase is ahead of the band. So those fingers merely broaden and swell the band volume to an important extent. Obviously, the effect on separation performance could be dramatic. An example of this VF phenomenon is illustrated by the chromatogram shown in Figure 1.

## PROTECT YOUR SEPARATION PERFORMANCE

HPLC practitioners should take all possible care to avoid a mismatch in viscosities between the solute injection plug and the mobile phase in 1D chromatography and between the two mobile phases used in the different dimensions of a 2D system. This is the simplest solution to the problem of viscous fingering. However, sometimes to gain the required degree of selectivity difference between the dimensions in a 2D system, using two phases with markedly different viscosities will be unavoidable. Likewise, proteins and polymers will affect the viscosity of injection plugs. A high solute concentration will do so as well. When there is a viscosity mismatch and chromatographic performance is poor, every effort should be made to overcome the problem and, with knowledge of the VF phenomena, to find a suitable alternative approach. This may be as simple as decreasing the sample load, which, if VF is the actual source of the problem, will easily restore separation performance.

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**Figure 3:** Chromatograms illustrating the effect of viscous fingering on the elution band profile. Curve (a) in each represents the elution profile of a band where the viscosities of the injection plug and mobile phase are matched. Curve (b) in each represents the elution profile of a band where the viscosity of the injection plug (0.38 cP) was lower than the mobile phase: Viscosity contrasts were (A) 0.05 cP, (B) 0.13 cP, (C) 1.30 cP.

