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Sample introduction for high performance separations

R. Andrew Shalliker [a,b,*], Jörgen Samuelsson [b], Torgny Fornstedt [b]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Science and Health, University of Western Sydney, North Parramatta, NSW 2150, Australia
[b] Department of Engineering and Chemical Sciences, INTERACT, Karlstad University, SE-651 88 Karlstad, Sweden

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ABSTRACT

In this review we discuss how viscosity contrasts between the injection plug and the mobile phase may lead to loss in separation performance, especially in UHPLC columns or SFC environments. Firstly, the wall effect is discussed, and how it can amplify viscosity contrast effects. We then illustrate how viscosity contrasts lead to the phenomenon known as viscous fingering, and we detail the destructive effects of this phenomenon. We expand on the viscous fingering component, however, demonstrating that viscosity contrast effects begin to deteriorate performance long before the conditions are such that viscous fingering occurs. Subtle changes in band-shape are apparent even with very low viscosity contrasts. Lastly we illustrate how viscosity contrast effects lead to severe peak distortions in SFC. Analysts who seek high efficiency separations must make every effort to eliminate, or at least minimise the viscosity contrast between the injection plug and the mobile phase.

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1. Introduction

Modern liquid chromatography, in either the guise of ultra-high performance liquid chromatography (UHPLC) or even HPLC itself, is nowadays so efficient that great care must be exercised to utilise the full potential of the chromatography column. This is especially so for sub-two micron particle packed columns, which are necessarily operated at high pressure. Complex adsorption dependence on the pressure at UHPLC conditions has recently been investigated and reported [1,2]. Earlier, even for ordinary HPLC systems, so called "pressure jumps" have been reported due to the injection valve switching [3]; it is easy to imagine that such effects might be even more important in UHPLC conditions. Thus, extra care must be taken on all instrumental aspects as the trend in chromatographic separations is moving towards higher throughput, efficiency and pressures [4].

Achieving high efficiency means that aspects, such as, extra column dead volume must be minimised, both prior to and after the column, and that any form of solvent mixing prior to the injector is done so efficiently with minimal gradient delay. Further, a substantial effort must be made to undertake properly the injection process itself so that the sample enters the column in the most efficient manner as is feasible. If care is not exercised in the attention to these details the highly efficient modern day column will not achieve the separation potential for which it was designed. It is the aim of this review paper to highlight an important aspect associated with sample introduction, that is, the mismatch in solvent viscosity between the injection plug and the mobile phase. We

* Corresponding author. Tel.: +61 2 96859951; Fax: +61 2 96859915.
E-mail address: r.shalliker@uws.edu.au (R.A. Shalliker).

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believe that now, more than ever before, the deleterious effects of poor sample introduction techniques on the modern column have a more serious consequence on separation performance than for the larger particle size, less efficient columns. Further, most recently, supercritical fluid chromatography (SFC) has undoubtedly established itself as one of the prime chromatographic techniques and has been incorporated into mainstream separation laboratories [5–8], a greater appreciation of the solvent injection process in modern SFC is also considered here, and discussed in this review.

Before we can discuss aspects of separation efficiency and solvent mismatch between injection plug and mobile phase we must first recognise the significance of wall effects, because wall effects greatly influence the displacement of an injection profile onto the column inlet. Later, the relationship between viscosity mismatch and wall effects will become clearer.

2. Wall effects in particle packed columns

Throughout the course of the development of HPLC or UHPLC, column technology has continually advanced. Particles sizes have systematically decreased and the dispersion of band profiles throughout the migration process has decreased. Nevertheless, for various reasons the packing density of particle packed columns is heterogeneous, both in the axial and radial directions [9–18]. This has been well understood since the first days that the modern column was developed [9], so there is no doubt as to the fact that uniform beds are not attained during the packing process. The most important consideration with respect to column bed heterogeneity is the variation in packing density in the radial direction, especially near the wall region. This is more important than the axial direction since variations in the radial direction result in changes in the velocity of the fluid flow as a function of the radial location [19]. As a result, the injection plug shape is not perfectly rectangular [19–21]; the radial central region of the bed is usually less densely packed than near the walls and as a consequence the fluid flow through the radial central region of the bed is faster than near the wall [19]. Hence the flow profile exhibits a parabolic-like shape [19]. A special case exists at the wall; neither the particles nor the wall itself can bend to accommodate the other and as a consequence the void space in this region of the column is at its highest, hence the permeability is at its highest [19]. At the same time, the packing density of the column is at its highest near the wall – note the distinction between 'at' the wall and 'near' the wall, so, near the wall the permeability is at its lowest [19]. These wall effects were discussed in detail by Shalliker et al. [19] and they have important consequences as to how an injection plug traverses a bed when there are viscosity differences between the injection plug and the mobile phase, which will become clearer in discussion that follows. An illustration of the bed heterogeneity is shown in the series of photographs in Fig. 1, where the migration of iodine in the immediate vicinity of the wall is detailed [19].

The photographs in Fig. 1 were recorded using the matched refractive index visualisation process developed by Shalliker et al. [19]. In this particular study a sample of iodine was injected at the wall, and as time passes it is apparent that at the wall the flow velocity is highest, while near the wall the flow velocity is lowest. Then the flow velocity increases systematically towards the radial centre of the column. An understanding of these aspects of column bed heterogeneity is important for understanding the context of viscosity differences between the injection plug and the mobile phase, and for that reason we will refer back to column bed heterogeneity after we next discuss phenomena related to the mismatch in viscosity between the injection plug and the mobile phase, starting firstly with a discussion on viscous fingering.

3. Viscosity contrasts and viscous fingering

Viscous fingering (VF) is a well-known flow instability phenomenon that could be detrimental to the separation, but it is fairly unfamiliar amongst practitioners of liquid chromatography [22]. Viscous fingering or Saffman Taylor instability [23] may occur at the interface between two fluids with different viscosity. Similar phenomena could also occur for density differences, however, the density contribution is usually negligible and is therefore not covered here,

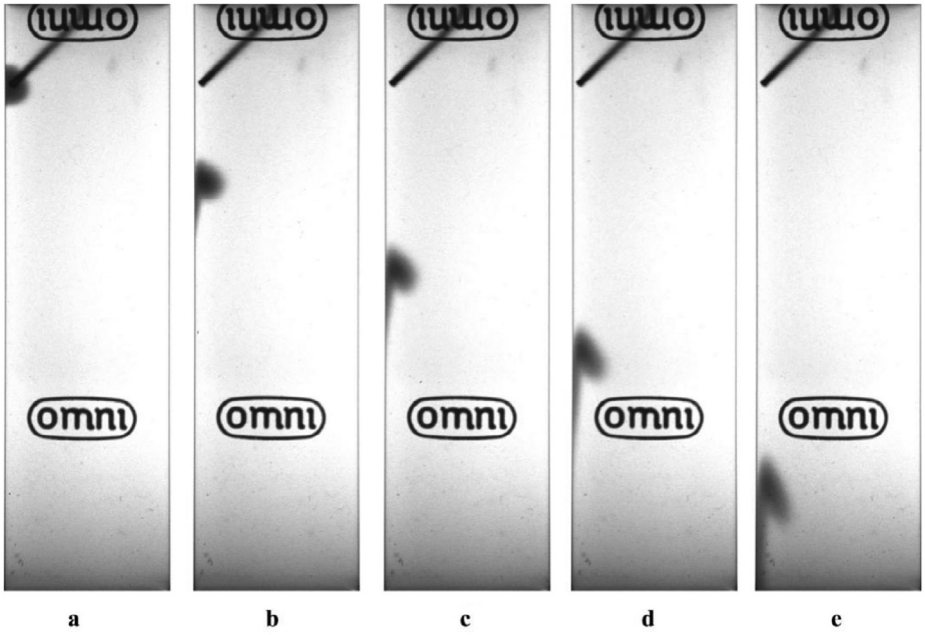

Fig. 1. Photograph of a 10 μL solution of iodine migrating along a chromatography column following an injection at the wall. Flow rate: 1/5 mL/min: (a) initial injection, time = 0, (b) time = 1.00 min., (c) time = 2.00 min., (d) time = 3.00 min., (e) time = 4.60 min. Note the two wall effects. The first leads to a very high flow velocity of the solute at the wall, the second to a decreasing flow velocity with increasing distance from the column radial centre. *Reprinted from Ref* [19]. *Copyright 2000, with permission from Elsevier.*