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Viscosity contrast effects in analytical scale chromatography - Evidence and impact

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ABSTRACT

The phenomenon known as viscous fingering has been shown to be very detrimental to separation performance in preparative and size exclusion chromatography, and also in multidimensional HPLC. However, there are few reports of viscous fingering in analytical scale high performance liquid chromatography (HPLC), despite samples very often being introduced to the analytical-size HPLC columns in a solvent have a substantially different viscosity than the mobile phase. With this study we aim at first hand to investigate if viscous fingering or any similar and related effects to these in preparative levels also take place in analytical scale HPLC and if so, what impact this have on the separation performance. We could show that not only viscous finger does occur in analytical scale columns but also that peak distortions are apparent already at viscosity ratios between eluent and sample solution approaching unity. The latter indicates that a pre-viscous phenomenon is occurring that could be more important than the viscous fingering itself at the analytical format. As the viscosity contrast increases, the leading edge of the sample band distorts and the band volume increases, both leading to a decrease in performance.
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1. Introduction

The widespread use of High performance liquid chromatography (HPLC) its successful trend towards higher pressures has come about because the science behind the technique is largely well understood and the technology that supports applications is continually being developed. HPLC are relatively straightforward; the sample is prepared by dissolution of the sample components in a solvent compatible with the mobile phase and then injected. However, this is not always the case since the sample may be difficult to dissolve in a solvent similar to the mobile phase due to the variety of components present in the sample. Sometimes the nature of the sample solvent, with respect to the properties of the mobile phase is often overlooked, yet it is in fact critically important. Ideally this solvent should have a solvent strength approximately equal to the mobile phase to avoid premature elution of any weakly retained species. Also, especially for analytes that are proteolytic a pH mismatch between the sample solution and mobile phase could lead to severe peak deformations [1–3]. Usually, when the sample contains just a few similar analyte components, dissolution of the sample in a solvent similar to the mobile phase is not difficult, but when the sample is more complex, and contains components with a wide range in solubilities, dissolution of the sample in the mobile phase may be difficult.

When initiating sample dissolution, sometimes the sample solvent has a different viscosity to the mobile phase, and if there is a mismatch a phenomenon known as viscous fingering or Saffman Taylor instability may occur [4]. Viscous fingering in chromatography is often present in preparative HPLC where the injection volumes usually are large and sample solvent is selected to maximize the solubility or in size exclusion chromatography where the viscosity of the sample is the limiting factor [5]. Viscous fingering is not only present in chromatography but also as well in many other processes that involve the flow of fluids through porous media e.g. river systems, underground water tables, hydrology and filtration, crude oil recovery from oilfields, and the irregular fingers of magma found in coal deposits [6–8]. Density difference between sample solvent and mobile phase could also result in flow instability similar to viscous fingering [6], but in pressurised beds, especially for columns in vertical alignment 'density fingering' effects are expected to be small in comparison to viscous fingering effects. As such, the density effect was not investigated in this study.

Despite the wide knowledge of viscous fingering in chemical engineering the HPLC community is largely ignorant of the phenomenon, probably since the phenomenon is difficult to observe; HPLC columns are generally made in stainless steel tubes, or from other non-transparent materials. Hence observation of the effect is generally possible only in an "indirect way", through a thorough understanding of the chromatographic traces. De Malsche et al. studied viscous fingering processes in perfectly ordered micro-pillar array columns instead of in the traditionally employed packed beds [9]. Their micro-pillar beds had a flat rectangular cross-section similar to classical Hele-Shaw cells. To

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visualise the viscous fingering process the cells were formed as a sandwich between Pyrex glasses. In cylindrical columns formats, containing a particle packed stationary phase, visualisation of the viscous fingering phenomena is more difficult, even for those well versed in the phenomenon. Fernandez and co-workers used NMR to visualise the effect in columns packed in plastic tubes [10–12], and then with a greater degree of visual clarity, Shalliker, et al. [13–17] used a matched refractive index technique that employed glass columns packed with C-18 silica. In these studies by Shalliker et al. [13–17] the mobile phase had the same refractive index as the C-18 silica; hence, the otherwise opaque column bed became perfectly transparent. The viscosity between the injection plug and the mobile phase could be adjusted and the viscous fingering effect visualised either with the aid of coloured samples or by injection of a solvent with a different refractive index to the mobile phase. Photographic equipment was used to record the migration process [13–17].

In the work undertaken by Shalliker et al., in references [15,16], a new viscosity contrast phenomenon was discovered, which was distinctly different to the viscous fingering effects, but influenced the geometrical distribution of the fingering pattern inside the column. The authors coined the term 'pre-viscous fingering' to describe this phenomenon. In short, the column bed heterogeneity in the region of the column wall [18] influenced the distribution of the high and the low viscosity components of the system – even for miscible solvents, such that the higher viscosity solvent penetrated preferentially into the higher void space along the column wall, and the lower viscosity solvent migrated into the region of higher packing density near the wall so as to facilitate this distribution process. Such an effect is unique to packed chromatography columns because of the geometrical constraints of the column design, i.e., a wall-packing interface and a heterogeneous, but uniform bed structure. This is not the case in Hele-Shaw cells. An important aspect of the viscous fingering phenomenon is that the interface between the solvents with different viscosities remains stable until a critical solvent ratio is reached, however, this interface is in fact destabilised by the pre-viscous fingering effect which occurs at very small viscosity contrasts; not in so much as fingers develop, rather, the leading and trailing edges of the solvent injection plug distort in alignment with the heterogeneous packing structure. This influenced the column efficiency, even though peak profiles showed no evidence of distortion – they simply broadened or compressed. Martin et al. recently summarized the state of the art of numerical calculations of viscous fingering in chromatography; here they theoretically demonstrated how the viscous fingering phenomenon develops inside the column and how it is ultimately manifested in a chromatogram [19]. Martin and his co-workers also studied the combined influences of viscous fingering and solvent effects between eluent and sample solvent on the peak shape for retained analytes using computational approaches [20–22], however, they did not account for the 'pre-viscous fingering' effects that occur even at very low viscosity contrasts.

While to date almost all studies on viscous fingering have been undertaken in column formats larger than analytical scale HPLC. However, recently Shalliker and Guiochon discussed that the viscous fingering phenomenon could be a possible and important effects also in analytical size separations [23] especially in 2DHPLC where the transport volume between dimensions is larger than the injection plug in regular HPLC [23,24]; the injection plug volume is important since the viscous fingering effect is enhanced at larger injection volume and on larger format I.D. columns. In their study on analytical scale, they reported changes in band shapes as a function of viscosity contrasts using HPLC columns made in stainless steel. They observed that viscosity contrast effects were important even when the retention factor of the solute was 5, and elution occurs much later than the injection plug. This was not surprising since visualisation of the viscous/pre-viscous fingering phenomena showed that the distortion of the band plug, and/or the development of the fingers occur the instant the sample enters the column, hence, even if strongly retained, the solute migration path has been altered as a consequence of the viscosity contrast, even if full blown viscous fingering is not established and only pre-viscous effects are apparent.

While Shalliker and Guiochon reported loss in efficiency as a result of "viscosity contrast" effects, they did not observe any peak splitting or serious distortion of bands, typical of viscous fingering, rather, peaks were broadened and efficiency generally decreased. With the knowledge that we now have at hand we suspect that pre-viscous fingering effects actually dominated their observed behaviour, rather than fully developed viscous fingering. Perhaps it is these combined effects that explains why the viscous fingering phenomenon is not well recognised amongst the HPLC community, changes may be subtle and unnoticed when separations are undertaken especially for complex samples where the injection solvent/mobile phase solvent mismatch is most likely to be most substantial. Many users may utilise HPLC for analyses of complex samples, but gradient elution may be employed and the effect is then further hidden, or their separation requirements may be simple requiring relatively low efficiency. On the other hand perhaps viscous fingering or viscosity contrast effects do not indeed occur or is strongly reduced in analytical scale columns since the effect is stabilised and fingers cannot fully develop and then propagate, although theory predicts that viscous fingering will occur in analytical scale columns also [25].

The aim of this study is to investigate if and then how viscous fingering viscous fingering and or pre-viscous fingering is manifested in standard analytical HPLC column formats, and to elaborate further on the impact of column performance when operated under conditions where there exist a viscosity contrast between the mobile phase and the solute injection solvent. For this purpose we explored systematic experiments with sample injections with different sample eluent viscosity mismatches in analytical scale HPLC columns using viscosity contrast solvent systems. The HPLC columns were made of glass and packed with C-18 silica. As well the solvents having a viscosity contrast, they also had the exact same refractive index as the stationary phase, and hence the otherwise opaque bed was perfectly transparent to the eye.

2. Experimental

2.1. Columns and reagents

Analytical scale (5 mm, I.D.) and a HR glass columns were supplied by Pharmacia (Sweden, Uppsala) and a 17 mm I.D. column, the glass tube were supplied from Omnifit with in-house made end-fittings. These columns were packed using an axial compression process consistent with the manufacturer's guidelines to a packed bed height of 5.4 cm for the narrow column (5 mm I.D.) and 6 cm for the 17 mm I.D. column. The packing material was Kromasil-100-5-C-18 (5 μm dp) AkzoNobel (Sweden, Bohus). The stationary phase was dispersed in acetone, stirred for 10 min, then ultra-sonicated for a further 10 min, prior to being poured into the column blank. Once the stationary phase had settled the column end-fitting was applied and the bed compressed.

The HPLC mobile phases and sample dissolution solvents were prepared from dichloromethane (DCM) and toluene, purchased from VWR International, in the proportion of 45:55 (DCM:Toluene). This composition had the exact same refractive index as the C-18 silica. The viscosity of the mobile phases was modified using cyclohexanol purchased from Sigma-Aldrich, which has the exact same refractive index as the C-18 silica. The exact compositions and subsequent viscosities are given in Table 1. The viscosity values reported in Table 1 are derived from prior work [15]; the solvents utilised here were prepared in the same manner as reference [15].

For visualisation of the viscous fingering effect, the injection sample was spiked with Oil-Red 'O′ dye purchased from Sigma-Aldrich, which is a bright red colour in the solvent systems employed here and is an un-retained marker.

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