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Viscous fingering induced flow instability in multidimensional liquid chromatography

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Abstract

Viscous fingering is a flow instability phenomenon that results in the destabilisation of the interface between two fluids of differing viscosities. The destabilised interface results in a complex mixing of the two fluids in a pattern that resembles fingers. The conditions that enhance this type of flow instability can be found in coupled chromatographic separation systems, even when the solvents used in each of the separation stages have seemingly similar chemical and physical properties (other than viscosity). For example, the viscosities of acetonitrile and methanol are sufficiently different that instability at the interface between these two solvents can be established and viscous fingering results. In coupled chromatographic systems, the volume of solvent transported from one separation dimension to the second often exceeds the injection volume by two or more orders of magnitude. As a consequence, viscous fingering may occur, when otherwise following the injection of normal analytical size injection plugs viscous fingering would not occur. The findings in this study illustrate the onset of viscous fingering in emulated coupled chromatographic systems and show the importance of correct solvent selection for optimum separation performance.

Keywords: Viscous fingering; Acetonitrile; Chromatographic system; Heart cutting

1. Introduction

Given the enormous number of chromatographic systems that are in operation, in everyday use for research, analytical, and preparative separations we find it somewhat surprising that there are so few documented reports of the flow instability phenomenon referred to as viscous fingering [1]. The chromatographic literature contains only a few papers describing experiments in which viscous fingering was encountered or even showing results that could be explained by this phenomenon [2–7]. Viscous fingering (VF) occurs at the interface between two different fluids percolating through a porous bed, when the low-viscosity fluid pushes the high-viscosity fluid. In a chromatographic system, these two fluids are the mobile phase and a sample plug. When a high-viscosity solute plug displaces a low-viscosity mobile phase, as when a concentrated feed sample is injected in preparative liquid chromatography, the interface remains sharp. However, at the rear end of the injection plug, the mobile phase pushes the solute plug and the low-viscosity mobile phase penetrates into the high-viscosity plug in a complex pattern resembling fingers, hence the name of the effect. When it takes place (e.g., in preparative size-exclusion chromatography), the impact of VF is very detrimental to chromatographic performance. The band shape is severely distorted and, in a worse case scenario, multiple bands are eluted when a single solute component was injected.

VF is unlikely to occur in analytical separations because sample plugs are dilute, their volumes small, and their viscosities almost identical to that of the mobile phase. However, there are only a few reports of viscous fingering at the

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preparative scale. This might be because the overloaded band profiles observed in preparative chromatography tend to be Langmuiran in shape. Hence, the leading edge of the solute plug is highly concentrated and the viscosity contrast causes the interface between the solute plug and the mobile phase to remain sharp. The trailing edge of the solute plug is dilute, the viscosity gradient is shallow, and these conditions do not favor a VF effect. Yet, even in the unusual cases of an anti-Langmuiran isotherm, VF has rarely if ever been reported.

More and more frequently, liquid chromatography is tackling more complex mixtures and there is a growing interest in multidimensional separations [8–12]. The development of these sophisticated separation methods tends to create experimental conditions under which VF will be favored, even at the analytical scale. Multidimensional high-performance liquid chromatography (LC/LC) consists of separation processes whereby the sample components are partially separated in one dimension, are transferred (all or in part) to a second, different and, if possible, orthogonal, separation dimension. This coupling affords an amplified separation space. In theory, the peak capacity for orthogonal systems is equal to the product of the peak capacities in each dimension. However, while information theory and factor analysis [13–15] provide a high value for the potential separation capacity, practical implementation requires the analyst to solve the many obstacles that prevent a full realization of this potential. For example, the coupling of two orthogonal systems, such as normal-phase reversed-phase (NP-RP) HPLC, creates obvious problems due to solvent immiscibility. Even the coupling of seemingly similar separation modes, such as that of two reversed-phase systems, may cause problems, despite the mobile phases used in each dimension being entirely miscible. In contrast to a one-dimensional system in which the sample solvent is selected to avoid a loss of performance due to an incompatibility with the mobile phase, multidimensional systems often require the use of significantly different mobile phases in each dimension in order to bring about an effective separation. The mobile phase component that is cut from the first dimension and moved into the second behaves as if it were the sample solvent for the separation undertaken in the second dimension. While incorporating stationary phases possessing different surface chemistries brings a high degree of independence between the two separations, the choice of mobile phase combinations, and the sequence of employing these mobile phases may be a determining factor in the overall performance of a two-dimensional separation. This may even be the case for those systems with similar stationary phase environments such as coupled reversed phase systems [10–12].

Acetonitrile and methanol are the most commonly used reversed-phase solvents. They are miscible in all proportions and have sufficiently different selectivities to be used in two-dimensional (2D) reversed phase separations [16]. The differences between these two solvents, combined with the selection of appropriate stationary phases, can afford a high degree of orthogonality to the 2D system. However, their different physical properties may also contribute to band broadening and to a separation loss [17]. For example, the very transfer of the peak(s) of interest from one separation dimension to the other may adversely affect resolution. Chromatography being a dilution method, the transfer volumes in multidimensional systems may be up to two orders of magnitude larger than conventional injection volumes. Flow instabilities can take place because, under these conditions, the differences between the chemical and the physical properties of the mobile phases used in each separation dimension are quite significant. The experimental conditions are favorable for VF to take place, unless the two mobile phases have nearly the same viscosity. Finally, the effects of VF are known to increase faster than the volume of the injection plug [18].

The aim of this study is to illustrate the effects of flow instabilities that may be observed in multidimensional RP–RP–HPLC processes that employ miscible mobile phase mixtures. Our intent was not to separate any mixture but simply to examine the peak shape resulting from the injection of a pure solute when combining two solvents. In this work, we emulate the transfer of a large solvent plug through its direct injection into what would ordinarily be the stream of another solvent, used in the second dimension of the separation process. In essence this emulates a heart cutting technique. The emulated 2D-system uses two binary aqueous/organic solvent, isocratic mobile phases. First, the sample plug was either methanol or acetonitrile. Subsequent studies employed binary aqueous mixtures of these two organic solvents.

A number of solutes were selected, based on their different functionalities, so as to determine whether the possible transfer problem was related to the chemical nature of the solute. A few aromatic compounds with a relatively high polarity (*p*-cresol, methoxybenzene, and ethoxybenzene) and a non-polar homologous series of alkyl benzenes were chosen. This group will serve to highlight whether solute/solvent interactions at the solvent interface might be responsible for a diminished separation performance. Duplicate injections of each solute were performed with four binary mobile phase combinations, emulating as many ‘two-dimensional’ systems.

While the mobile phase combinations selected are by no means exhaustive, the process highlights the problems that may occur when attempting to mix any compatible miscible solvents. It must be emphasized that viscous fingering is a chaotic phenomenon (like the weather). Although the phenomenon itself is highly reproducible, the details of the viscous fingers formed under a given set of experimental conditions are less so. In fact the degree of reproducibility of the fingers in successive experiments depends much on the experimental conditions, particularly on the flow rate [19]. So little is known regarding the effects of VF on the shape of band profiles in chromatography that a systematic study of the influence of all the parameters would be premature.

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile and methanol were purchased from Mallinckrodt Australia. Milli-Q water was obtained in-house. These solvents and all the mobile phases were filtered through a 0.2 μm filter. The sample solutes, toluene, ethylbenzene, propylbenzene, butylbenzene, *p*-cresol, anisole, and phenetole, were purchased from Aldrich (Milwaukee, WI, USA). All mobile phases were sparged with helium. For experiments involving direct visualization of viscous fingering, HPLC grade dichloromethane, and toluene were obtained from LabScan (Putumwan, Bangkok, Thailand). Cyclohexanol (Refractive index 1.465 at 20 °C; Unilab grade, Ajax Chemicals, Sydney, Australia). Oil Red O dye was used to visualise the viscous fingering and was purchased from Sigma (St. Louis, MO, USA). The stationary phase used was Nucleosil C18 silica (Alltech Associates, Deerfield, IL, USA). The particles of this packing material are spherical, with an average particle size of 10 μm. The mobile phase flow rate was 2 mL/min for the experiments reported in Figs. 6 and 7.

2.2. Equipment

All chromatographic experiments were conducted using a Varian LC system (Walnut Creek, CA, USA), employing a 9012 pumping system, a 9050 UV detector (set at 254 nm), and a Valco injection valve (VICI model EHMA). Data acquisition was achieved using a Lawson Labs model 203 serially interfaced 20-bit data acquisition system with a custom ±1 V gain range operated at 10 Hz (Lawson Labs, Malvern, PA, USA). Two Valuepak C18 (250 mm × 4.6 mm) 5 μm P_d columns were employed for studies on the analytical scale development of viscous fingering in the emulated coupled systems. For the visualization of VF in glass columns, a Waters model 600 multisolvent delivery system (Milford, MA, USA) was used to deliver the dichloromethane/toluene/cyclohexanol mobile phase. A Valco VICI EHMA 6-port, 2-position switching valve allowed sample introduction into a glass tube (100 mm × 17 mm) packed with Nucleosil C18 silica (Alltech Associates). This column was submersed in a dichloromethane reservoir in order to remove the cylindrical lens effect caused by the curvature of the glass column. The viscous fingering was recorded 'on-column' using a Pentax ZXM SLR 35 mm camera fitted with a Tamaron 90 mm macro lens. Kodak Professional (PORTA, 160 VC) (160 ASA) film was used throughout. The photographic images were digitized using a Nikon CoolScan III (Nikon Inc. Melville, New York, NY, USA) film scanner. All images were acquired at the maximum resolution of the scanner (2700 dots per inch). Adobe Photoshop 5.0 (Adobe Systems, San Jose, CA, USA) was used to perform image manipulation. Further details of the visualization experiments can be found in references [18,20,21]. Post-column detection was recorded using a GBC, LC1200 UV/Vis detector set at 575 nm. Data was collected on a Lawsons lab A/D converter set at 2 Hz.

2.3. Chromatographic separations

The seven sample solutes used to test the simulated two-dimensional system were each dissolved separately in 100% methanol or acetonitrile, at appropriate concentrations so as to maintain a constant sample load of 1 mg/mL, irrespective of the sample injection volume. Separations were conducted using 20/80 water/methanol or 30/70 water/acetonitrile mobile phases. These solvent combinations have approximately the same solvent strength. All flow rates were 1.0 mL/min unless stated otherwise. All injections were performed in duplicate and the results averaged. Injections were performed for each of the following sample solvent/mobile phase combinations:

System	Sample solvent	Mobile phase
A	100% Methanol	20/80 Water/methanol
B	100% Acetonitrile	20/80 Water/methanol
C	100% Acetonitrile	30/70 Water/acetonitrile
D	100% Methanol	30/70 Water/acetonitrile

The solutes *p*-cresol and butyl benzene were further injected as solutions in a variety of mobile phase compositions, as noted in the appropriate sections of the text.

3. Results and discussion

The samples were dissolved in either methanol or acetonitrile and injected into streams of either water/methanol or water/acetonitrile. A selection of band profiles recorded with these four solvent combinations is shown in Fig. 1. Note that the peak heights were normalized and the peak maximum aligned for visual clarity. Fig. 1(a) shows bands of ethylbenzene, representative of the homologous alkylbenzene series selected for this study. Ethylbenzene always gives sharp and narrow peaks, except when its solution in acetonitrile is injected into a water/methanol stream. The peak shape was significantly broader in this last case than in the other three and it exhibited a modest degree of fronting. The best peak shape was obtained when the methanol solution was injected into a water/methanol stream. The two other combinations gave bands only slightly broader with no determinable difference between them. Similar behavior was observed for all the alkylbenzenes, irrespective of their retention factor. The results obtained with the other substituted aromatic compounds were similar, except that the degree of band fronting of *p*-cresol is so large that its band exhibited a series of three partially resolved peaks when its solution in pure acetonitrile was injected into a methanol/water stream (Fig. 1b). In general the band shape of more strongly retained species was less affected while the less retained solutes exhibited more severe band distortions. A significant increase of the width of the

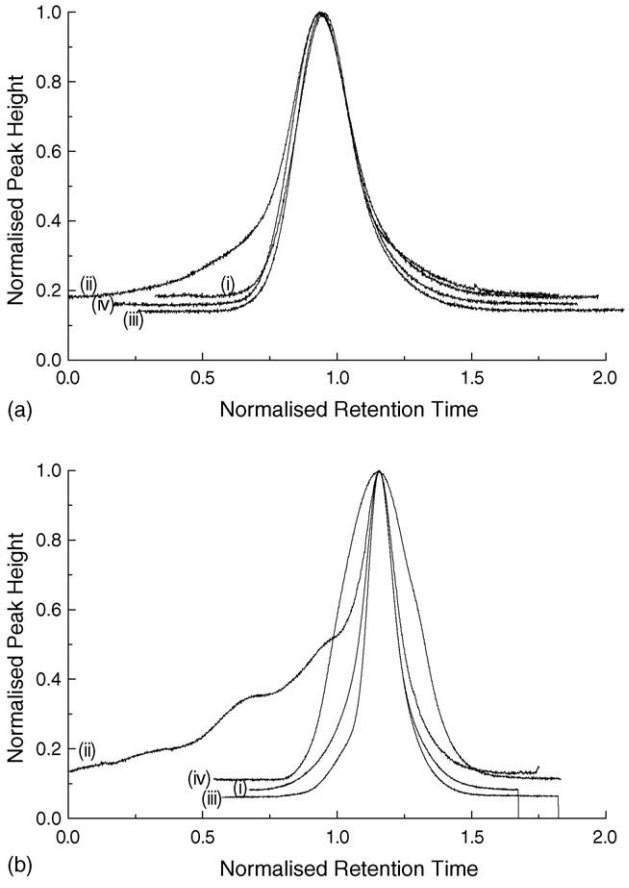

Fig. 1. Band profiles of (a) ethylbenzene and (b) *p*-cresol injected into solvent injection plug and mobile phase combinations of: (i) solvent plug 100% methanol, mobile phase (water/methanol), (ii) solvent plug 100% acetonitrile, mobile phase (water/methanol), (iii) solvent plug 100% acetonitrile, mobile phase (water/acetonitrile) and (iv) solvent plug 100% methanol, mobile phase (water/acetonitrile). Injection volume 200 μL.

p-cresol band is apparent when its solution in pure methanol is injected in the stream of water/acetonitrile.

The reproducibility of the two irregular profiles in Fig. 1b was poor. The peak fronting observed with alkylbenzene (Fig. 1a) was reproducible in the duplicate test whereas the reproducibility of the band fronting observed for the polar solutes was poor. As an example, Fig. 2 shows duplicate profiles recorded upon successive injections of the solution of *p*-cresol in acetonitrile into a stream of water/methanol. While the reproducibility of the peak front was poor, the reproducibility of the peak maximum and of the band tail was excellent.

In order to understand better the process causing band distortion, we changed the composition of the stream, increasing its water content from 20 to 50%. This has several simultaneous effects. First, it increases the retention of the solute, driving the solute from the solvent plug (acetonitrile) to the stream of the second mobile phase (water/methanol). Secondly, it increases the viscosity of the second mobile phase [22]. *p*-Cresol was selected because it has a short retention time and was the compound that displayed the greatest degree

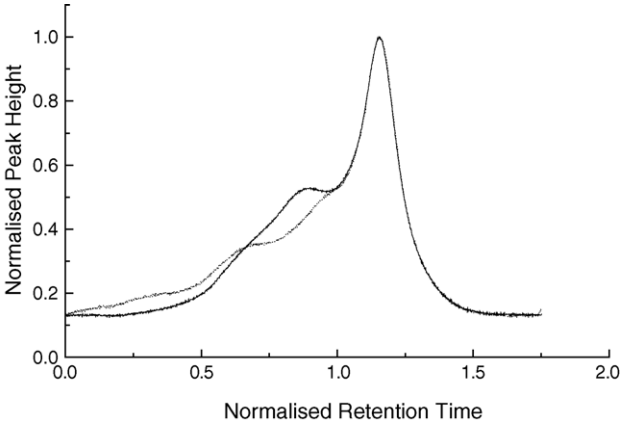

Fig. 2. Duplicate injection profiles following the injection of *p*-cresol dissolved in 100% acetonitrile into a stream of water/methanol (20/80). Injection volume 200 μL.

of peak fronting in this experiment. The results are shown in Fig. 3. The peak maximums are aligned and the data normalized, to enable an easy comparison. (Note: the difference in the general nature of the band distortions compared to those observed in Figs. 1 and 2 is explained by the results for Fig. 3 being acquired on a different Value-Pak column). The extent of peak distortion increases with increasing water content and therefore with decreasing solubility in the sample solvent and increasing viscosity contrast between the two solvents, which would explain an increasing degree of viscous fingering. It might seem that, in actual 2D separations, since its retention on the stationary phase increases with decreasing solubility, the solute would spend less time in the solvent, the volume of the transfer plug would increase and the fingering effect should decrease. However, the results of chromatographic experiments carried out in glass columns (to be discussed later) show that the viscous fingering process begins as soon as the sample plug enters the column. Consequently, if the experimental conditions allow viscous fingering to take place, it

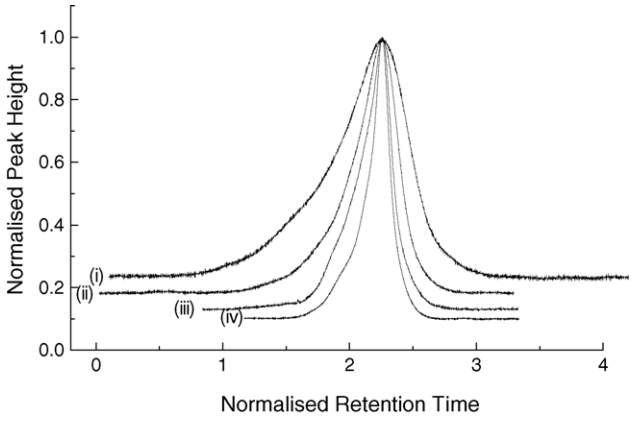

Fig. 3. Diagram illustrating the change in elution band shape of *p*-cresol as the ratio of water/methanol is changed in the mobile phase. Sample plug 100% acetonitrile, injection volume 200 μL; (i) 50/50 water/methanol, (ii) 40/60 water/methanol, (iii) 30/70 water/methanol and (iv) 20/80 water/methanol.

will and, regardless of whether or not the solute is retained, its peak shape will be distorted. The driving force for viscous fingering is the viscosity contrast. It is independent of the interactions of the solute with the mobile and stationary phases.

The converse effect was investigated with the most retained solute, butyl benzene. Reducing the water content of the mobile phase decreased its retention. The band shape (not shown) was less distorted when the plug was transferred into the stronger solvent, with which the retention is lower and the viscosity contrast also lower. The important difference between the viscosity of the solute plug (100% acetonitrile, $\eta = 0.37$ cP) and of the mobile phase stream (20/80 water/methanol, $\eta = 1.25$ cP [22]) provides conditions that are favorable for viscous fingering and cause significant band distortion. In contrast, band profiles remain conventional when the viscosity difference between the solute plug and the mobile phase is small.

To further test the hypothesis that viscous fingering explains the band distortion observed in Figs. 1–3, we prepared solute plugs from mixtures of water and acetonitrile in three compositions, all having a viscosity near that of the mobile phase. Fig. 4a illustrates the band profiles of *p*-cresol solutions in sample solvents of different compositions, injected into a stream of 40/60 water/methanol. The band profile improves when the viscosity of the sample injection plug approached that of the mobile phase. A very irregular band profile was observed upon the injection of pure acetonitrile (less viscous than water/methanol, 40/60) into the mobile phase. The profile improves significantly when the sample solvent was 20/80 water/acetonitrile and an almost Gaussian band with only a small hump at its leading edge was observed when the sample solvent was 40/60 water/acetonitrile. Similar results were observed for butyl benzene (Fig. 4b).

Band distortion resulting from viscous fingering is exacerbated with an increase in the injection volume [18]. The influence of the injection volume was investigated for the same two solutes as studied earlier. As an example, the change in band distortion is illustrated in Fig. 5 for *p*-cresol. The band distortion increased with increasing injection volume. Virtually no band distortion was apparent for a 30 μL injection volume, a distinct peak fronting becomes obvious for a 100 μL injection and strong band distortion is apparent for a 200 μL injection. Typical cut volumes in two-dimensional LC, using conventional analytical scale columns, may be in the order of 1 mL [10,11], hence the practical importance of this result.

In contrast to what is normally expected for a VF effect in a chromatographic system, the band irregularities take place this time at the peak front, not at the rear boundary. This change in VF location is simply explained because, in this case, the large injection plug that emulates a heart-cut solvent is less viscous than the mobile phase. The VF effect always takes place when the less viscous fluid pushes the more viscous one, only then. Thus, the inversion of the side of the band on which VF takes place when the viscosity contrast is

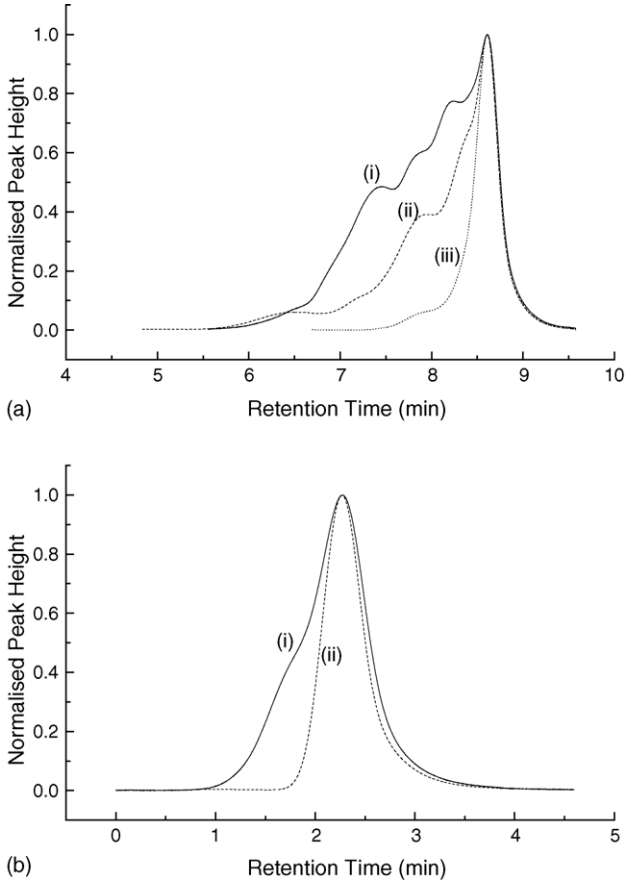

Fig. 4. Illustration of band profiles as a function of the sample solvent composition: (a) *p*-cresol, mobile phase 40/60 water/methanol and (b) butyl benzene, mobile phase 20/80 water/methanol. Sample solvent composition: (i) 100% acetonitrile, (ii) 20/80 water/acetonitrile, and (iii) 40/60 water acetonitrile. Injection volumes 200 μL.

reversed proves that VF is involved in this band dispersion. The VF process is poorly reproducible, if at all, at high mobile phase velocities but it is very reproducible at low velocities [19]. However, even a small degree of irreproducibility of

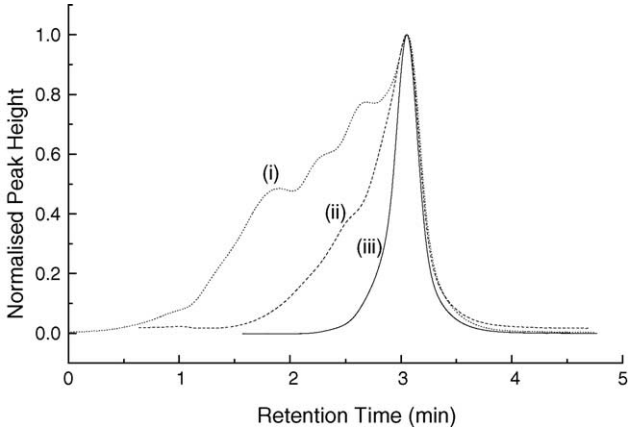

Fig. 5. Illustration of the change in band profile as a function of sample injection volume: (i) 200 μL, (ii) 100 μL and (iii) 30 μL. Sample *p*-cresol prepared in 100% acetonitrile, mobile phase 40/60 water/methanol.

the VF pattern causes most different elution profiles on conventional chromatograms. In a previous study where VF was visualised 'on-column' [18], the profiles of bands exhibiting VF on column were recorded post-column. The replicates of these profiles were, for the most part, irreproducible as observed in the current study. This supports the occurrence of VF.

To illustrate more conclusively the VF effects, we undertook a series of experiments in glass columns. When the refractive indices of the mobile and the stationary phase match exactly, the otherwise opaque stationary phase becomes invisible to the eye. Under such conditions, VF is clearly observed when a colored plug of a solvent having a viscosity that is sufficiently different from that of the mobile phase is injected into the column. More extensive details of this experiment are found in previous studies [18–21]. A solvent system consisting of dichloromethane, toluene and cyclohexanol in the correct proportions has the exact refractive index as the C18 silica. The viscosity can be varied by increasing the concentration of cyclohexanol (which has exactly the same refractive index as the C18 silica used) and then adjusting the ratio of dichloromethane and toluene to maintain the refractive index match. A dye marker incorporated into the sample solvent can be used to observe the fingering process.

Fig. 6a illustrates the VF effect observed when a plug of sample solvent less viscous than the mobile phase is injected into the column. The viscosity difference is in the order of 0.48 cP. That is, the sample solvent viscosity was 0.38 cP and the mobile phase was 0.86 cP. The sample solvent being less viscous than the mobile phase, fingers form at the leading edge of the sample band. These fingers are highly prominent and become more so as the viscosity difference increases. Fig. 6b illustrates the band profile that would ordinarily be observed when the viscosities of the sample solvent and the mobile phase match exactly. No fingering is apparent. The post-column chromatograms recorded for the bands photographed in Fig. 6 are shown in Fig. 7. Quite clearly, viscous fingering distorts the profiles, most significantly their leading edge, increases the band width, and reduces their height. In this instance the difference in viscosity between the mobile phase and the sample solvent is not as large as in our studies on stainless steel columns where it was almost 1 cP instead of 0.48 cP. This is a reason why the band profiles on the stainless steel columns were more severely distorted. Another explanation is simply that the analytical column is more efficient than the larger glass 'home-made' column. As a consequence, axial dispersion being lower on the analytical column, the fingering pattern is more readily detected. Yet another reason for the difference in the post-column detection chromatograms is that we do not know what is the effect of the column diameter on the fingering pattern. The analytical column had an internal diameter of 4.6 mm, while the internal diameter on our glass column was 17 mm. As we have not been able to undertake visualization experiments in narrow diameter glass columns, we have no way of comparing the fingering patterns.

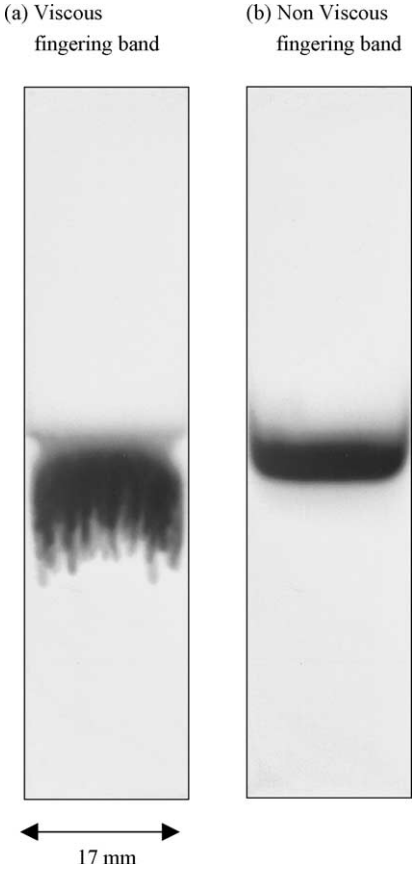

Fig. 6. Photographs illustrating the magnitude of the band distortion as the sample migrates along the chromatographic column following: (a) viscous fingering band profile, (b) non viscous fingering band profile observed when the viscosities of the sample solvent and mobile phase match exactly. Column internal diameter 17 mm.

We repeated these 'on-column' visualisation experiments on a number of different columns and also using a wide variety of mobile phases by variation in the cyclohexanol composition. We carried out also similar experiments using pure

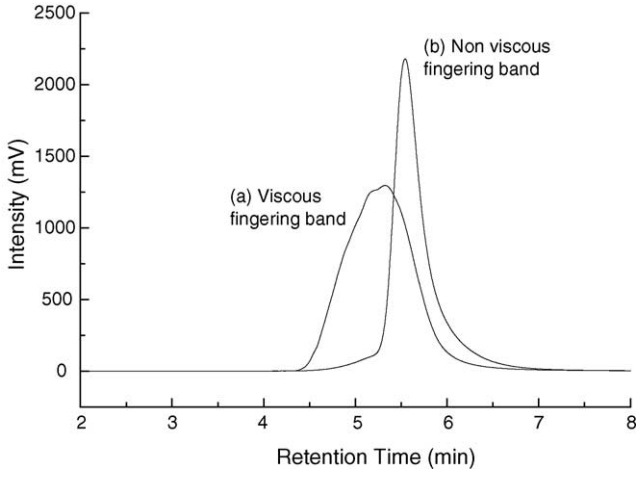

Fig. 7. Post column detector elution profiles corresponding to the photographs illustrated in Fig. 6. Detection 575 nm, flow rate 2 mL/min.

carbon tetrachloride. Whether or not a mobile phase is a pure solvent, a binary or a ternary mixture, VF was always observed if the viscosity contrast between the injection plug and the sample solvent was appropriate. In fact, VF effects become apparent in a very mild form when the viscosity difference between the sample solvent plug ($\eta = 0.38$ cP) and the mobile phase ($\eta = 0.47$ cP) is less than 0.1 cP. This is substantially less than the difference observed for the systems reported earlier in this work. However, at such small viscosity differences, post-column chromatograms do not exhibit VF effects, primarily, we believe because band dispersion over-rides it. Details of these studies are not included in this work for the sake of brevity.

The results reported earlier reflect the occurrence of VF and, for the most part, can be explained by this phenomenon. However, there remain some ambiguities. An important anomaly is that VF does not seem to take place when a plug of methanol is injected into a stream of water/methanol, even at relatively high water concentration for which the viscosity difference is significant. In contrast, a strong VF effect takes place when the same plug is injected into a water/acetonitrile solution (Fig. 1b). It might be that methanol dilutes very fast into the methanol/water solution, or that the viscosity difference is not as great as between acetonitrile and the same water/methanol stream, or that the effects that we observed and report in this manuscript are more complex than simple VF. Solvation effects may be an underlying factor that could exacerbate the band distortions observed in this study. It is important to note, however, that this last case was the only instance in which band distortions occurred at both the leading edge and the rear boundary of the band. This may indicate the difference between VF and solvation effects. Or perhaps to further complicate the viscous fingering process the disturbance at the rear boundary may be an effect that is observed in the presence of nonmonotonic viscosity profiles, such as those observed by Manickam and Homsy [23]. Manickam and Homsy have studied the nonlinear evolution of VF instabilities in miscible displacements with nonmonotonic viscosity profiles [23,24]. In this situation, the fluid that is pushed has a concentration gradient away from the interface. Aqueous solutions of 1- and 2-propanol that were taken as cases in point in the simulations [23], or of methanol [22], have a nonmonotonic viscosity. The viscosity of these solutions is maximum for concentrations of alcohol around 60% (nearly the same for all three alcohols). They have showed that while an unfavorable viscosity contrast always leads to VF instability for monotonic fluids, this is not true for nonmonotonic fluids. Under certain conditions, and for a certain time, a stable displacement can take place when the viscosity contrast is unfavorable and conversely, VF instabilities may eventually develop with an initially favorable viscosity contrast. This is due to the effects of diffusion (and the faster dispersion in HPLC) that slowly affects the viscosity profile. The condition for instability in a nonmonotonic viscosity profile is $((\mathrm{d}:/\mathrm{d}C)_{C=0} + (\mathrm{d}:/\mathrm{d}C)_{C=1}) < 0$ (with :, local viscosity and C, local eluite concentration). In our experiments, the concentration of methanol or acetonitrile in the mobile phase was kept constant but it varies during the elution of a peak. During the elution of a peak of pure methanol by a 20/80 methanol/water mobile phase or during the elution of a peak of pure acetonitrile by a 30/60 water/acetonitrile solution, we do have formation of a monotonic fluid. However, the elution of a water zone by a 20/80 methanol/water mobile phase leads to a nonmonotonic fluid. The situation might be more complex during the elution of peaks of pure methanol by a 30/60 water/acetonitrile solution or of pure acetonitrile by a 20/80 methanol/water since we do not know the viscosity of the ternary solutions.

Finally, the work of Homsy on VF instability in nonmonotonic fluids [23,24] may provide an answer to the question of why VF does not plague preparative HPLC. A high-concentration band profile generally constitutes a nonmonotonic fluid, with a maximum viscosity at the band apex. In most cases, the stable region is downstream. Under such conditions, the band propagation is stable initially and instability will arise too late to cause serious troubles.

4. Conclusion

Multidimensional reversed-phase/reversed-phase HPLC will eventually become an effective, flexible approach for the rapid and concise separation of complex samples. However, flow instabilities arising from the transfer-injection of a fraction of the eluent from the stream of products separated in the first dimension into the stream of mobile phase used in the second dimension may only serve to decrease the performance of the 2D system and render it less efficient and less viable.

The phase systems studied here are not inclusive of those that may be encountered in the various possible implementations of RPLC × RPLC. They are particularly simple, fully miscible, and have very similar physico-chemical properties. So, they illustrate well that flow instabilities do occur, even in the simplest cases. The effect of these flow instabilities may be complicated by interference with solvation. Whatever their origin, however, effects that decrease the resolution of the sample components must be avoided or, if that is not possible, alleviated by suitable means to minimize the performance loss. Suitable mixing chambers may be needed. We found also that one particular solvent combination resulted in severely distorted peak behavior but that merely reversing the sequence of the two separations may be all that was required to dramatically improve peak resolution. In cases in which severe VF effects are harmful, the adjustment of the viscosities of both mobile phases by the addition of a proper concentration of water may bring about improved peak shape. Of course, the trade-off between selectivity and resolution will determine which system is better and what sequence should be selected.

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References

[1] P.G. Saffmann, G. Taylor, Proc. R. Soc. London Ser. A 245 (1958) 312.
[2] L. Plante, P. Romano, E. Fernandez, Chem. Eng. Sci. 49 (1994) 2229.
[3] M. Dickson, T. Norton, E. Fernandez, AIChE J. 43 (1997) 409.
[4] E. Fernandez, T. Norton, W. Jung, J. Tsavalas, Biotechnol. Prog. 12 (1996) 480.
[5] E. Fernandez, C. Grotegut, G. Braun, Phys. Fluids 7 (1995) 468.
[6] D. Cherrak, E. Guernet, P. Cardot, C. Herrenknecht, M. Czok, Chromatographia 46 (1997) 647.
[7] M. Czok, A.M. Katti, G. Guiochon, J. Chromatogr. 550 (1991) 705.
[8] J. Dallüge, J. Beens, U.A.Th. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[9] P. Schoemakers, P. Marriott, J. Beens, LC–GC Eur 16 (2003) 335.
[10] M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, J. Chromatogr. A 1028 (2004) 247.
[11] M. Gray, A.P. Sweeney, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, Analyst 128 (6) (2003) 598.
[12] V. Wong, A.P. Sweeney, R.A. Shalliker, J. Sep. Sci. 27 (2004) 47.
[13] P.J. Slonecker, X. Li, T.H. Ridgway, J.G. Dorsey, Anal Chem. 68 (1996) 682.
[14] M. Gray, G.R. Dennis, P. Wormell, R.A. Shalliker, J. Chromatogr. A. 975 (2002) 285.
[15] M.J. Gray, G.R. Dennis, P.J. Slonecker, R.A. Shalliker, J. Chromatogr. A. 1015 (2003) 89.
[16] L.R. Snyder, P.W. Carr, S.C. Rutan, J. Chromatogr. A. 656 (1993) 537.
[17] A.P. Sweeney, PhD Thesis, University of Western Sydney, 2003.
[18] B.S. Broyles, R.A. Shalliker, D.E. Cherrak, G. Guiochon, J. Chromatogr. A. 822 (1998) 173.
[19] R.A. Shalliker, V. Wong, G. Guiochon, submitted for publication.
[20] R.A. Shalliker, B.S. Broyles, G. Guiochon, Anal. Chem. 72 (2000) 323.
[21] B.S. Broyles, R.A. Shalliker, G. Guiochon, J. Chromatogr. A 867 (2000) 71.
[22] H. Colin, J.C. Diez-asa, T. Czaychowska, I. Miedziak, G. Guiochon, J. Chromatogr. 167 (1978) 41.
[23] O. Macinkam, G.M. Homsy, Phys. Fluids 5 (1993) 1356.
[24] O. Macinkam, G.M. Homsy, Phys. Fluids 6 (1994) 95.