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Journal of Chromatography A, 822 (1998) 173–187

JOURNAL OF CHROMATOGRAPHY A

Visualization of viscous fingering in chromatographic columns

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Received 20 March 1998; received in revised form 16 June 1998; accepted 1 July 1998

Abstract

A 17 mm I.D. glass column, packed with YMC-15 (spherical C_{18} silica, 30 μm particles) as the stationary phase and used with carbon tetrachloride as the mobile phase provided a suitable system for the visual observation of viscous fingering inside the packed bed, after the cylindrical lens effect had been canceled. Such a system appears nearly transparent due to the close matching of the refractive indices of the mobile and the stationary phases. The profiles of the bands obtained upon the injection of a flat pulses of a solvent of different refractive index were readily observed. On-column detection using a 35 mm photographic camera was employed for the direct recording of band profiles. A toluene UV marker was used to record subsequent elution profiles via UV detection. The influence of the sample viscosity, the mobile phase flow-rate, and the injection volume on the extent of viscous fingering and band broadening were studied. Viscous fingering was shown to increase with increasing difference between the viscosity of the sample injected and the mobile phase. Viscous fingers were more clearly delineated at higher flow-rates, but band distortion was observed even at flow-rates as low as 0.2 ml min^{-1}. The degree of band distortion was observed to increase with increasing volume injected. Finally, viscous fingering was found to be a reversible process, as previously reported.

Keywords: Viscous fingering; Preparative chromatography

1. Introduction

Viscous fingering is the flow instability resulting from a less viscous solvent displacing a more viscous solvent. The nature and consequences of the instability of the interface between these two solvents have been investigated in considerable detail in the last decade [1–12]. Of particular interest to the chromatographer is viscous fingering in packed beds and the resulting effects on the separation performance in cases in which the introduction of the sample pulse alters markedly the local viscosity of the mobile phase. Such a case is most often encountered in the analysis of macromolecules by size-exclusion chromatography (SEC), since the sample viscosity may be dramatically increased due to the size of the analytes while, the residence times being small, the bands tend to remain narrow. It occurs more frequently in SEC separations carried out by preparative chromatography, because the sample concentrations and the volumes injected tend to be large. In a worse case situation, viscous fingering causes the solute band to fragment into smaller bands occupying only a fraction of the column cross-section and migrating at different velocities. These bands propagate more or less independently of each other, resulting into the

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00568-8

formation of multiple elution peaks. At best, when limited viscous fingering occurs, band profiles are non-uniform in the radial direction, their elution widths are increased and band resolution is substantially decreased.

Viscous fingering has been studied in detail by Cherrak et al. [2,3] who found that a significant efficiency loss and random peak deformations were caused by greater than 10% viscosity differences between the sample and the mobile phase. They observed that in a system undergoing viscous fingering the elution profiles obtained exhibited a smooth front and an irregular rear boundary whenever the viscosity of the sample was greater than that of the mobile phase. The opposite held true whenever the viscosity of the sample was less than that of the mobile phase. When the viscosities of the sample and the mobile phase matched, both the front and the rear boundaries of the profiles were stable and a characteristic Gaussian-shaped profile was observed for small injection volumes.

With proteins in SEC, Czok et al. [1] illustrated the principle of two techniques which could minimize the effect of viscous fingering. They used a SEC system with which ovalbumin samples of low concentrations were eluted as symmetrical peaks in a phosphate buffered mobile phase. At high concentrations, however, ovalbumin samples were eluted as substantially broader bands exhibiting multiple peaks on the rear boundary. The effects of viscous fingering were first minimized by matching the viscosity of the mobile phase to that of the sample solution. When samples of concentrated ovalbumin solutions were eluted by a mobile phase containing 15% glycerol, almost symmetrical peaks were observed, similar to those of the low concentration samples. Peak profiles could also be improved by using the original low viscosity mobile phase but injecting, immediately after the sample pulse, a wide pulse of a solution of glycerol in the buffer having a viscosity at least equal to that of the sample. The band profiles obtained were almost identical to those of the low concentration samples for which viscous fingering was not observed.

So, the results of several investigations of the behavior of elution bands in chromatography support the occurrence of viscous fingering. However, they do so indirectly and they cannot demonstrate directly the existence of the viscous fingers. This demonstration requires the visualization of the bands inside chromatographic columns. This is difficult, even when using a glass column, because the silica bed is opaque and the particles scatter light. In a recent series of papers, Fernandez and co-workers [4–7] illustrated the formation of viscous 'fingers' using nuclear magnetic resonance imaging (MRI). This method provided a detailed representation of the flow stream inside a chromatographic column. Dramatic fingering of the less viscous solvent (a phosphate buffer) into the rear of a viscous band of glycerol was observed. The extent of the fingering was such that the sample band was highly distorted [4]. Similar results were observed with samples of bovine serum albumin, with important peak distortion at concentrations in excess of 5 mg ml^{-1}. Even though distortions of the band profiles were not observed at lower concentrations, HETP measurements indicated that viscous fingering may have had a deleterious effect on column performance.

Dickson et al. [5] also examined viscous fingering with binary samples of butanediol and polyethylene glycol (PEG) of different molecular masses. They showed that the phenomenon was more intense with a multicomponent sample than with a single component one when each individual concentration remained constant. In this case, the total concentration of analytes was initially greater, hence the viscosity of the sample and of the yet unresolved band was higher. Using a higher molecular mass PEG also increased the extent of fingering, due to a further increase in the sample viscosity. However, unlike the band profiles observed in single component systems, the profile of the butanediol peak (eluted after PEG) exhibited distortion along its leading edge. This was explained by some butanediol being carried into the fingering zones of the solvent during the early step of the elution, before band separation could take place to any significant extent.

Finally, Fernandez et al. [6] attempted to design columns in which viscous fingering would be minimized. Because the extent of viscous fingering was shown to increase with increasing column diameter [3–6], they incorporated into the bed of large diameter columns bundles of capillary tubes parallel to the column axis. The resulting columns displayed significantly less viscous fingering, but also a mark-

edly lower efficiency than conventional columns. Thus, the need to find a satisfactory trade-off between the column efficiency and the extent of viscous fingering arose. The loss of column efficiency observed was largely a result of an increased wall effect and an increase in the heterogeneity of the column packing. Further studies in this area need to be performed before this method proves to be acceptable for avoiding viscous fingering.

MRI has proven successful in providing excellent interpretations of the flow distribution in a chromatographic column and of the concentration profiles in bands migrating along columns in cases in which viscous fingering takes place. Some doubts linger, however, as to the exact meaning of the values measured. MRI visualization is achieved through the exceptional magnetic properties of the gadolinium ion, Gd^{3+}. This ion catalyzes the relaxation of the excited protons. In the work reported above [3–6], the sample consists of a solution of Gd^{3+} and of the viscous additives desired. The latter are retained and rapidly separated from the inorganic ions which are not retained. Other similar MRI investigations of band migration along chromatographic columns have been performed to illustrate the heterogeneity of the packed bed inside the column [13,15]. These studies used gadolinium chelates both as the visualization agent and as the chromatographic analyte which allows the direct determination of important chromatographic data [13]. In all these MRI studies, the visualization of the band profiles remains still two-dimensional unless three dimensional image analysis software is employed to produce a representation of a three-dimensional image [7]. The amount of NMR signal required limits drastically the spatial resolution in the third dimension (i.e., the thickness of the slice remains important). Consequently, the image clarity is moderate. As a result, the fingers observed in the nonuniform flow along the column are somewhat diffuse and because of the relatively long acquisition times necessary to obtain an image, the resolution is quoted to be around 2 mm, which is somewhat limiting for detailed quantitative analysis [7]. In the present paper we discuss the results obtained with a simple optical method of visualization of the viscous fingering in silica based columns on a semi-quantitative level. However, that is not to say that the visualization method cannot be made quantitative. A forthcoming study will show how [14]. Even as it is used here, however, this optical method has advantages and drawbacks which make it complement favorably those of MRI.

2. Experimental

2.1. Chemicals

Reagent grade carbon tetrachloride and HPLC grade toluene were obtained from Sigma (St. Louis, MO, USA); reagent grade *n*-propanol and dichloromethane from Mallinckrodt (Paris, KY, USA).; reagent grade methanol, *n*-butanol and HPLC grade acetone from Fisher (Fair Lawn, KY, USA); 100% ethanol from AAPER Alcohol (Shelbyville, KY, USA); and reagent grade cyclohexanol from Fluka (Buchs, Switzerland). All the mobile phases used were sparged with helium during the chromatographic experiments. The viscosities and refraction indices of all solvents used are summarized in Table 1.

Carbon tetrachloride is a toxic chemical which should be handled carefully. All experiments were carried out under a closed, well ventilated hood. A container was located under the column to collect leaks (most resulting from column breakage). Carbon tetrachloride was recycled by collecting separately the bands eluted and the pure solvent. Wastes were discarded in compliance with University policy.

2.2. Columns

All chromatographic experiments were performed on a 100×17 mm borosilicate glass (Pyrex) column supplied by OMNI (Cambridge, UK). Actual bed lengths varied from 6.20 cm to 6.62 cm. Column end fittings were prepared by the University of Tennessee workshop. These fittings included a fixed length outlet fitting and an adjustable inlet fitting which allowed axial compression of the bed. The stationary phase used was from YMC (Kyoto-Fu 613 Japan). This material is a spherical C_{18} bonded silica, 15–30 μm average particle diameter. The column was slurry packed in a downward configuration. The empty column was filled with dichloromethane. The packing material was slurried in methanol and the

Table 1
Physical characteristics of the solvents and column materials used

Solvent	Viscosity (cP) at 25°C [23]	Refractive index at 20°C [24]
Acetone	0.30	1.359
Borosilicate glass column (Pyrex)	–	1.473[a]
Carbon tetrachloride	0.86	1.460
Cyclohexanol	68 at 20°C	1.466
Dichloromethane	0.40	1.424
Ethanol	1.04	1.361
Methanol	0.55	1.328
n-Propanol	1.94	1.386
YMC-15 C_{18} silica	–	~1.460

From Ref. [22].

slurry was pushed into the column and consolidated by a steady stream of methanol.

Visualization of the viscous fingering was markedly improved by enclosing the glass column inside a rectangular reservoir filled with carbon tetrachloride. The reservoir was machined in-house from a 7.6 cm (diameter)×7.6 cm (width)×12.6 cm (height) block of black Delrin plastic (inert to CCl_4). The inside of the block was bored out to accommodate the glass column and its end fittings. For viewing the column, 3.1 cm×8.4 cm windows were cut in all four vertical sides of the block. The windows were sealed along with the bottom hole which allowed an outlet tube to be connected to the bottom of the column. A small hole in the top of the reservoir serves as a fill and drain hole for the carbon tetrachloride. A metallic ruler is fixed to the side of the column, to allow precise measurements of distances along the column axis. The top column end fitting protrudes from the top of the reservoir making the adjustable end fitting accessible for adjustment.

This design practically cancels out the cylindrical lens effect created by the column which otherwise would cause considerable distortion of the band features. Because the refraction index of the column (n_D=1.473) is different from those of C_{18} bonded silica (ca. 1.460) and carbon tetrachloride (1.460), the column is faintly visible but the residual lens effect is negligible. Thus, imaging of the viscous fingers inside the packed bed was most clear and it became possible to photograph these fingers without optical distortions of the image.

2.3. Equipment

The chromatographic system consisted of two high-performance liquid chromatographic (HPLC) pumps (model 510, Waters Associates, Milford, MA, USA) controlled by a Waters automated gradient controller. Solvent switching and sample injection were achieved using one of two switching valves (Models 7100 and 7000, Rheodyne, Cotati, CA, USA). A variable-wavelength UV detector (Linear) was set at 275 nm. Data acquisition was performed by a 486 IBM PC running a DOS based software (PEAKSIMPLE II v. 3.54, SRI Instruments, Torrance, CA, USA). Photographs were recorded using a Canon T50 35 mm camera (Canon USA, NY, USA), with a macro lens adapter and Kodak 200 ASA 35 mm film.

2.4. Column preparation

Because of the nature of the experiments, i.e. operating the glass columns at pressures nearing their limits of design, breakages of column tubing and end fittings took place several times. Consequently this work required the preparation of several columns. The first column (column a) when tested with toluene, an unretained test solute, gave an unsymmetrical peak exhibiting a significant degree of distortion on its trailing edge. This prevented any meaningful information being obtained regarding column efficiency. The peak shape from the second column (column b), although yielding a similar band

profile to the first column, produced significantly less tailing and the reduced plate height measured from half-height at 1.0 ml min^{-1} was in the order of 11.2. This flow-rate corresponded to a reduced velocity in the order of 1.6 for toluene. We believe that this band distortion[1] is due largely to the design of the inlet frit, although there may be a contribution from the valve injection device. It is not due to the packing of a poor quality bed. In order to demonstrate that point a third column was packed (column c). Following a valve injection, the reduced plate height was determined to be 6.4 from peak width at half height (asymmetry factor 5.54), whilst central point injection verified that the actual column reduced plate height without inlet frit or injection valve contributions was 1.4 (based on peak width at half height). In addition the peak asymmetry factor was 1.00.

While it is conceivable that the packing conditions (and particularly the limited packing pressure) required for these glass columns could lead to a poorly packed bed, all care was taken to consolidate the packing over a suitable period of time. Furthermore, the use of an axial compression inlet fitting allowed the bed head to be further compressed. Even so, the packing conditions did not allow for a radially homogeneous bed, as a wall effect is clearly evident following valve injections on all three columns (see e.g., Fig. 2c).

3. Results and discussion

3.1. Visualization of viscous fingering

For all practical purposes, our instrumental design is a Hele-Shaw cell [17]. Viscous fingering could be observed easily, with almost perfect clarity, in real time, as the mobile phase flowed along the packed bed, carrying the injected band downstream. This required only a good match between the refraction indices of the packed bed and of the solvent flowing through the bed. This match is achieved with C_{18} silica and carbon tetrachloride[2]. Then, the bed becomes transparent to the eye. The injection of a plug of solvent having a different refractive index makes the bed turn cloudy, milky, or white locally, during the passage of the band, depending on the local refraction index, i.e., on the relative concentration of the mobile phase and the sample. The migration, the broadening, and the dilution of the sample band can be followed easily. Viscous fingering is easily generated and its extent adjusted by appropriate changes of the viscosity of the sample injected. Unfortunately, still photographs give a poor rendition of the visual observation.

Fig. 1 compares the viscous fingering observed on two different columns (a and c), showing that viscous fingering occurs to a similar extent, irrespective of the column efficiency measured on the bulk effluent. The injection of acetone following a central point injection into column c avoided the inefficiency associated with the inlet frit and the valve injection device. This result clearly indicates that while heterogeneity of the column head may lead to variations in the onset of viscous fingering [7], the actual phenomenon of viscous fingering itself occurs in a highly efficient column as well as in a poorly packed bed. This is despite the fact that a poorly packed bed would increase mixing and hence dilute the interfacial region between the viscosity extremes which would then decrease the tendency for viscous fingering. Furthermore, Fernandez et al. showed that permeability differences beyond the column inlet do not substantially affect the development of fingers [7].

Fig. 2a–d compare the extent of viscous fingering achieved when 500 μl samples of four different solvents, acetone, methanol, ethanol and *n*-propanol, are injected (see viscosity and refractive index of each solvent in Table 1). Fig. 3a–d illustrate the influence of the flow-rate on the extent of the viscous fingering obtained with samples of dichloromethane. The injection of the least viscous solvent (acetone) produced a most intense viscous fingering (Fig. 2a). As predicted by theory [17], the interface between

[1] Being able to see what happens inside the column has its drawbacks too, as aptly stated by H. Eyring, 'What the eyes do not see does not bother the mind' [16].

[2] NB. The match is not as good between neat silica and CCl_4.

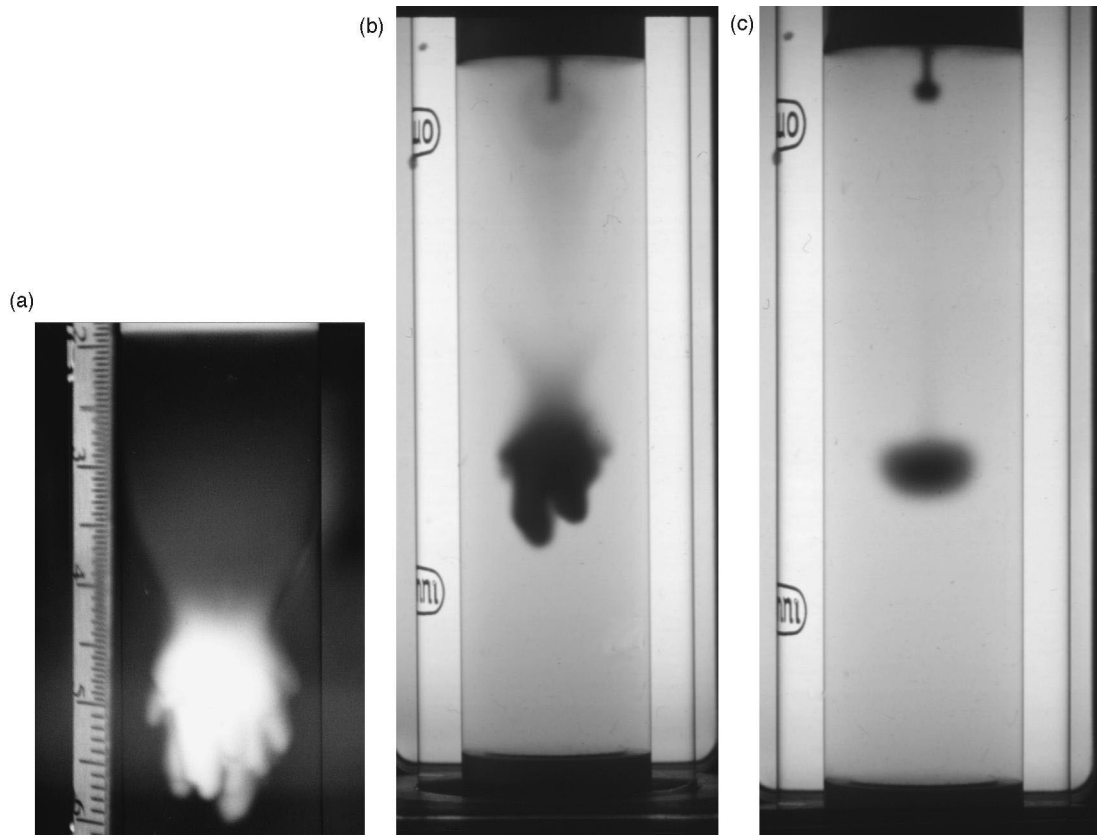

Fig. 1. Injection of neat solvent and saturated iodine solution. (a) Sample: acetone; flow-rate 2.0 ml min^{-1}. Column a. Valve injection. (b) Sample: acetone; flow-rate 1.0 ml min^{-1}. Column c. Central point injection. (c) Sample: saturated iodine in carbon tetrachloride; flow-rate 1.0 ml min^{-1}. Column c. Central point injection.

the front of the low viscosity acetone (η=0.30 cP) band and the more viscous carbon tetrachloride (η= 0.86 cP) was unstable. The sample front penetrated into the mobile phase, producing the fingering effect at the leading edge of the plug. The rear portion of the injection pulse remained uniform throughout the entire elution of the band.

When the viscosity of the solvent injected became closer to that of the mobile phase, the extent of viscous fingering decreased (Fig. 2b–d). With methanol (η=0.55 cP), flow instability was still observed, but the fingers were no longer resolved (Fig. 2b). Instead the band profile adopted the shape of a central knob keeping away from the wall region. It has been shown that slurry packed columns are heterogeneous, with a wall region in which the column permeability is lower than in the core region [18]. The viscosity difference causes an amplification of the consequences of a radial distribution of the local bed permeability [17]. With ethanol (η=1.04 cP), the front interface was stable while the rear interface was no longer so [17]. There was no viscous fingering in the front (Fig. 2c). Viscous fingerings in the rear of the band profiles are more difficult to illustrate because the low viscosity solvent (now, carbon tetrachloride) penetrates into the opaque region of the column. The refraction index of ethanol is too different from that of silica and the phenomenon, faintly visible to the eye cannot be photographed properly. A further increase in the viscosity of the sample injected produced a smooth band front for *n*-propanol (Fig. 2d) and some viscous

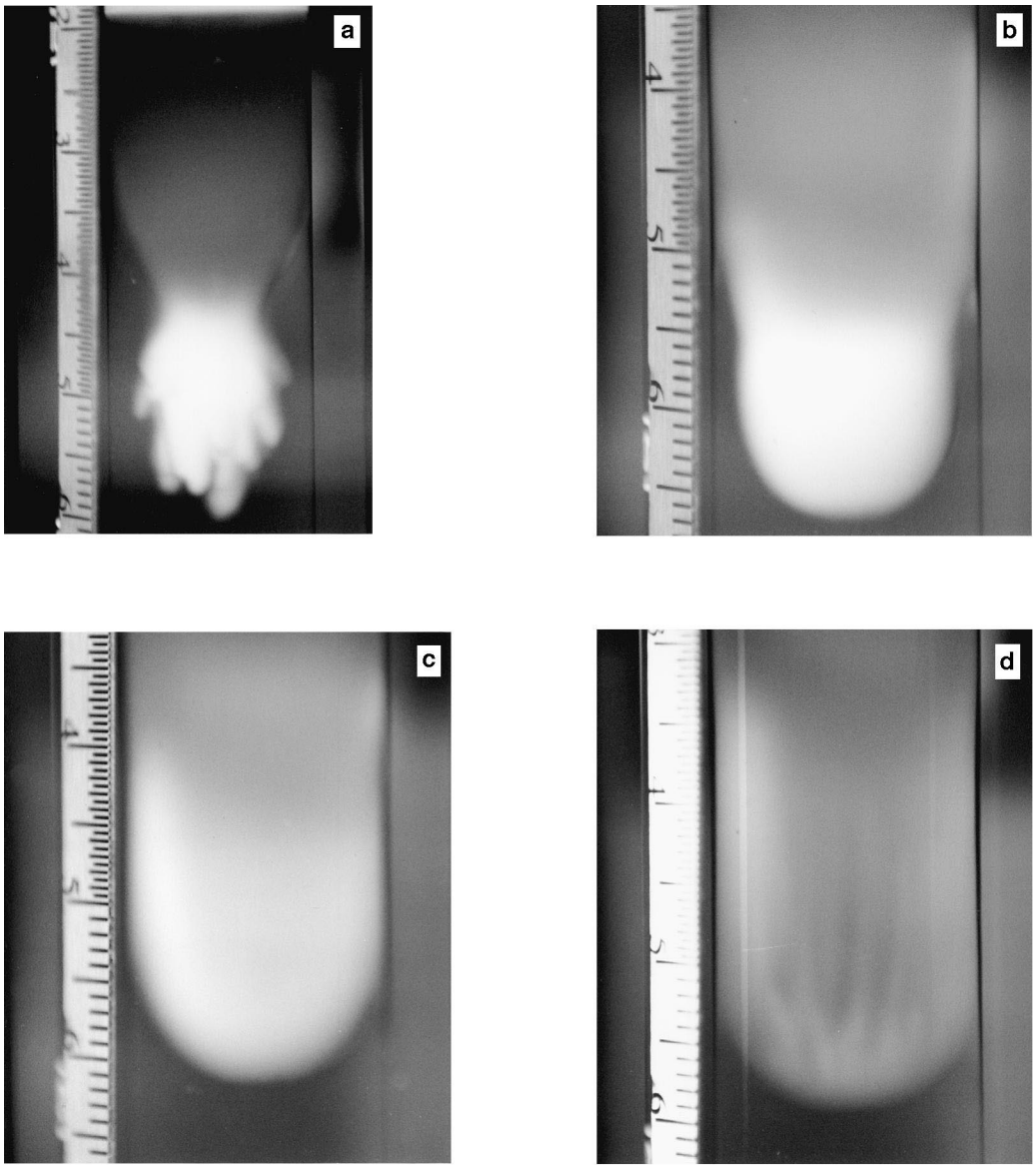

Fig. 2. Injection of 500 μl of neat solvent. Mobile phase: CCl_4; flow-rate 2.0 ml min^{-1}. Column a. The scale is graduated in centimeters; (a) acetone (b) methanol (c) ethanol (d) *n*-propanol.

fingering in the rear of the injection band. These fingers are only faintly visible, but slightly more so than for the case of ethanol.

Measurement of the resulting chromatographic profile was not possible due to a large refractive index effect in the detector flow cell (see Table 1). The difference between the refractive index of the mobile phase and of the four solvents used here is of the order of 0.10. Because of the large volume of the samples injected, the apparent aperture of the UV beam changes considerably during the elution of the band and the signal of the UV detector is unreliable. As a result of this optical effect, accurate information on the band widths could not be observed with the

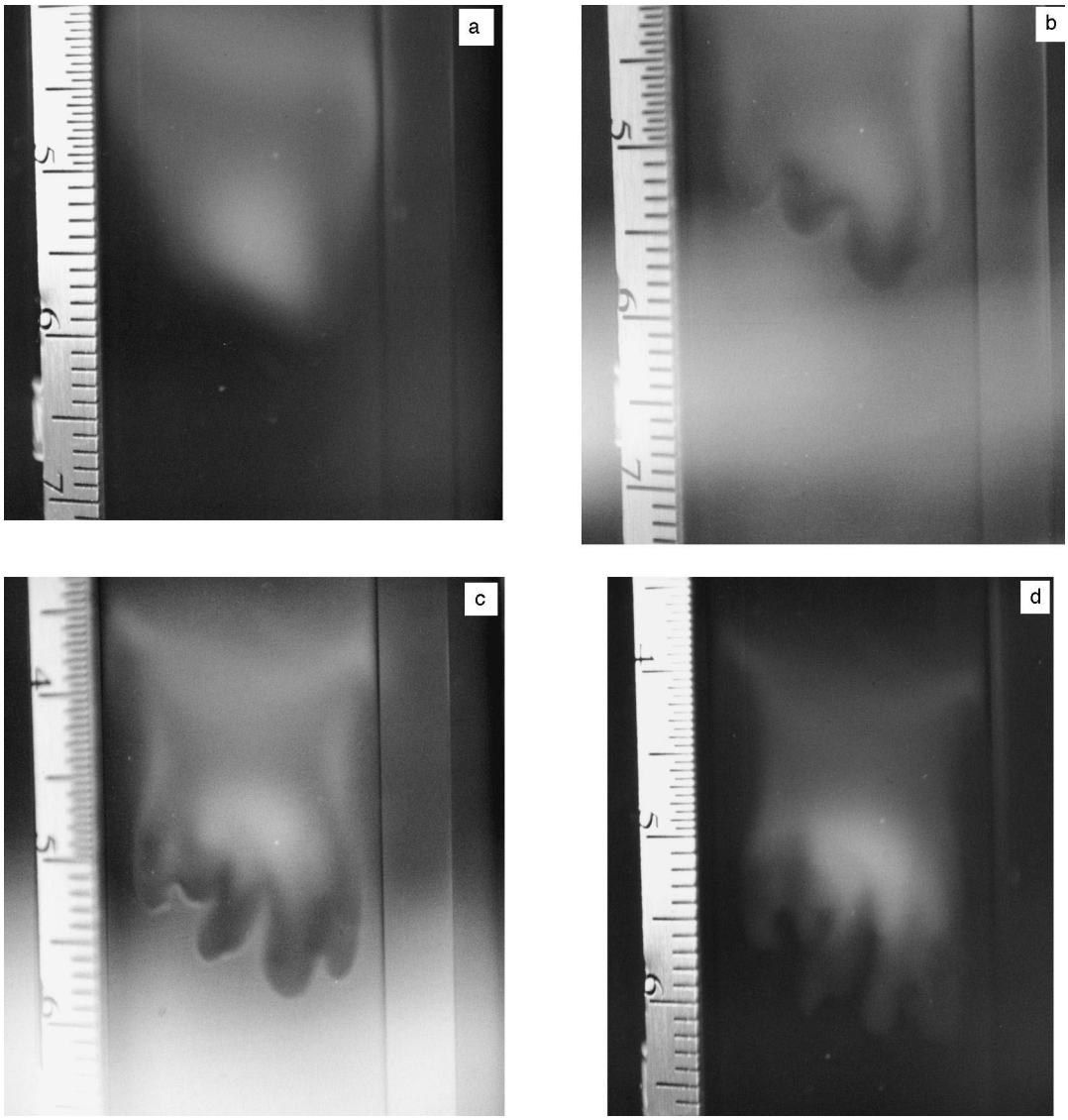

Fig. 3. Injection of 500 μl of dichloromethane containing 0.04% v/v of toluene. Mobile phase: CCl_4. Column b. Flow-rates: (a) 0.2 ml min^{-1} (b) 0.5 ml min^{-1} (c) 1.0 ml·min^{-1} (d) 1.3 ml min^{-1}.

conventional means of chromatography. Band widths were measured instead from the photographs, after an elution time of 2.60 min. The migration distances of the band front and rear are given in Table 2. These results show that the band width increased with increasing extent of viscous fingering. It should be noted, however, that, in the case of methanol in which flow instability caused only band distortion rather than the formation of resolved fingers, the band width was smaller than that of ethanol which gave no frontal viscous fingering. This could possibly be explained by the band distortion (Fig. 2b) causing the band to migrate in the core region of the bed, at an average velocity higher than when the band samples the whole cross-section area of the column. This could be confirmed by the fact that the retention times of the samples pulses decreased with decreasing sample viscosity (Table 2, the migration

Table 2
Width of the bands shown in Fig. 2a–d

Sample	Peak front[a] position (cm)	Peak rear[a,b] position (cm)	Peak width (cm)	Time (s)	Linear velocity of peak front (m/s)
Acetone	6.1	4.0	2.1	110	$3.8 \cdot 10^{-4}$
Methanol	6.25	4.2	2.05	144	$3.0 \cdot 10^{-4}$
Ethanol	6.1	4.0	2.1	156	$2.7 \cdot 10^{-4}$
n-Propanol	6.0	3.5	2.5	156	$2.6 \cdot 10^{-4}$

[a] Column head=1.9 cm.
[b] This position was determined as the center of the rear of the band taken subjectively from the developed photograph taken at the time specified. Peak tailing of the band is significant in all cases along the wall, however, this constitutes a minor portion of the overall band volume and width.

distances at a given time increased with increasing solvent viscosity).

Note that, in all our experiments, the low density solvent was above the high density solvent (carbon tetrachloride). This maintained hydrostatic stability. In the rear of the band, this stability was not achieved when carbon tetrachloride follows the light solvent, but this region has not been examined carefully because the clear fingers are inside the cloudy region where the refraction indices of the solvent and the packing materials do not match. Hydrostatic instability could be a problem when chlorinated solvents are used as the mobile phase. Because of various regulations, this tends to be an unusual case. We did not pursue its investigation.

3.2. Effect of the flow-rate on viscous fingering

The influence of the mobile phase flow-rate on viscous fingering was examined on both columns. In order to correlate the photographs of the bands (i.e., whole column refractive index detection) and the conventional chromatographic peak profiles, conventional chromatograms were recorded, using the response of the UV detector. The influence of the variation of the refractive index during the elution of the large solvent band was reported above. This effect was minimized by using dichloromethane as the sample solvent and adding a small concentration of toluene as a UV marker. Toluene eluted with exactly the same retention time in both dichloromethane and carbon tetrachloride. It was assumed that it was unretained under these non-adsorptive retention process (NARP) conditions and thereby was distributed homogeneously within the band volume, its concentration being proportional to that of the sample solvent, everywhere, all the time.

The photographs in Fig. 3a–d show an increasing extent of flow instability with increasing flow-rate, from 0.2 to 1.3 ml min^{-1}. This result is more dramatically illustrated in the degree of fingering observed in the photographs (Fig. 3a–d) than in the chromatograms (Fig. 4). The four photographs were recorded at nearly the same migration distance. Fingering was barely visible at the lowest flow-rate of 0.2 ml min^{-1} (reduced velocity, $\nu=0.30$), although the band profile was severely distorted. Although an efficiency curve was not obtained for this column, we believe that this flow-rate corresponds to the minimum reduced plate height of this column. As a result, molecular diffusion may explain the lack of resolution between adjacent fingers. By

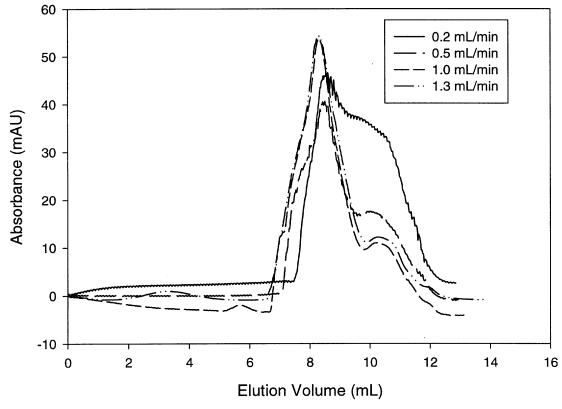

Fig. 4. Conventional chromatograms obtained as detector response at 275 nm at different flow-rates. Sample injected: 500 μl of a 0.04% (v/v) solution of toluene in dichloromethane. Mobile phase: CCl_4. Column b.

contrast an extensive degree of fingering was visible at 1.3 ml min^{-1} (reduced velocity approximately 2). In this last case a clear indication of partial band splitting and considerable band distortions are seen in the chromatograms (Fig. 4). This phenomenon is consistent with the observation that, in the photographs, the concentration of sample solvent appears to be highest at the tip of the fingers. The chromatograms in Fig. 4 also illustrate that the dependence of viscous fingering on the flow-rate decreases with increasing flow-rate. The largest deviation between the chromatograms occurred between 0.2 and 0.5 ml min^{-1}, while only a small difference in the elution profiles was observed between 1.0 and 1.3 ml min^{-1}. Over the range of reduced velocities tested (from approximately 0.3 to 2), the extent of

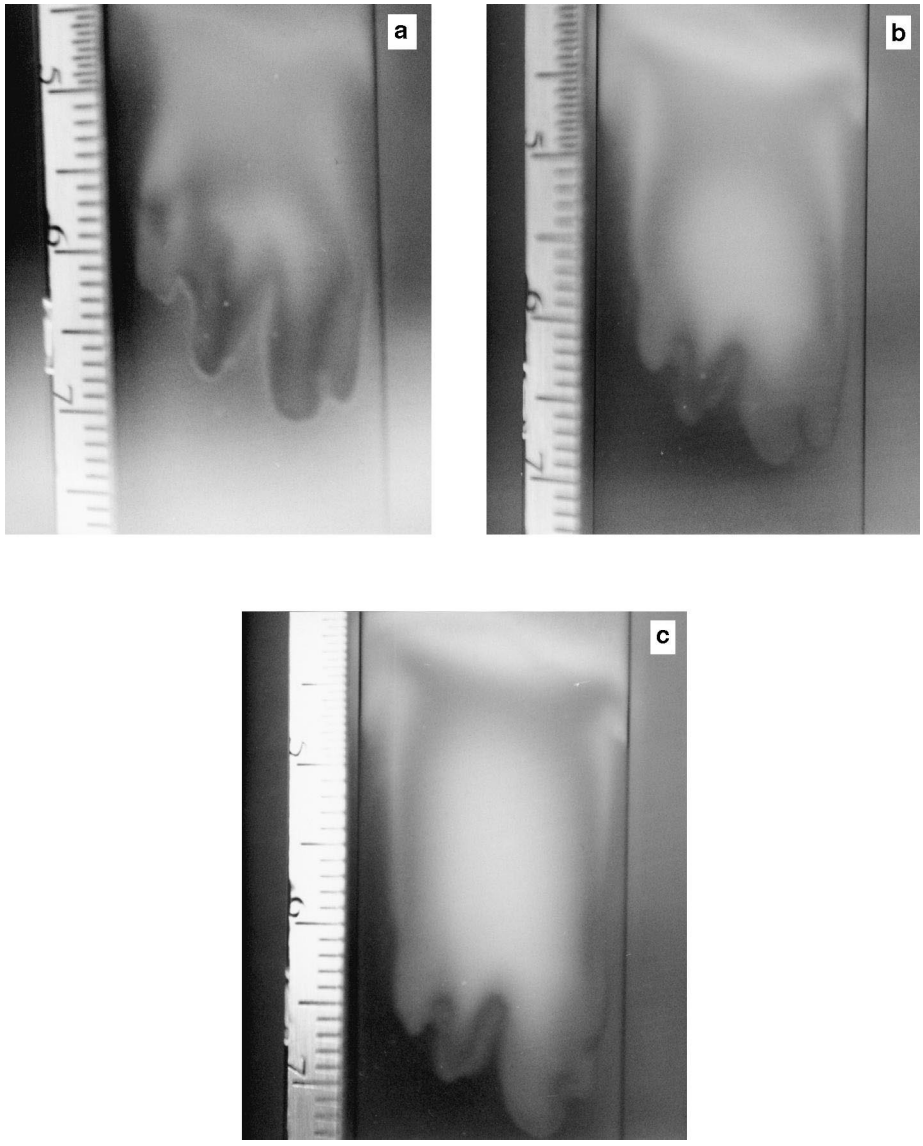

Fig. 5. Influence of the sample volume on the extent of viscous fingering. Sample: 0.04% toluene in dichloromethane. Mobile phase: CCl_4. Column b. Sample volumes (a) 500 μl (b) 1000 μl (c) 1500 μl.

viscous fingering was observed to be dependent upon the flow-rate, but regardless of this extent or of the finger resolution, chromatographic performance was severely affected. Finally, note in Fig. 3c and d that the average length of the viscous fingers is approximately equal to the column radius and that the average diameter of these fingers is slightly less than half the column radius.

The chromatograms (Fig. 4) suggest that the retention volumes of the bands decreased slightly with increasing flow-rate. Note also that the relative intensity of the rear shoulder of these bands decreased with increasing flow-rate. The broadest shoulder was observed for the lowest flow-rate of 0.2 ml min^{-1}. Also, the increase in the extent of fingering observed at high flow-rates (see Fig. 3a–d) explains the marked decrease in the breakthrough volume of the front of the bands seen in the elution profiles (Fig. 4) when the flow-rate increased.

3.3. Effect of the volume of solvent injected

The influence of the injection volume on the extent of viscous fingering is illustrated in Fig. 5a–c which show photographs of the bands obtained at nearly the same migration distance, upon the injection of volumes of 500, 1000 and 1500 μl of a solution of 0.04% v/v toluene in dichloromethane. These volumes are typical of those injected in preparative scale chromatography on a column the size of the one used here (17 mm I.D.). The corresponding band profiles are shown in Fig. 6. As these results show, the lengths and sizes of the viscous fingers tend to decrease slightly with increasing sample size but the intensity of the distortion of the band profiles increased with increasing injection volume. The retention volume of the band front increased from approximately 6.7 ml to approximately 7.4 ml when the injection volume was increased from 500 μl to 1500 μl. The band front became more distorted and the band maximum shifted toward higher elution volumes when the injection volume was increased. Interestingly, however, the extent of peak splitting, which is a general characteristic of viscous fingering, appeared to decrease slightly with increasing injection volume as can be seen by the smoothing trend of the rear of the elution profile. This improvement in the rear of the peak shape, however, is more than offset by the increase in the irregularity of the band front.

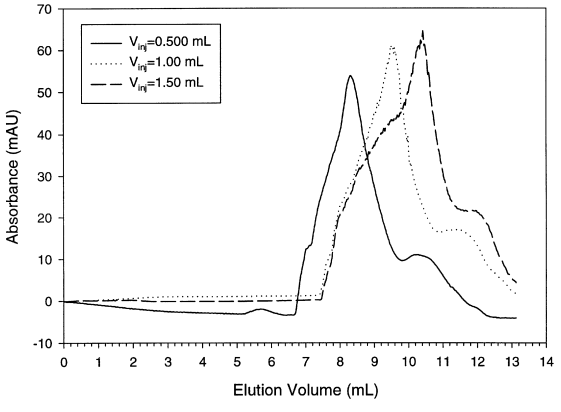

Fig. 6. Conventional chromatograms obtained as detector response at 275 nm, for different sample volumes. Sample: 0.04% (v/v) solution of toluene in dichloromethane. Mobile phase: CCl_4 . Flow-rate 1.0 ml min^{-1}. Column b.

The average length of the viscous fingers in Fig. 5a–c is close to the column radius. Although they are more clearly resolved in Fig. 5a–c than in Fig. 3a–d, their average diameter remains approximately the same, between a quarter and half of the column radius. These values are comparable to those reported by Fernandez et al. [6].

3.4. Reproducibility of viscous fingering

Several authors have reported the lack of reproducibility of the elution bands recorded under experimental conditions which lead to the formation of viscous fingering [2,3,19]. These studies have mainly investigated the most common situation in chromatography, the one in which the sample solution injected is more viscous than the mobile phase. So far in this study, by contrast, the injection pulse contains a solution less viscous than the mobile phase, because the photographs of viscous fingers are easier to make and of a higher quality. However, in order to study the reproducibility of viscous fingering under the conditions most relevant to preparative chromatography, a highly viscous injection solvent was used.

Most of the useful solvents that are less viscous than carbon tetrachloride have refraction indices that are very different from that of carbon tetrachloride

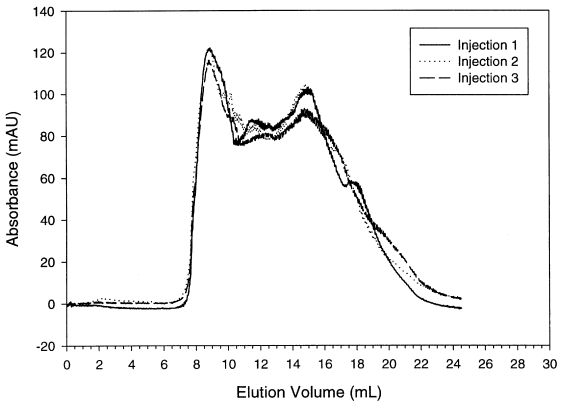

Fig. 7. Reproducibility of viscous fingering. Conventional chromatograms (UV detector at λ=275 nm) for triplicate 500 μl injections of a 0.08% (v/v) solution of toluene in cyclohexanol. Mobile phase: CCl_4. Flow-rate 2.0 ml min^{-1}. Column a.

(Table 1). As explained earlier, this leads to easy visualization but also to refraction index problems in the UV detector. On the other hand, solvents that have a higher viscosity than CCl_4 have a close refraction index and cannot be visualized as easily because of their transparent nature. The injection of polymers should be avoided because the viscous fingering observed would be a convolution of the viscous fingering of the injection plug itself and of the separation of the excluded band. The reproducibility had to be tested from the profile of an unretained elution band. Cyclohexanol (η=68 cP at 20°C) was chosen as the injection solvent, and a low concentration of toluene was added as a UV marker. This compound is most viscous and had to be warmed up before handling. Fig. 7 illustrates the elution profile of triplicate injections of cyclohexanol. The extent of band distortion and the lack of reproducibility of the profile indicate that a great deal of viscous fingering has occurred. This result confirms those of previous studies [2,3,19].

3.5. Column reversal and viscous fingering

In a classical study of the flow velocity distribution and the dispersion in an horizontal, rectangular channel partially obstructed by vertical cylindrical rods (thus, simulating the planar cross-section of a packed bed with a low packing density), Hiby [20] reported that dispersion in a packed bed is reversible. The flow velocity in his experiments was such that the Reynolds number (Re) was small and the flow was laminar and creeping. Hiby injected a narrow ribbon of a dye marker across the flow stream, the dye ribbon being perpendicular to the direction of the flow in the channel. Because of the uneven distribution of the flow velocity associated with each streampath across the different channels between the vertical rods (which simulate the packing of spherical particles), the ribbon rapidly became a complex, strongly curved line. When the flow was reversed, however, the dye marker traced its way back around the cylinders and returned to the exact location from where it originated. Diffusion caused only a moderate broadening of the straight ribbon.

It was important to determine whether this reversibility, which is universally accepted now in all studies of creeping flow, is also valid in the case of dispersion caused by the onset of viscous fingers. In order to examine the reversibility of viscous fingering in packed beds, it was very important to use a symmetrical column assembly with identical inlet and outlet frit geometry, so that the radial distribution of the flow velocity should be the same in either direction. The experimental design was adjusted accordingly.

Acetone was chosen as the injection solvent because of its low viscosity, its refraction index very different from that of carbon tetrachloride, and the impressive fingering obtained (see Fig. 2a). After the sample was injected, flow was allowed in the downward direction until the migration distance of the solvent band reached the six centimeter mark. At that point the flow was reversed using a switching valve. The series of photographs in Fig. 8 illustrate how, at first, viscous fingering develops during the band migration in the downward direction (Fig. 8a–d) and then how the viscous fingers retrace their path when the flow takes place in the reverse direction (Fig. 8e–h). The only residual change between the profiles of the original injection pulse and of the final pulse, when, after flow reversal, it reaches back the former column inlet, appears to be the result of axial dispersion (axial diffusion and the contribution of the local unevenness of the channels).

This result was not entirely expected. By contrast with creeping laminar flow which is steady and, obviously, reversible, viscous fingering takes place

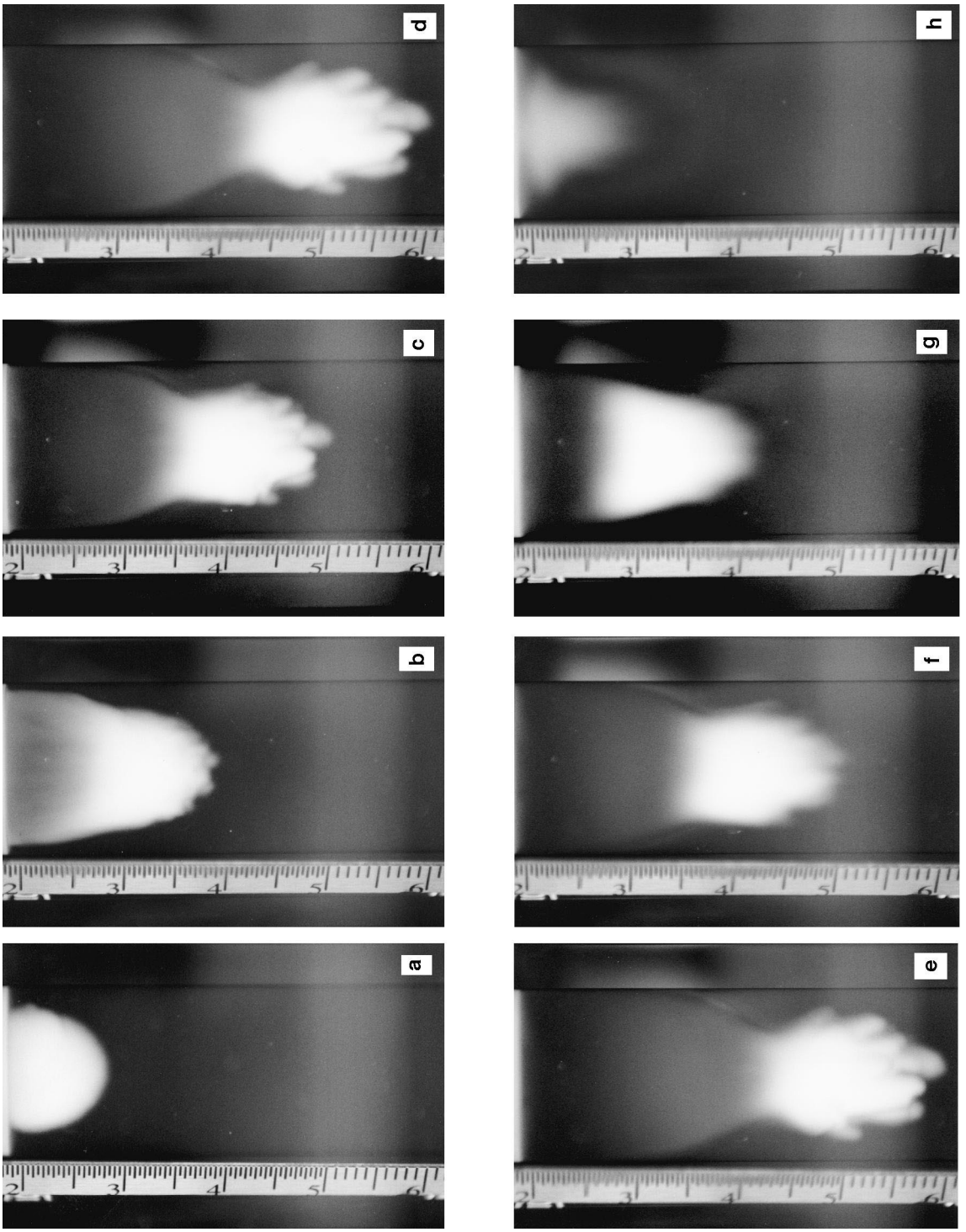

Fig. 8. Effect of flow reversal on viscous fingering. Sample: pure acetone; Mobile phase: CCl_4; Flow-rate 2.0 ml min^{-1}; Column a. Top row (a–d). Flow direction from top to bottom. Bottom row (e–h). Flow direction from bottom to top.

as the result of a flow instability [17]. In such a case, any little local fluctuation of the flow velocity is amplified. However, these fluctuations result from the local fluctuations of column permeability, which are related to the local variations of the packing density. The bed being itself stable, they are reversible because the experiment is carried out under conditions of creeping flow ($Re<0.01$). The streampaths along which the average viscosity is lower are those along which the velocity is higher. Those paths make the channels along which the fingers grow. When the flow is reversed, they are also the channels along which the reverse velocity is higher (because the average viscosity remains lower) but it is along these streampaths that the migration distances are also longer. When the migration time in the reverse direction is the same as it was in the forward direction at the time of flow reversal, the migration distances on all the streampaths are the same and a flat band is returned. A certain amount of irreversibilty results from axial diffusion and local eddy dispersion and explains the broadening of the final band. On the other hand, the large scale hydrodynamic contribution to dispersion is reversible. An interesting aspect of the phenomenon profile is that, as illustrated earlier (e.g., Figs. 2 and 3) viscous fingering occurred mainly in the central region of the column, not along the column wall. The band profile seemed to migrate away from the wall when fingering was initiated. Upon reversal of the flow, the band migrated back toward the wall.

4. Conclusion

Flow instability and the resulting detrimental effect on column performance caused by viscous fingering is an important concern in preparative chromatography because the use of high flow-rates, large injection volumes and high concentration samples is required. This can cause an important increase of the viscosity of the injection band compared to that of the mobile phase. As a matter of fact, an important increase in the viscosity of the mobile phase following an injection is accompanied by an increase in the head pressure required to maintain a constant flow-rate [21]. However, although observed more than infrequently, this effect has not become a universal plague of preparative liquid chromatography.

Admittedly, the effect cannot be observed directly and documented, columns being most often metallic and, when in glass or silica, used with solvents the refraction index of which rarely matches that of the packing material. Viscous fingering then is observed by its consequences, excessive band broadening and irregular elution profiles with a string of peaks on one side of the profile [1,19]. In most practical cases of preparative chromatography, the solution of the sample components in the mobile phase is more viscous than the mobile phase, so the leading edge is stable, the tail edge, unstable. However, in most cases of column overloading, the isotherms are convex upward. This causes the front of the band to be self-sharpening and its tail to be diffuse. Then, the region in which the viscosity varies rapidly is stable. In the region in which the less viscous fluid follows the more viscous one and actually pushes it, the composition varies progressively and, although we do have an unstable situation, the consequences of this instability are less dramatic. In most cases, it takes a high sample concentration to cause a large enough variation of the viscosity and the onset of viscous fingering. In such a case, a marked increase of the head pressure will be observed at constant flow-rate [21]. When the mobile phase is a solution of water and methanol or acetonitrile, the situation is more complex and the variation in viscosity remains probably moderate.

This observation and the results of this study allow the following conclusions. If sample injection causes a variation of the head pressure at constant flow-rate (or of the flow-rate at constant head pressure) which is less than 10–15% of the value observed with the pure mobile phase, there is no significant risk of viscous fingering effect. If this increase is between 10 and 25% and the equilibrium isotherms are convex upward (e.g., Langmuirian), viscous fingering, if any, should not cause much concern. Finally, if the excursion in head pressure is high and viscous fingering results in a severe perturbation of the band profiles and large losses of the production rate or the recovery yield, the flow-rate should be decreased and/or the sample injected in a larger volume of as a more dilute solution or in a solution in a solvent less viscous (but not stronger) than the mobile phase.

Acknowledgements

This work was supported in part by Grant DE-FG05-88ER13859 of the US Department of Energy and by the cooperative agreement between the University of Tennessee and the Oak Ridge National Laboratory. We thank Joseph J. Kirkland (Hewlett-Packard) for suggesting the method of band visualization used in this work. In addition, we thank Carol Broyles and Gary Bowman for the loan of the camera equipment.

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