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# Peak distortion in reversed-phase liquid chromatography as a consequence of viscosity differences between sample solvent and mobile phase

Cecilia B. Castells[a,b], Reynaldo C. Castells[a,b,*]

[a]*CIDEPINT, 52 e/121 y 122, 1900 La Plata, Argentina*

[b]*División de Química Analítica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentina*

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**Abstract**

Solute peaks may suffer severe distortions when their solutions in solvents whose viscosities differ from those of the mobile phase are injected in typical RPLC columns. A series of experiments with carefully chosen systems were performed, employing octyl silica columns, sample solvents whose viscosities were higher or lower than those of the corresponding mobile phases, and solutes that were more or less retained then the corresponding sample solvents. For a given viscosity difference, the importance of the distortions increases as the difference between solute and sample solvent retention times decreases. Chromatographic systems can be classified into four categories, and chromatograms illustrative of all of them were obtained. © 1998 Elsevier Science B.V.

*Keywords:* Peak shape; Viscosity; Mobile phase composition

## 1. Introduction

Viscous fingering has been made responsible for the distortion of size-exclusion chromatographic peaks in several cases. The viscosity drop at the rear boundary of a viscous sample plug results in hydrodynamic instability; fingers of mobile phase penetrate into the sample while portions of sample solution are delayed. The solute band broadens, tails and peaks with several maxima may eventually be detected. Viscosity gradients are relaxed by chromatographic dispersion, and the importance of fingering shall be the result of these two opposite factors. Moore [1] was the first author to associate peak distortion in SEC with viscous fingering. Czok et al. [2] studied the effects of sample and mobile phase viscosities on peak shape, and detected that instabilities occur at the front boundary of the sample plug when this is less viscous than the mobile phase, in accordance with the predictions of an instability criterion proposed several years before in a nonchromatographic context [3,4]. As an irrefutable proof of its existence, viscous fingering was recently visualized by magnetic resonance imaging [5].

It is proper to ask if viscous fingering is possible when using HPLC modes different from SEC. Samples most usually analyzed in these cases are diluted solutions of substances of low to medium molecular weight, and hydrodynamic instabilities cannot be expected when mobile phase is employed as sample

*Corresponding author.

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solvent. However, in many cases nonpolymeric solutes must be injected in solvents whose viscosities are very different from that of the mobile phase; choice of those solvents can be dictated by their efficiency and/or selectivity to extract some analyte from a complex matrix, because of advantages of those solvents as the medium to perform some precolumn derivatization reaction, etc.

Under the circumstances mentioned, the sample solvent plug shall suffer the effects of hydrodynamic instability, although usually this is not perceived because the conditions at the detector are chosen to optimize the solutes signal. However, so long as both travel together along the column for a sufficient time, the solute band shall be deformed by action of the fingered solvent band, thus providing an indirect proof of viscous fingering. It can be predicted that distortions of the solute band shall be affected not only by differences between sample solvent and mobile phase viscosities but also by the solute and the sample solvent migration velocities. Several studies of peak distortion in RPLC that may be explained in terms of viscosity mismatch were detected in a nonexhaustive survey of the literature [6–12]; some of their authors, however, suggested explanations based on differences between the solvent strengths of mobile phase and sample solvent.

Peak distortions generated in an aminopropyl silica column using arbutin as probe solute were studied in a recent work [13]. Distortions appeared at the rear of the solute band when water was used as the mobile phase and arbutin was injected in 2-propanol–water mixtures; the severity of the distortions increased when the viscosity of the sample solvent or the injected volume were increased and when the flow-rate was diminished. On the other side, the front of the solute band distorted when 2-propanol–water (50:50) was the mobile phase and arbutin was injected in less viscous solvents such as water or acetonitrile–water. Symmetric peaks were always obtained when the mobile phase was used as sample solvent. An experimental fact of the utmost importance was that successive injections, under identical conditions, may produce different chromatograms; this is a strong indication of the non-equilibrium origins of the phenomenon and enables us to discard explanations based on solvent strength differences, at least for this experimental setup.

Arbutin and 2-propanol elute together from aminopropyl silica columns and are poorly retained, thus fulfilling the best conditions for the manifestation of fingering. The present paper is a consequence of the previous work; in it the response of an RPLC column to the injection of samples whose viscosities differ from that of the mobile phase and where solute and sample solvent have different retention times are investigated.

## 2. Experimental

The HPLC instrument (Shimadzu LC-10A) consisted of a DGU-2A helium degassing unit, a LC-10AD pump, a Sil-10A autoinjector with a 50-μl sample loop and a SPD-M10A diode array detector connected to a Class-LC10 workstation. Detection of phenol, *o*-nitrophenol and 2-methylanisole was carried out at 280 nm and benzene was detected at 260 nm. Disturbances in the base line were monitored at 200 nm as indicative of methanol, 2-propanol or acetonitrile elution.

RPLC columns were Beckmann Ultrasphere octyl silica (250×4.6 mm I.D., 5-μm spherical particles). Column temperature was adjusted to 30°C by means of a water jacket.

HPLC-grade acetonitrile, methanol and 2-propanol were purchased from E.M. Science. All other reagents were of analytical grade: benzene and toluene were obtained from Merck (Darmstadt, Germany), 2-octanol and 2-methylanisole were purchased from Aldrich and phenol was purchased from Mallinckrodt. Water was purified by passing distilled water through a Milli-Q System (Millipore). All the solutions were filtered through 0.22-μm Nylon membrane filters (Micron Separations) before injecting.

## 3. Results and discussion

The chromatograms shown in Fig. 1 were obtained in two successive injections of phenol dissolved in methanol–water (78:22) using acetonitrile–water (75:25) as the mobile phase. The unrepeatibility shown in Fig. 1 is an important characteristic of the phenomenon under study: once instability is manifested by an elution profile, it is not possible to

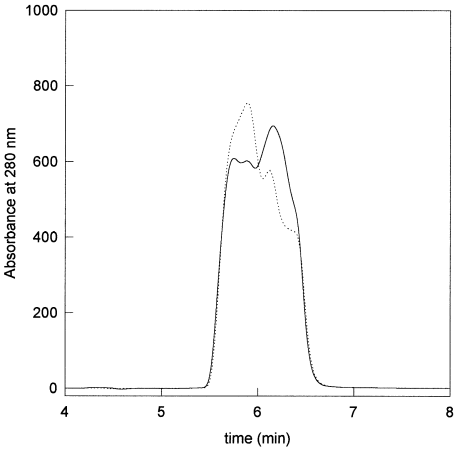


Fig. 1. Two successive injections under identical conditions. Mobile phase: acetonitrile–water (75:25). Sample: 20 μl, phenol, 0.33 mg/ml dissolved in methanol–water (78:22).

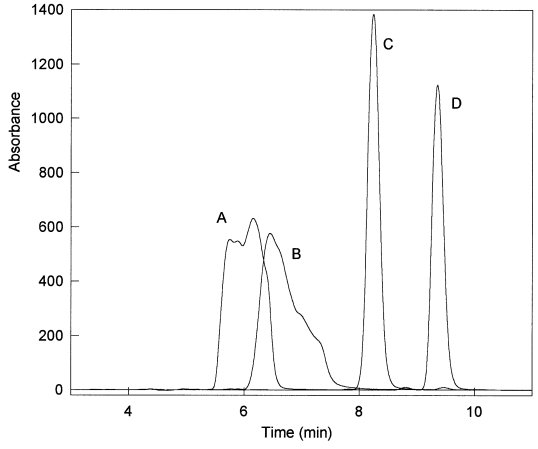


Fig. 2. Superposition of four chromatograms. Mobile and sample solvent: as in Fig. 1. A: 20 μl, phenol, 0.33 mg/ml. B: 50 μl, *o*-nitrophenol, 0.12 mg/ml. C: 50 μl, benzene, 1.52 mg/ml. D: 25 μl, 2-methylanisole, 0.60 mg/ml. Detection wavelengths: A, B and D at 280 nm; C at 260 nm.

reproduce the exact location of its maxima and shoulders. This would not be expected in case peak deformation were caused by differences between the sample solvent and the mobile phase solvent strengths; furthermore, in this particular case Snyder's solvent strength parameters [14] were matched.

In an attempt to demonstrate that the distortion of the elution profiles are determined by differences between sample solvent and mobile phase viscosities and between solute and sample solvent retention times, the chromatograms obtained in the present work have been classified into four cases that shall be discussed separately. Mobile phases and sample solvents viscosities at 30°C have been gathered in Table 1 to facilitate the reading. Chromatograms were obtained in the 250×4.6 mm column, at a flow-rate of 0.5 ml/min, unless otherwise stated. Viscosity and retention time are denoted by the usual symbols $\eta$ and $t_R$, respectively.

### 3.1. Case I: $\eta_{sample\ solvent} > \eta_{mobile\ phase}$; $t_{R,\ solute} > t_{R,\ sample\ solvent}$

Chromatograms separately obtained for four solutes with different $t_R$ have been superposed in Fig. 2; the mobile phase (acetonitrile–water, 75:25) and the sample solvent (methanol–water, 78:22) were the same in the four opportunities; base-line perturbation registered between 4.3 and 5.3 min, is produced by

Table 1
Viscosities at 30°C of mobile phases and sample solvents

| | Mobile phase | | Sample solvent | |
|---|---|---|---|---|
| Case | Composition | Viscosity | Composition | Viscosity |
| I | ACN/W 75:25 | 0.48 | MeOH/W 78:22 | 1.05 |
| I | ACN/W 75:25 | 0.48 | 2-PrOH/W 57:43 | 2.50 |
| I | ACN/W 90:10 | 0.43 | MeOH/W 78:22 | 1.05 |
| II | ACN/W 75:25 | 0.48 | 2-Octanol | 4.80 |
| III | MeOH/W 78:22 | 1.05 | ACN/W 75:25 | 0.48 |
| IV | 2-PrOH/W 65:35 | 2.47 | Toluene | 0.52 |

Units: Composition, % v/v. Viscosity, cp.
Symbols: W=water; ACN=acetonitrile; MeOH=methanol; 2-PrOH=2-propanol; Viscosities were taken from: ACN/W and MeOH/W, Ref. [15]; 2-PrOH/W, Ref. [13]; toluene and 2-octanol, Ref. [16].

elution of methanol and disturbance of the distribution equilibrium of acetonitrile. Phenol and *o*-nitrophenol, eluting very near from the sample solvent, show highly distorted elution bands; benzene and 2-methylanisole are separated from the sample solvent in the first segments of the column and as a consequence elute as normal peaks. Retained solutes separate from the sample solvent in the first portions of the column, obviously because of a more intense interaction with the stationary phase; poorly retained solutes, on the opposite side, travel through longer column lengths accompanying the sample solvent band.

The chromatograms obtained by injecting phenol dissolved in mobile phase (acetonitrile–water, 75:25), in methanol–water (78:22) and in 2-propanol–water (57:43) are compared in Fig. 3. The instability criterion developed by Hill [3] and by Saffman and Taylor [4] (as well as previous chromatographic experience [2,13]) predicts that the rear boundary of a viscous plug shall be fingered by the less viscous mobile phase; distortions at the rear of the solute peaks in chromatograms B and C are thus accounted for by the fingering of the eluent into the sample and become more evident as the viscosity mismatch increases. However, deformation at the front of these two peaks is also evident, although the aforementioned criterion predicts a stable front for a viscous plug. To understand this behaviour it is necessary to realize that the front of the solute band must be passed through by the fingered rear of the less retained sample solvent band. Therefore the shapes of both the front and the rear of the solute peak may be affected by the fingering of the sample solvent.

The chromatograms shown in Fig. 4 were obtained by injecting *o*-nitrophenol dissolved in the same solvents and using the same mobile phase as in the former figure. The rear of the solute peaks are less distorted than those in Fig. 3, although the injected volume was 2.5 times larger. The larger retention time of *o*-nitrophenol determines that the rear of its peaks remain in contact with the rear of the sample solvent band for a shorter time than in the case of phenol. On the other side, the chances of smoothing out distortions by the action of chromatographic dispersion are larger, the longer the retention time.

These trends are more notorious in the case of the even better retained benzene. Fig. 5 shows the coincidence between the rear boundaries of the chromatograms obtained when this solute is injected, dissolved in mobile phase, in methanol–water and in 2-propanol–water (benzene separates very rapidly from the sample solvents, and any residual distortion produced in this part of its peaks is smoothed out by chromatographic dispersion). The fronts of the peaks

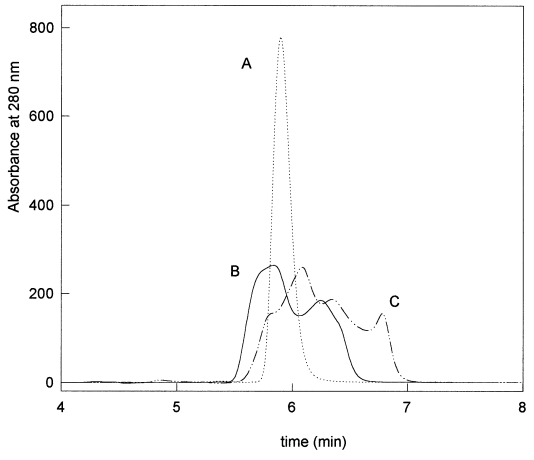


Fig. 3. Superposition of three chromatograms for phenol. Mobile phase: as in Fig. 1. A: 20 μl, 0.09 mg/ml in mobile phase. B: 20 μl, 0.10 mg/ml in methanol–water (78:22). C: 20 μl, 0.11 mg/ml in 2-propanol–water (57:43).

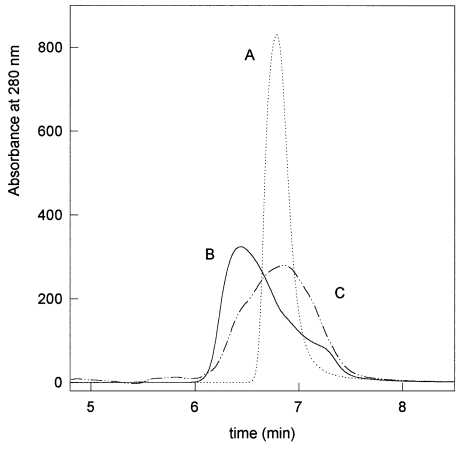


Fig. 4. Superposition of three chromatograms for *o*-nitrophenol. Mobile phase: as in Fig. 1. A: 50 μl, 0.04 mg/ml in mobile phase. B: 50 μl, 0.05 mg/ml in methanol–water (78:22). C: 50 μl, 0.04 mg/ml in 2-propanol–water (57:43).

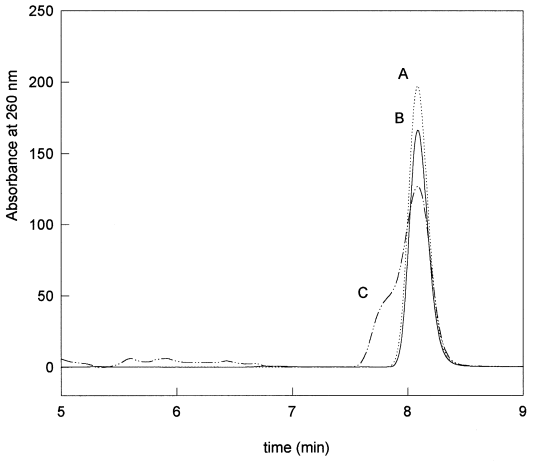


Fig. 5. Superposition of three chromatograms for benzene. Mobile phase: as in Fig. 1. A: 20 μl, 0.68 mg/ml in mobile phase. B: 20 μl, 0.55 mg/ml in methanol–water (78:22). C: 20 μl, 0.90 mg/ml in 2-propanol–water (57:43).

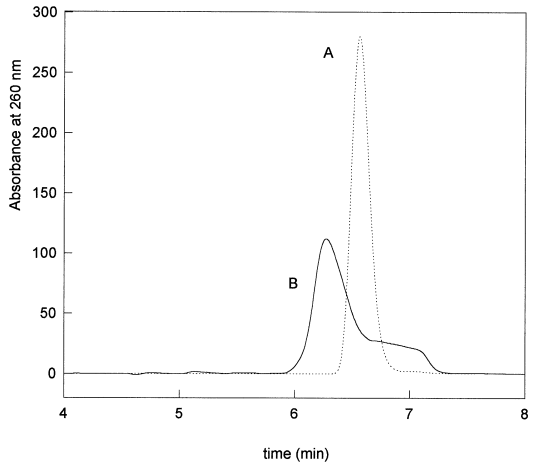


Fig. 6. Superposition of two chromatograms for benzene. Mobile phase: acetonitrile–water (90:10). A: 40 μl, 0.59 mg/ml in mobile phase. B: 40 μl, 0.55 mg/ml in methanol–water (78:22).

obtained using the mobile phase and methanol–water are also coincident, but not that corresponding to the more viscous 2-propanol–water solvent mixture. The retention time of benzene is diminished to a value intermediate between those corresponding to phenol and to *o*-nitrophenol in the former situation by changing the mobile phase to acetonitrile–water (90:10). Under this new condition both the front and the rear boundaries of the peak, produced by injecting benzene dissolved in methanol–water (78:22), are distorted as a consequence of a larger contact time between the solute and the sample solvent band (see Fig. 6).

### 3.2. Case II: $\eta_{sample\ solvent} < \eta_{mobile\ phase}$; $t_{R,\ solute} > t_{R,\ sample\ solvent}$

The instability criterion predicts that the forward interface of the sample plug can penetrate into the mobile phase; solute molecules shall be carried by the sample solvent, invading the precedent eluent stream. Since the sample solvent band is faster and its rear boundary is hydrodynamically stable, the rear of the solute band remains unperturbed. These trends are clearly shown by the chromatograms in Fig. 7, obtained using methanol–water (78:22) as mobile phase and injecting solutions of phenol in the mobile phase and in acetonitrile–water (75:25). The chromatograms obtained under the same conditions with *o*-nitrophenol and with benzene indicate that as the solute retention time increases, the effects at the front boundary become weaker, because of a shorter contact time between solute molecules and the fingered solvent and because chromatographic dispersion has a longer time at its disposal to erode

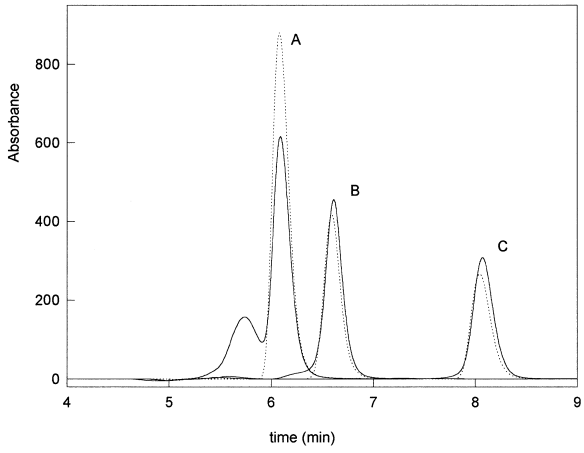


Fig. 7. Superposition of the elution profiles of three solutes, each dissolved in two solvents. Mobile phase: methanol–water (78:22). Dashed lines: solutions in mobile phase. Filled lines: solutions in acetonitrile–water (75:25). A: 20 μl, phenol, 0.09 and 0.10 mg/ml, respectively. B: 20 μl, *o*-nitrophenol, 0.05 and 0.06 mg/ml, respectively. C: 50 μl, benzene, 0.57 and 0.54 mg/ml, respectively. Detection wavelengths as in Fig. 2.

fingering effects produced at the first segments of the column.

### 3.3. Case III: $\eta_{sample\ solvent} > \eta_{mobile\ phase}$; $t_{R,\ solute} < t_{R,\ sample\ solvent}$

This, as well as the fourth case, is a less usual but not impossible situation in regular chromatographic practice; they were included to further demonstrate the correctness of the predictive scheme. Since the front of the sample solvent plug is stable and its rear is fingered by the mobile phase, and as the solvent lags behind the solute band, instabilities only at the rear of the solute elution profile are predictable. Fig. 8 shows the chromatograms of phenol when injected dissolved in the mobile phase and in 2-octanol; the characteristics of the sample solvent make the simultaneous record of its elution profile with a shape that coincides with that of the solute possible.

### 3.4. Case IV: $\eta_{sample\ solvent} < \eta_{mobile\ phase}$; $t_{R,\ solute} < t_{R,\ sample\ solvent}$

The front of the solvent band fingers into the leading mobile phase, and its rear is stable. Deformation only of the solute peak front could in principle be expected; however, the rear of the solute peak must pass through the distorted front of the solvent peak during the bands uncoupling process that leads to their separation. Fig. 9 shows the spoiling of both fronts of the phenol peak and the fingering of the front of the toluene peak; a highly viscous 2-propanol–water mixture (65:35) was the mobile phase.

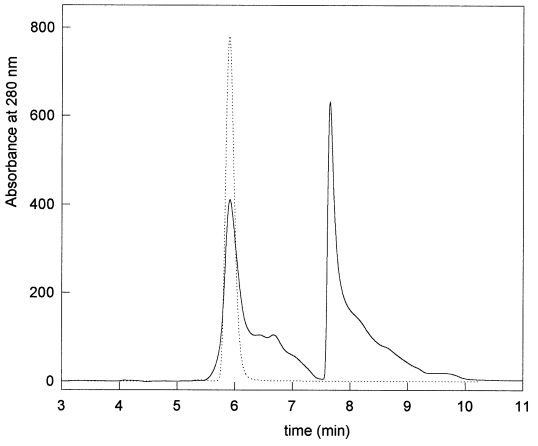


Fig. 8. Two chromatograms for phenol. Mobile phase: as in Fig. 1. Dashed line: 40 μl, 0.04 mg/ml in mobile phase. Filled line: 40 μl, 0.06 mg/ml in 2-octanol; the second peak corresponds to 2-octanol.

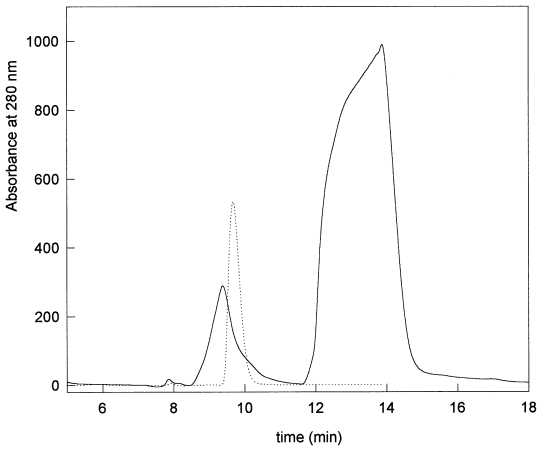


Fig. 9. Two chromatograms for phenol. Mobile phase: 2-propanol–water (65:35). Dashed line: 40 μl, 0.06 mg/ml in mobile phase. Filled line: 40 μl, 0.07 mg/ml in toluene.

## 4. Conclusions

Hydrodynamic instability of a sample solvent plug, arising from viscosity differences with the mobile phase, can produce distortions in the concentration profile of a solute eluting from an RPLC column. The intensity of the phenomenon depends on the degree of viscosity mismatch and on differences between solute and sample solvent migration velocities; for a given viscosity difference, the larger effects shall occur when solute and sample solvent elute together at a short retention time. This is probably the reason why, up to now, the phenomenon has only been reported in connection with SEC, a chromatographic mode where the viscosity mismatch is produced by the solute itself and the larger retention volumes correspond to the column hold-up volume.

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