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# Influence of differences between sample and mobile phase viscosities on the shape of chromatographic elution profiles

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**Abstract**

The injection of samples whose viscosities are appreciably different from that of the mobile phase can result in highly distorted elution profiles, showing several maxima. The distortions are produced at the rear of the band when the viscosity of the sample solvent is higher than that of the mobile phase. It is shown that the distortion of the peaks produced on injecting arbutin in columns of aminopropyl silica, using water as mobile phase and mixtures of 2-propanol and water as the sample solvent, grows in importance when the sample size increases, when the viscosity of the sample solvent increases and when the flow rate is decreased. On the other side distortions at the band front are observed when arbutin dissolved in water or in acetonitrile–water mixtures is injected in a 2-propanol–water (50:50) mobile phase of higher viscosity. These trends are coincident with the predictions of a hydrodynamic instability criterion postulated several years ago and up to now almost entirely ignored in chromatography, except in relation to size-exclusion chromatography. © 1997 Elsevier Science B.V.

*Keywords:* Viscosity; Mobile phase composition; Elution profiles; Arbutin

## 1. Introduction

The injection of samples dissolved in solvents different from the mobile phase can result in severe peak distortions. The peak for a single component can show a front or a rear shoulder, and in extreme situations double or multiple peaks are generated. This is a surprising behaviour since it has been shown for solutes that are easily soluble in the mobile phase and in the sample solvent and, furthermore, for eluents and solvents that are totally miscible.

Tseng and Rogers [1] observed that the dihydroxybenzenes gave simple symmetric peaks when water or methanol were used as sample solvent and as mobile phase; however each isomer produced a double peak when their solutions in water were injected in methanol as the mobile phase, and peaks with front shoulders resulted when methanol was the sample solvent and water was the eluent. Similar distortions and/or multiplication of peaks were reported by Kirschbaum et al. [2], Khachik et al. [3], Hoffman et al. [4] and by Vukmanic and Chiba [5]. Some of the quoted authors suggested theoretical explanations based on differences between the solvent strengths of the mobile phase and of the injection solvent, and peak splitting was simulated by means of a model that took into account changes

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in the capacity factor as the injection solvent moved through the column [6]. Zapata and Garrido [7] demonstrated that this explanation was highly questionable: chlorophyls produced single symmetric peaks when methanol–water (95:5) was the injection solvent, but multiple peaks were obtained when solutions in acetone–water (90:10) were injected in the same eluent. The solvent strengths of both sample solvents differed by only 7% according to Snyder's scale [8] and, furthermore, multiple peaks were produced by employing a acetone–water (69:31) mixture whose solvent strength matches exactly that of the methanol–water (95:5) mixture.

A totally different approach to the relationship between peak shape and sample solvent composition was followed by Czok et al. [9]. Using a high-performance size-exclusion chromatography column and an aqueous phosphate buffer as the mobile phase they observed that the shapes of the peaks produced by injecting uracil dissolved in several water–glycerol mixtures depended dramatically on glycerol concentration. A single, almost symmetric peak was obtained when 100% water was the injection solvent, but shoulders were generated at the rear of the peaks when solutions containing 10 and 17% glycerol were injected, and very broad peaks with several maxima were obtained for higher glycerol concentrations; all the chromatograms showed a maximum at a retention time coincident with that of the peak obtained by using the aqueous buffer as injection solvent. A single peak was obtained when a phosphate buffer containing 33% glycerol was the eluent and the sample solvent; peak deformations at the band front were produced by injecting uracil solutions containing less than 33% glycerol, and distortions at the band rear appeared for injection solvents with glycerol concentrations higher than 33%. The authors attributed this behaviour to hydrodynamic instabilities resulting from viscosity differences between the injected pulse and the mobile phase.

These effects have been described by the term "fingering", and a criterion of stability has been established in terms of viscosity and density differences [10,11]. The interface between two fluids that are moving vertically upwards within a packed bed with uniform velocity $u$ shall be stable if

$$(\mu_2 - \mu_1)u/k - (\rho_2 - \rho_1)g > 0 \qquad (1)$$

and unstable otherwise. Suffix 1 refers to the upper fluid and suffix 2 to the lower; $\mu$ denotes viscosity, $\rho$ the density, $k$ is the permeability of the bed and $g$ the acceleration due to gravity. When instability conditions are given and any packing fluctuation has caused the boundary to bulge in the flow direction, the bulge will grow giving rise to "fingers" of the less viscous fluid (suffix 2) that penetrate into the leading fluid. This effect has long been known in connection with the displacement of immiscible fluids in porous media; mixing tends to relax the boundary, thus restricting the possibilities of occurrence of fingering in systems composed of miscible fluids.

The displacement of a sample band through a chromatographic column shows some obvious peculiarities in connection with the phenomenon under discussion. Firstly, the permeability of the chromatographic bed is low, about $5 \cdot 10^{-10}$ cm$^2$ for 5 μm spherical porous particles [12]; this makes the density terms of Eq. (1) negligible compared to the viscosity terms. Secondly, since there are two boundaries between a chromatographic band and the mobile phase, any band whose viscosity is different from that of the eluent would become unstable; instabilities would occur at the band rear if the band is more viscous than the eluent, and conversely at the band front if the eluent is more visccous than the band. This would transform fingering into a rather ubiquitous phenomenon, present each time that a sample dissolved in a solvent different from the mobile phase is injected; the facts do not agree with this prediction. The reason is that the injected pulse suffers a rapid dilution process as it moves through the first section of the column and, since mixing acts in the direction of removing the conditions for the onset of fingering, the occurrence of the phenomenon shall depend not only on viscosity differences but also on the velocity of the dilution process. It can be predicted that sample size and flow-rate shall have a profound influence. Fingering can also pass unnoticed when a very fast separation between solute and sample solvent occurs in the very first portions of the column and the solute has a long retention time. The severely distorted peaks detected by Czok et al. [9] can in part be attributed to the fact that uracil and glycerol elute together; solute peak distortion is favoured by maximizing the residence time of

its molecules within the fingered sample solvent band. On the other hand uracil and glycerol retention volumes were coincident with the volume of mobile phase contained in the column; although a short retention time limits the extent of dilution, it is not a critical requisite for the distortion of the solute elution profile.

Multiple peaks and shoulders quoted in Refs. [1–5] may be considered as distortions of the single, unperturbed peak obtained by using mobile phase as the injection solvent; furthermore, it can be shown that these distortions are formed at the side of the sample band that could be predicted on the basis of eluent and sample solvent viscosities. For example, when Tseng and Rogers [1] injected any of the three dihydroxybenzenes dissolved in water ($\mu = 0.90$ cP) using methanol ($\mu = 0.51$ cP) as mobile phase, the retention time of the first peak was coincident with that of the only peak produced when methanol was the sample solvent, and a second, partially resolved peak appeared at the band rear (the pulse, with a larger viscosity, is penetrated by the mobile phase on its rear portion). Conversely, a front shoulder was produced when the roles of methanol and water were inverted (the pulse, with a lower viscosity, penetrates the mobile phase).

The incidence of sample viscosity on peaks shape has only been mentioned in the chromatographic literature in connection with the injection of highly viscous samples in size-exclusion columns [13]. The objective of the present paper is to demonstrate that under some conditions fingering can complicate or spoil results obtained in other forms of HPLC, and to study the effects of sample size, solvent composition and flow-rate on the phenomenon. Arbutin dissolved in water or in 2-propanol–water mixtures of different compositions is injected into an aminopropyl silica column using water as the mobile phase at several flow-rates. The viscosity of 2-propanol at 30°C is more than twice that of water, and the viscosity of 2-propanol–water mixtures shows a maximum close to the 50:50 composition. Some experiments were performed using this highly viscous mixture as the mobile phase. A very important property of the chosen experimental system is that arbutin and 2-propanol elute together from the aminopropyl silica column. Several more complex behaviours are possible when solute and sample solvent are separated by the column; the study of those more complicated systems demands, as a previous stage, the understanding of the system chosen for the present paper.

## 2. Experimental

### 2.1. Equipment

The HPLC experiments were performed with a Shimadzu LC-10A System, which consisted of a DGU-2A helium degassing unit, an LC-10AD pump, a Sil-10A autoinjector (50-μl loop), an SPD-M10A diode array detector and a CLASS-LC10 workstation. Detection of arbutin was performed at 285 nm.

### 2.2. Columns and reagents

All the chromatographic runs were performed with a Supelcosil LC-NH2, 5 μm spherical particles, 25 cm×4.6 mm I.D. column. The column was placed in a jacket through which water at 30°C was circulated. 2-Propanol and acetonitrile were purchased from E.M. Science, and arbutin was from Sigma. Water was obtained by means of a Milli-Q Waters Purification System (Millipore).

### 2.3. Viscosity measurements

Densities and kinematic viscosities of 2-propanol–water mixtures at 30°C were measured by means of a 5-ml pycnometer and of a No. 50 Cannon-Fenske viscometer, respectively. Relative viscosities, $\mu/\mu_{water}$, are plotted against composition in Fig. 1.

## 3. Results and discussion

Fig. 2 is a typical manifestation of fingering: arbutin, that elutes as a single symmetrical peak when the mobile phase is used as injection solvent, produces a peak with two maxima when a solution in a 2-propanol–water mixture is injected. The retention time of the most important peak in profile B is coincident with that of the peak in A; since the viscosity of the injection solvent in B is 3.2 times that of water, the rear of the band is fingered by the eluent. Several conditions for the manifestation of

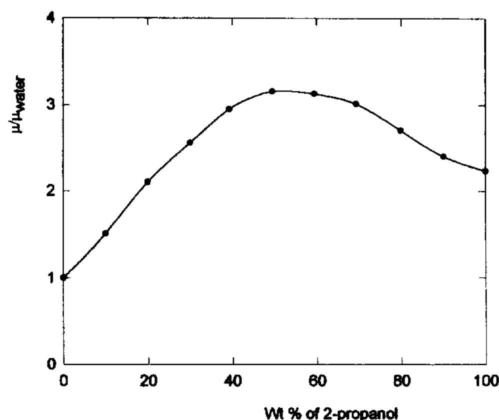


Fig. 1. Relative viscosities of 2-propanol–water mixtures at 30°C against composition.

fingering are combined in this system: (a) a large viscosity difference between the sample solvent and the mobile phase; (b) solute and sample solvent elute almost together (see peak C for pure 2-propanol); (c) since the solute has a short retention time, the possibilities of blurring the distortions in its concentration profile as a consequence of the several dispersion processes are minimized.

An important characteristic of the elution profiles produced by injecting arbutin dissolved in 2-propanol–water mixtures, with sufficient alcohol concentration, using water as mobile phase, is that all of them show a maximum in common. It is the first maximum of the profile and its retention time coincides with that of the symmetrical peak obtained by injecting arbutin dissolved in the mobile phase; however, it is not necessarily the most important maximum. This is the only repetitive characteristic and, in the cases of manifest instability, the rest of the profile is completely unpredictable. Furthermore, totally different elution profiles may be obtained under identical experimental conditions; the two chromatograms shown in Fig. 3 were obtained by two successive injections separated by a 10 min interval at 2 ml/min. These characteristics of the phenomenon are a clear indication of its origins; one could expect repeatability in case peak distortions were produced by differences between the sample solvent and the mobile phase solvent strengths.

The unrepeatability of the elution profiles obtained under instability conditions restricts the possibilities of studying the phenomenon. The effects of flow-rate, of sample size and of injecting a constant mass of arbutin dissolved in 2-propanol–water mixtures of

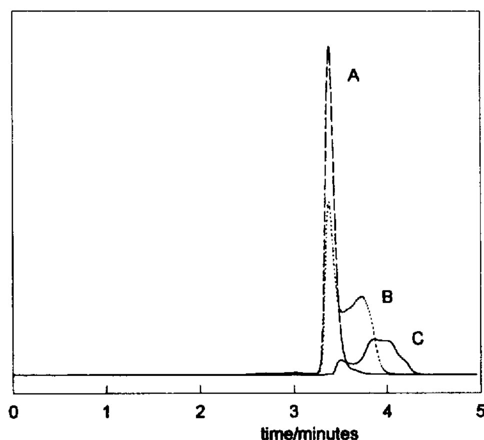


Fig. 2. Elution profiles of arbutin dissolved in two different solvents and of pure 2-propanol. (A) Arbutin dissolved in water (1.3 mg/ml), 5 μl; detection at 285 nm. (B) Arbutin dissolved in 2-propanol–water mixture (67:33; 1.5 mg/ml), 5 μl; detection at 285 nm. (C) 2-Propanol, 10 μl; detection at 200 nm. Mobile phase: water, 1.0 ml/min.

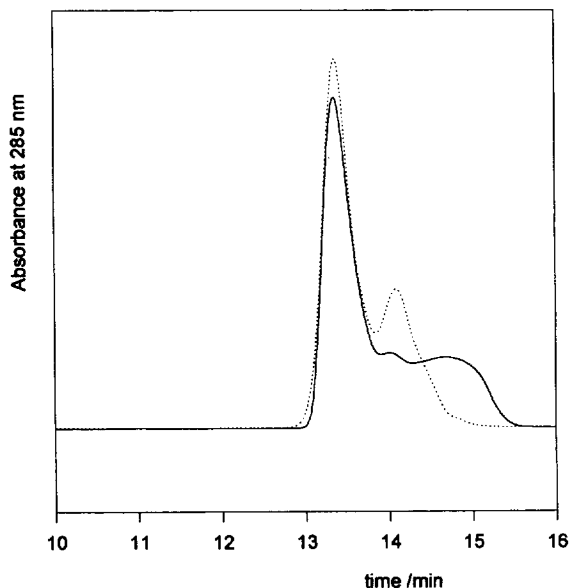


Fig. 3. Unrepeatibility of elution profiles. Two successive 5-μl injections of a solution of arbutin (1.6 mg/ml) in 2-propanol–water (65:35). Mobile phase: water, 0.25 ml/min.

Table 1
Sample size and flow-rate effects on the elution profiles of arbutin using water as mobile phase

| Sample size[a] (μl) | Flow-rate (ml/min) | | | | |
|---|---|---|---|---|---|
| | 2.0 | 1.5 | 1.0 | 0.5 | 0.25 |
| 1 | NF | NF | NF | NF | NF |
| 2 | NF | NF | NF | NF | NF |
| 4 | NF | NF | F | F | F |
| 5 | NF | F | F | F | F |
| 10 | F | F | F | F | F |
| 15 | F | F | F | F | F |

[a] The solution injected contained 1.6 mg/ml of arbutin in a 2-propanol–water (65:35) solvent.
NF: Fingering not manifested.
F: Fingering manifested.

different compositions were studied in the present work.

Sample size and flow-rate effects when water is used as mobile phase are summarized in Table 1 where results obtained by injecting different volumes of a solution containing 1.6 mg/ml of arbutin in a 2-propanol–water (65:35) mixture are gathered. Normal peaks are produced at small sample size and high flow-rate; the shapes of the elution profiles become more complicated as the injected volume is increased and/or the flow-rate is decreased and, at adequate values of these two parameters, very complex profiles, showing several maxima are obtained. The behaviour is well represented in Fig. 4, where the results obtained for 5 μl samples are shown. It should be stressed that the product (peak area × flow-rate) is constant within experimental error for the elution profiles shown in Fig. 4; the coefficient of variation is only 2.2%, a very small value on account of the errors in integrating the irregular peaks obtained at the smaller flow-rates. Therefore, the shapes of the profiles cannot be attributed to solute degradation or to any other chemical artifact occurring within the column.

A series of experiments was performed in order to detect relationships between peak shape and sample solvent composition at constant quantity of arbutin injected. All the chromatograms appearing in Fig. 5 were obtained by injecting 10 μl of solutions of arbutin containing 1.4 mg/ml; sample solvents contained from 10 to 60% (v/v) 2-propanol in water. Parts A and B were run at 1.0 ml/min and at 0.5 ml/min, respectively. The chromatograms clearly show the constancy of the retention time of the first maximum, as well as the increase of sample pulse penetration by the mobile phase as the sample viscosity increases. The areas under the peaks are again constant, with coefficients of variation smaller than 2% at both flow-rates.

Up to this point only systems in which the sample is more viscous than the mobile phase were considered; therefore only chromatograms showing constancy in the retention time of the first maximum and fingering of the mobile phase into the rear of the

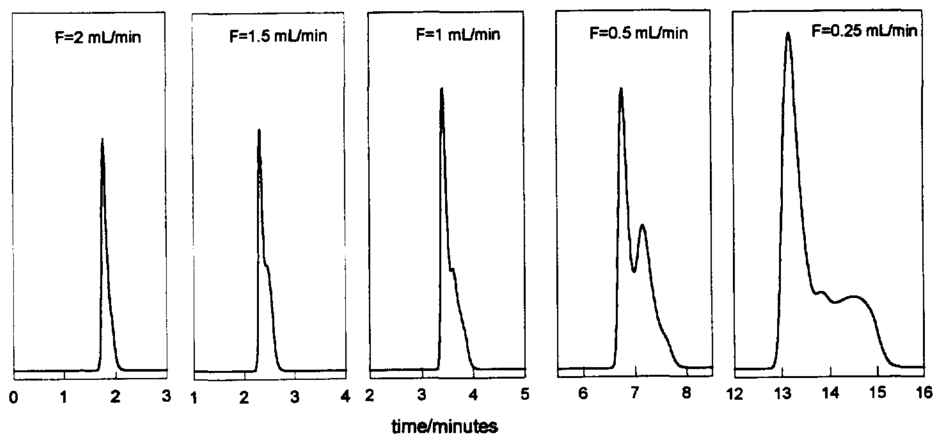


Fig. 4. Effects of flow-rate. Elution profiles for 5 μl of a solution of arbutin (1.6 mg/ml) in 2-propanol–water (65:35). Mobile phase: water, at the flow-rates indicated in the figure.

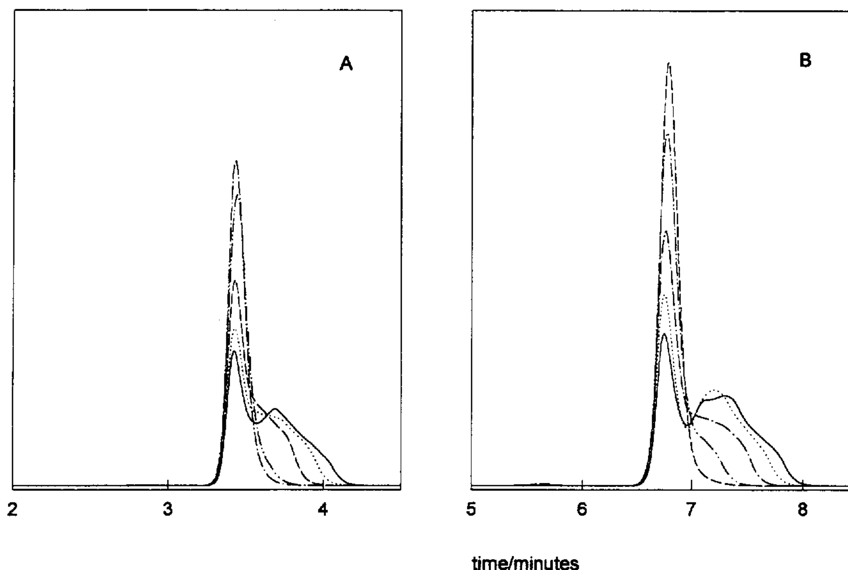


Fig. 5. Effect of sample solvent composition. Injected solutions: arbutin, 1.4 mg/ml, dissolved in 2-propanol–water containing (from top to bottom) 10, 20, 40, 50 and 60% (v/v) 2-propanol in water. Sample size: 10 μl. Mobile phase: water, at 1 ml/min in A and at 0.5 ml/min in B.

sample pulse have been shown. The chromatograms shown in Fig. 6 were run using a 2-propanol–water (50:50) mobile phase ($\mu$=2.50 cP at 30°C) at 0.5 ml/min. The three chromatograms correspond to the injection of 10 μl of solutions containing similar (although not exactly equal) concentrations of arbutin. The sample solvents were mobile phase in A, water ($\mu$=0.79 cP) in B, and acetonitrile–water (90:10) ($\mu$=0.43 cP) in C. The fingering of the less viscous pulses into the mobile phase is evident in B, and more notoriously in C. In this last case a maximum with a retention time coincident with that of peak A can be noticed; however profile C shows that when viscosity differences are very large other peaks of larger height can be produced. The chromatograms in Fig. 6 confirm the predictions of Eq. (1).

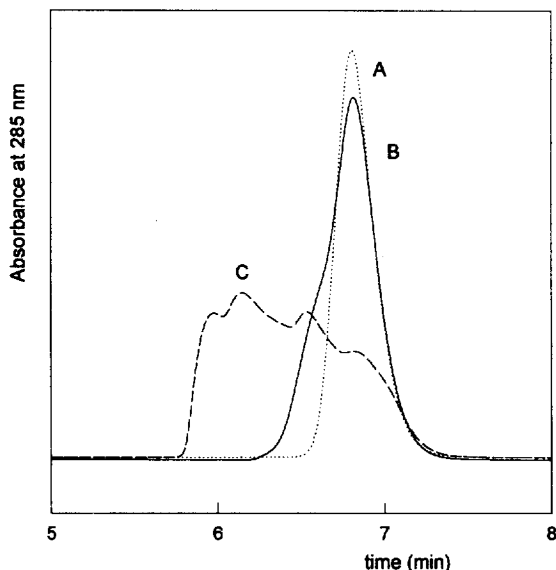


Fig. 6. Elution profiles obtained by using a 2-propanol–water (50:50) mobile phase at 0.50 ml/min. (A) 1.1 mg/ml arbutin in mobile phase. (B) 1.3 mg/ml arbutin in water. (C) 1.5 mg/ml arbutin in acetonitrile–water (90:10).

## 4. Conclusions

Fingering, a phenomenon originated in hydrodynamic instabilities in the boundary between two moving fluids of different viscosity, can produce important distortions in the shape of the chromatographic peaks. This constitutes a new artifact to be taken into account during the design, development and analysis of chromatographic separations since its

effects can easily be mistaken for unexpected components in the sample, can mask expected peaks or otherwise spoil chromatographic results. It constitutes another reason to obey the long practised rule of chromatographers of using the mobile phase as the injection solvent, as far as this practice is feasible.

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