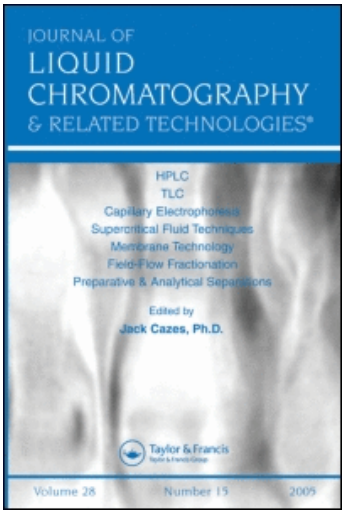

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B. Porsch[a]
[a] Institute of Macromolecuiar Chemistry Czechoslovak Academy of Sciences, Prague, Czechoslovakia

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EXPERIMENTAL CONSEQUENCES OF DIFFERENCES BETWEEN COMPOSITION OF THE SAMPLE SOLVENT AND OF THE MOBILE PHASE IN HPLC

B. PORSCH
Institute of Macromolecular Chemistry
Czechoslovak Academy of Sciences
162 06 Prague 6, Czechoslovakia

ABSTRACT

Anomalous peaks may be expected to occur even in cases when sample solubility in mobile phase is sufficient if sample solvent and mobile phase differ considerably in viscosity and/or elution power of sample solvent exceeds mobile phase strength substantially. Then, to improve peak shapes, the sample solvent — mobile phase viscosity ratio should be kept fairly below two and too high elution power of the sample solvent should be decreased by mixing with mobile phase prior to injection.

INTRODUCTION

The deleterious effects of injecting samples of appreciable volume into liquid chromatography systems where the sample solvent and mobile phase are different have been well known[1,2] for a long time. It is generally agreed that the two solvents should be the same in order to realize the best chromatographic performance. This general rule is frequently infringed in practice[3-12]. Among

other reasons, samples are often obtained in solvents different from mobile phase composition and the sample solubility becomes sometimes paramount in reversed phase chromatography, especially in preparative systems[13]. Therefore, the effect of difference between composition of the sample solvent and of the mobile phase on the chromatographic behavior of compound undergoing analysis has been studied with increasing interest[3]. Contradictory observations as anomalous peak shapes[3] and splitting of peaks[3-5] have been reported, and it has also been found that these effects differ depending on the chromatograph used[6]. An explanation was sought in interactions between compounds undergoing separation and solvents[7,8]. It seems, however, that this is a natural consequence of the dynamic gradient between composition of the sample solvent and of the mobile phase at the beginning of the column[9,10]. It was found[11], moreover, that these effects depend on the injected volume, on the configuration of the apparatus between the injection valve and column, and that they can be restricted by additional mixing with the eluent before the column is entered.

This indicates that a different extent of partial mixing is responsible for most of the observed contradictions. Recently described[14] HPLC preparative columns guarantee no mixing of sample with mobile phase before the column and at the column inlet due to the use of stop-flow injection of the sample into the sorbent bed. In fact, this system differs from conventional injection[16] in that the column packing exists on both sides of the injection plane. The main advantage[14] is then symmetrical peaks even in the case of higher loadings, where conventional column configuration already exhibits considerable peak asymmetry[17]. Other features of the injection into the sorbent bed are used in this paper to collect reproducible data concerning effects resulting from differences between sample solvent and mobile phase composition. Extra column mixing of sample with mobile phase is impossible, provided the sample injection tube is flushed out with sample solution. Any additional mixing and/or distortion of the sample zone at the column top, possible with conventional columns[2], are also eliminated.

EXPERIMENTAL

Preparative columns 250×43 mm I.D. with injection into the sorbent bed, packed with a spherical reversed phase Separon SGX C 18 (mean particle diameter d_p = 15μm, Tessek Ltd., Prague, Czechoslovakia) have been described elsewhere[14]; the injection tube is introduced centrally through the bottom end-fitting to 4/5 of the column length, so that its effective length is 200 mm. The preparative liquid chromatograph consisted of a dual-action membrane pump of the Orlita type of our own production (maximum flow rate 50 ml/min), a membrane pump VCM 300 (Development Workshop of the Czechoslovak Academy of Sciences, Prague) for sample injection (flow rate 4 ml/min), a four-way switching valve PK 1 (Development Workshop of the Czechoslovak Academy of Sciences, Prague), a UV detector UVF 254 with a preparative cell (Development Workshop of the Czechoslovak Academy of Sciences, Prague), and a recorder TZ 4620 (Laboratory Instruments, Prague). Samples were injected in the stop-flow mode[14] with closed inlet of mobile phase and bypassed pump. The sample introduction tube was first flushed out and filled with sample solution; only the next injections of the sample completely free of any dilution were used.

Reference glass analytical columns of the CGC system, size 150×3.3 mm I.D., packed with the spherical reversed phase Separon SGX C18 d_p = 7μm, were supplied by Tessek Ltd., Prague. The analytical chromatograph consisted of a positive displacement pump of our own construction, an injection valve Rheodyne 7125 (Rheodyne Inc., CA, USA) with 20 μl loop, a UV VIS detector LCD 2563 (Laboratory Instruments, Prague) and a recorder Servogor 2S (Goerz Electro, Vienna, Austria).

RESULTS AND DISCUSSION

The unconventional injection system used deserves short explanation. McDonald and Bidlingmeyer[2] have shown that loop injection into the stream of mobile phase gives very poor (parabolic) sample distribution, if mobile phase enters the column top centrally through narrow bore due to high momentum

TABLE 1

Dependence of Efficiency of the Preparative Column at Various Loadings of Benzene on the Composition (v/v) of Methanol/Water Sample Solvent

Loading		Efficiency, N			
ml	mg	2/1	3/1	4/1	6/1
8	120	782	568	346	179
4	60	1294	1029	609	351
2	30	1660	1300	878	542
1	15	2491	1763	1388	840

Mobile phase: methanol/water = 3/1 (v/v)

of the liquid stream with very high linear velocity. Direct stop-flow injection of the sample into the column top was shown to give approximately spherical sample zone[15]. Our system has a different geometry[14]; stop-flow injection is accomplished in the direction opposite to the mobile phase flow (with closed column inlet) and sample displaces the corresponding volume of mobile phase through the bottom outlet. To see the hydrodynamic pattern during injection, glass column of the same diameter[14] packed with spherical silica (d_p =15μm) was made transparent; toluene/heptane mobile phase compostion was adjusted to match the refractive index of the packing and azobenzene solutions were injected. The visually observed sample zones formed oblate ellipsoids with approximate axis ratios 4:1 and most of the column diameter was occupied when the sample volume reached about 8 ml. Apparently, the specific configuration of this injection system determines the observed hydrodynamics of zone formation.

Table 1 summarizes efficiencies of the preparative column reached with various sample-solvent compositions at varying loading. Benzene at a concentration of 15 mg/ml in the respective solvent, having $k' = 1.3$ and solubility 90 mg/ml in the methanol/water = 3/1 (v/v) mobile phase used, was a simple solute. It can be seen in agrement with results of Evans and McGuffin[18] that the highest efficiency is reached when the injection is made in a solvent weaker than the mobile phase. This is a natural consequence

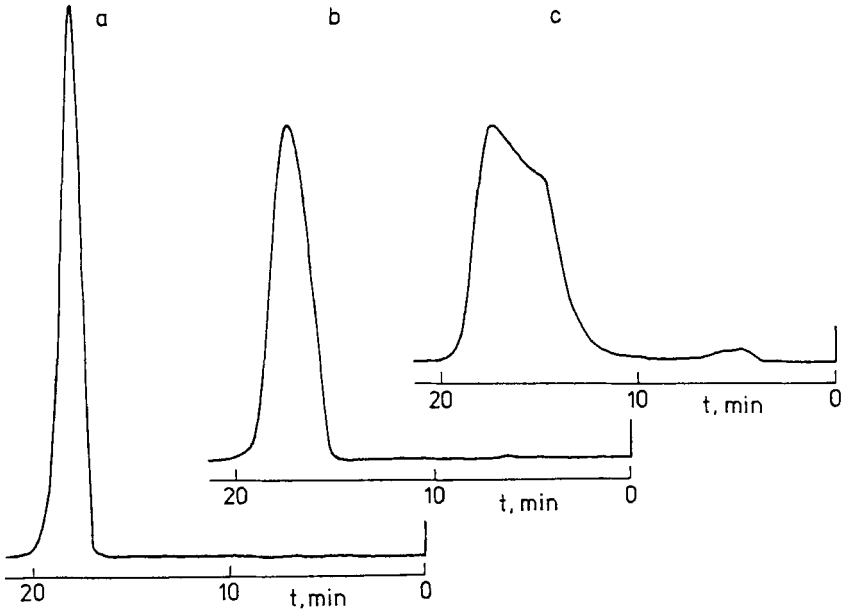

FIGURE 1. Peak shape corresponding to the injection of 60 mg benzene in 4 ml of methanol/water, composition in volume ratio: (a) 2/1, (b) 6/1 and (c) pure methanol. Column: 250 × 43 mm I.D., mobile phase: methanol/water = 3/1 (v/v), flow rate: 23.5 ml/min, UV detection.

of the well-known on-column enrichment principle[19], i.e., concentrating the solute injected under conditions of higher retention. When increasing the elution strength of the solvent with respect to that of the mobile phase, the efficiency was observed to decrease. An explanation obviously consists in that in the sample zone a dynamic gradient arises between sample solvent and mobile phase[10]. These effects are illustrated in Fig. 1a by the peak of benzene dissolved in methanol/water = 2/1; a change in the composition of the sample solvent to methanol/water = 6/1 causes a drop in the efficiency (Fig. 1b), but the shape and symmetry of the peak still fulfils the chromatographic requirements. A pronounced change is produced only by injection in pure methanol (Fig. 1c) which is in agreement with the observations found in Ref.[13].

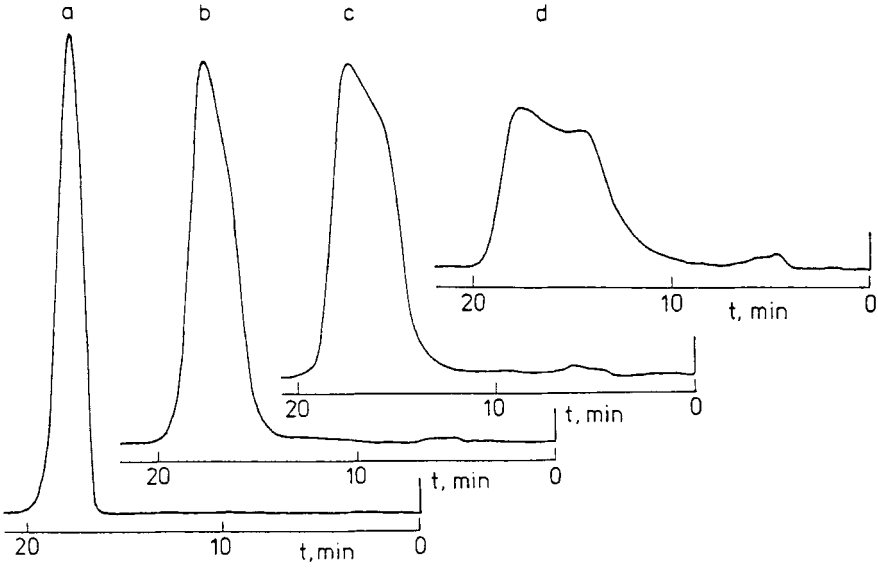

FIGURE 2. Peak shape corresponding to the injection of 90 mg benzene in 6 ml of: (a) mobile phase and (b) 0.6 ml, (c) 1.5 ml, (d) 6 ml methanol. Conditions as in Figure 1.

In addition to the leading edge and an indication of double peak formation, yet another peak appears in the solvent zone. Recently, Hofman and Rahman[20] have computer-modelled sample injection in a strong solvent into a weaker mobile phase. The column band distributions obtained by them are in a good agreement with our experiments (cf. Fig. 1c and Fig. 7 in Ref.[20]). Also, their study implies that these effects should decrease with increasing k' of the solute[18]; hence, the low retention chosen in our experiments gives the most pronounced effect. The influence of the injected sample volume in pure methanol is illustrated in Figs 2b-d (reference injection in the mobile phase is in Fig. 2a); it can be seen that with decreasing injected volume deformation of the benzene peak is reduced, but does not disappear (cf.[20], Fig. 4). Kaminski and Reusch[12] who observed similar effects in preparative HPLC, pointed out the possible influence of viscosity of the injected sample, and showed that an

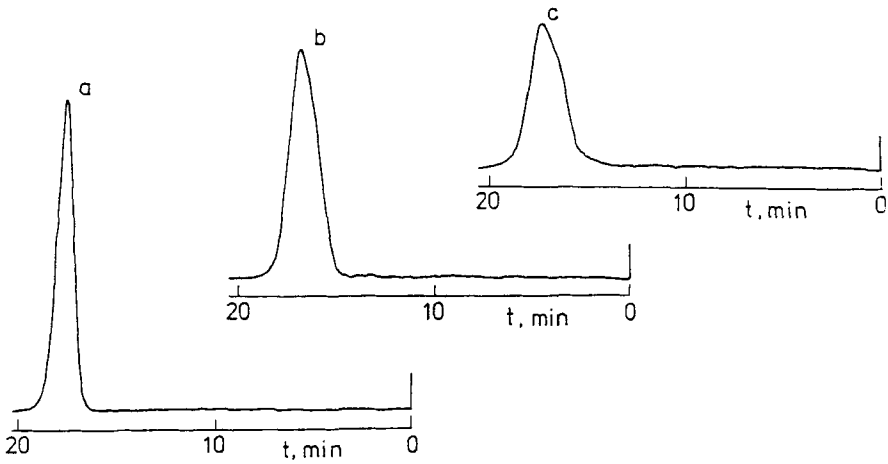

FIGURE 3. Peak shape corresponding to the injection of 120 mg benzene in 8 ml of: (a) mobile phase, (b) mixture methanol/glycerol = 86/14 (v/v), (c) 1-propanol, (d) methanol. Conditions as in Figure 1.

increase of flow rate improves the peak shape due to increased mixing of the sample with the mobile phase in the upper packing layer. The effect of sample viscosity is well known from GPC as viscous fingering[21]. Czok, Katti and Guiochon[22] have shown that fingering effect may be observed with both higher and lower viscosity of the sample than that of the eluent. In our case, mobile phase-methanol viscosity ratio $\simeq$ 2.3 and 1-propanol-mobile phase viscosity ratio $\simeq$ 1.5 may be compared to common GPC criterion[22] which suggests that sample-mobile phase viscosity ratio should be less than two to avoid viscous fingering. Fig. 3 shows chromatograms which illustrate the effect of viscosity of benzene solutions (with the reference injection in the mobile phase, Fig. 3a) for the same injected volume and mass loading. It can be seen that, on passing from methanol as the sample solvent (Fig. 3d) to 1-propanol as a solvent with a higher elution power but lower viscosity difference against the mobile phase (Fig. 3c), the peak shape is fairly improved, while the leading edge is merely reduced, but does not disappear. In Fig. 3b we have the benzene peak from injection in a methanol/glycerol mixture 86/14 (v/v) having approximately

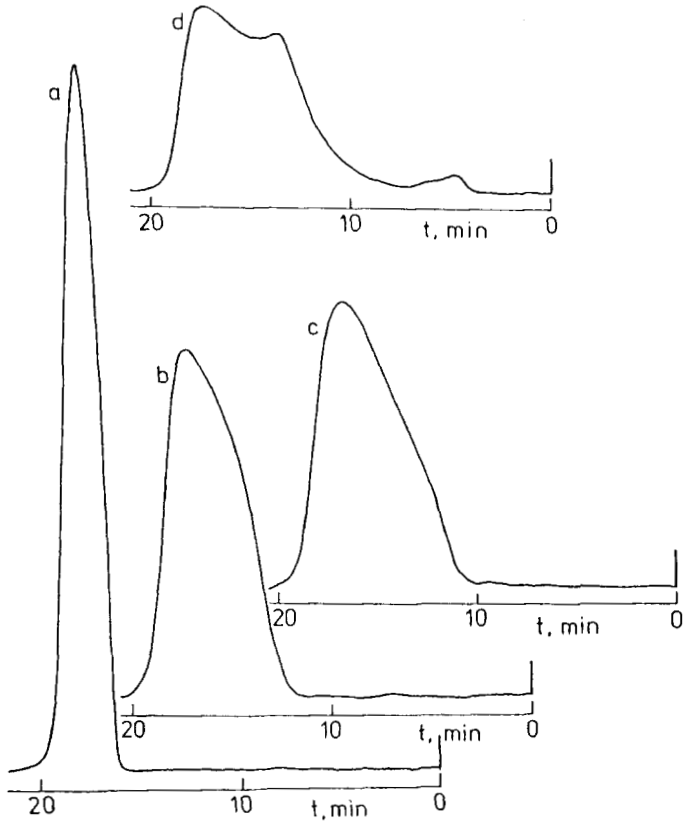

FIGURE 4. Peak shape corresponding to the injection of 7.5 mg of benzene in 0.5 ml of: (a) mobile phase, (b) 1-propanol, (c) methanol. Conditions as in Figure 1.

the same viscosity as the mobile phase. The elution power of this solution should be somewhat weaker than that of methanol, but still higher than that of the mobile phase; the peak distortion remains. It can be assumed, therefore, that in real cases two effects are superimposed, i.e., the higher elution power and the different viscosity of the sample solvent as compared to that of the mobile phase. Fig. 4 shows that a decrease in the injected volume and in the

TABLE 2

Dependence of Efficiency of the Preparative Column at Various Loadings of Benzene on the Sample Solvent

Loading		Efficiency, N				
		Sample solvent: 1-propanol/water				methanol/water
ml	mg	55.5/44.5	2/1	3/1	6/1	3/1
8	120	700	543	355	242	390
4	60	1085	914	612	410	566
2	30	1969	1537	1157	771	721
1	15	2102	1923	1369	1052	1266
0.5	7.5	2623	2309	1694	1474	1676
0.25	3.75	2851	2459	2118	1638	2065

Mobile phase: methanol/water = 3/1 (v/v)

mass loading of the column to 7.5 mg does not remove these effects, but only makes them weaker[3,23].

Table 2 summarizes the efficiencies of another preparative column obtained using various compositions of the 1-propanol/water mixtures as the sample solvent at various mass loadings. Methanol/water = 3/1 (v/v) was again the mobile phase, with benzene at a concentration 15 mg/ml used as the solute. The elution power of the mixture 1-propanol/water = 55.5/44.5 (v/v) should approach that of the mobile phase; it forms a saturated benzene solution at the same concentration (90 mg/ml) as the mobile phase. We can see that such composition does indeed give the highest efficiencies and that with increasing elution power of the sample solvent the column efficiencies decrease similarly to Table 1. A comparison between the methanol/water (3/1, the last column) and the 1-propanol/water sample solvent composition, equal to 55.5/44.5, reveals that it is more advantageous to use the more viscous mixture 1-propanol/water as the sample solvent. Although this conclusion may be valid only for our injection system, it clearly shows the importance of the sample introduction hydrodynamics.

Similarly to Table 1, the shapes of the peaks within the whole range of compositions of the sample solvent in Table 2 are completely acceptable

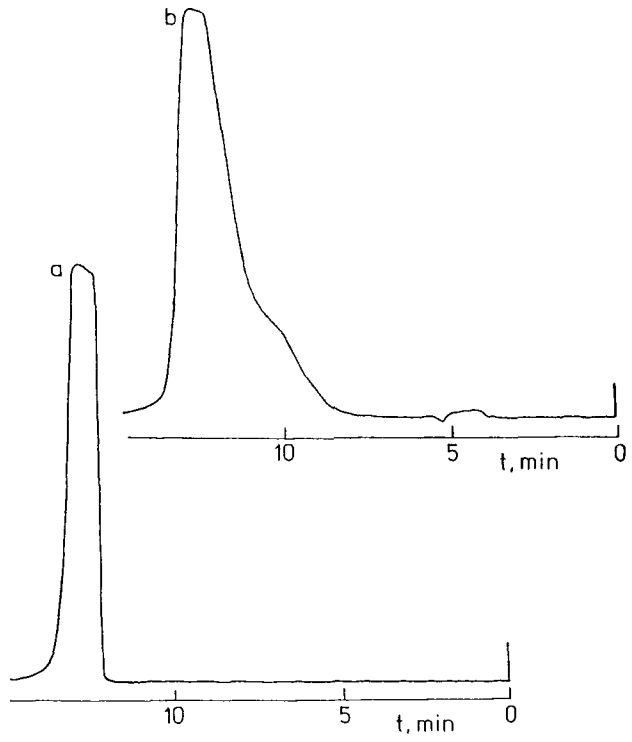

FIGURE 5. Peak shape corresponding to the injection of 20 μl benzene solution (15 mg/ml) in: (a) mobile phase and (b) methanol on a CGC column 150 × 3.3 mm I.D. Mobile phase: methanol/water = 3/1 (v/v), flow rate 0.15 ml/min, UV detection (identical conditions, nonlinear response).

and the differences are reflected in the efficiencies only. A similar decrease in efficiency has been observed in analytical columns[3,23]. Our data show that partial dilution of the sample dissolved in pure methanol or 1-propanol by the mobile phase removes both the leading edge of the peak and the secondary peak in the solvent zone. This is in agreement with the finding[11] that the shape of the peak can be considerably improved by diluting the sample with the mobile phase between the injection valve and column. In analytical HPLC equipments the injected sample is probably always mixed to some extent with the mobile

phase due to the occurrence of the parabolic profile of the zone[1] caused by the laminar flow through the injector loop and the connecting tube to the column, and also due to changes in linear flow rate[2] in the tube fittings and at the column inlet. These effects are well known from the flow injection analysis[24] and chromatographic measurements of the diffusion coefficients[25]. Fig. 5 shows the shapes of the peak of benzene dissolved in methanol and the mobile phase resulting from the injection of 20 μl (equivalent to 3.4 ml in our preparative column) on our analytical apparatus where certain dilution can be anticipated, especially, if the loop is not filled completely. The leading edge of the peak in the case of 20 μl injection in methanol (Fig. 5b) appears also in this case, but at injections below 10 μl (equivalent to 1.7 ml in our preparative column) it virtually diappears and no difference against the injection in the mobile phase (Fig. 5a) is observed. If pure methanol is diluted with methanol/water = 3/1 in the ratio 3/4, the resulting composition of the mixture is 6/1 (on our analytical scale e.g., 9 and 12 μl) and then, as illustrated in Fig. 1b,c, the double peak becomes a normal (single) peak. Assuming various dilution of the injected sample in a strong solvent, it is thus possible to explain the differences observed between various chromatographs[6] which probably differ in the configuration injection valve — connecting capillaries — column inlet. Our data show that the highest sensitivity to the difference between sample solvent and mobile phase composition should be expected when sample/mobile phase mixing effects are minimized, i.e., with chromatographic systems exhibiting the lowest extra column band broadening.

All experiments in this paper were done below the solubility limit of benzene in the mobile phase used. The only exception is Fig. 2b where benzene precipitation may occur. No pronounced change in the peak shape is observed, indicating that slight increase of the sample concentration above the corresponding solubility limit in the mobile phase might be acceptable. Nevertheless, Jandera and Guiochon[13] studied oversaturation effects and have clearly shown that the use of sample concentrations above the solubility limit in mobile phase should be avoided whenever possible.

It may be said, in conclusion, that anomalous peak shapes occur even in cases when sample solubility in mobile phase is sufficient if sample solvent and

mobile phase differ considerably in viscosity and/or sample solvent strength is considerably higher. If sample preparation procedure leads to the solution in a solvent different from mobile phase, some useful hints can be drawn. The difference between both the viscosity and solvent strength (if higher) of the sample solvent and of the mobile phase should be kept as small as possible. The sample solvent-mobile phase viscosity ratio should be kept fairly below two, similarly, like in GPC experiments. Even the addition of another solvent as a viscosity modifier may be advantageous if properly selected. Too high elution power of the sample solvent should be decreased by a simple mixing with mobile phase prior to injection.

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