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INFLUENCE OF SAMPLE SOLVENT AND STATIONARY PHASE POLARITY ON PEAK BROADENING, DISTORTION AND SPLITTING DUE TO THE "FLOODING EFFECT"

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SUMMARY

Experimental data and rough theoretical calculations show that the residence time of liquid solvent inside a column is related to the amount of solvent vapours transported at equilibrium outside the column. The evaporation time is independent of the nature of the stationary phase. Liquid sample migration inside the column depends on the polarities of both the sample solvent and stationary phase. On non-polar immobilized stationary phases, highly polar solvents produce much larger flooded zones than non-polar ones. The dependence of column separating power on solvent polarity (mixtures of chloroform and methanol with different compositions) shows an S-shaped curve, suggesting a change of liquid migration mechanism for highly polar solvents. Visual observations support this conclusion. At column temperatures higher than the boiling point of the solvent these differences practically disappear. On polar immobilized stationary phases, similar wet zones are obtained for polar or non-polar solvents. Their lengths are similar to those obtained on non-polar stationary phases when non-polar or slightly polar solvents are used. However, peak-broadening, distortion and splitting phenomena are less evident, suggesting that the distribution of sample components along the flooded zone depends on the nature of the stationary phase.

INTRODUCTION

The movement of a liquid sample inside a capillary column upon cold on-column injection was described previously[1,2]. Later it was shown[3,4] that the spreading of the liquid sample along the inlet part of the column produces broadening, distortion and splitting of chromatographic peaks. This process has negative consequences on column separating power and quantitative results, both in on-column and splitless injection techniques.

In order to avoid the consequences of this process, Grob[5] proposed the creation of a "retention gap", which generally represents a good solution, provided a satisfactory method is found for connecting the precolumn to the main capillary[6–8]. However, for highly polar solvents on non-polar stationary phases, producing flooded zones of 5–6 m, the usual retention gap hardly redresses the peaks[9,10].

While studying the liquid migration and solvent evaporation mechanism inside capillary columns[9–12], we have observed that the liquid injected forms a plug, which quickly travels along 10–20 cm of the column inlet. It leaves a thick film of sample behind it. The geometric characteristics of this "initial flooded zone" can be approximated by the Fairbrother–Stubbs equation[13].

The instability of this annular thread of liquid produces liquid lenses, which further migrate along the column, leading to the "final flooded zone". At room temperature, this is generally 2–3 times longer than the initial zone. However, for polar solvents on non-polar phases the final zone length is much more important.

The mechanisms of liquid sample migration and solvent evaporation have some similarities with the phenomena encountered in the dynamic coating of capillary columns. Therefore, some ideas and results from publications concerning this technique[14–21] can be used for a better understanding of the importance of different factors to the flooding effect. However, in one case the solid surface is generally etched glass, while in the other case it is the immobilized stationary phase.

The sample solvent influences peak broadening, distortion and splitting not only through the flooding effect, but also by the solvent effect. Peak distortion produced by a "partial solvent trapping effect" has recently been investigated[22–24]. Fortunately, the contributions of these processes can be separated. The partial solvent-trapping effect is especially important for volatile components, while the flooding effect mainly affects less volatile ones. The influence of solvent composition on peak shapes in the on-column injection technique has been investigated by Jenkins[25]. As the chromatograms reported in his paper show that heavier components give better peak shapes than the more volatile ones, we believe that the peak distortions reported by him are mainly due to the partial solvent-trapping effect.

Sauter et al.[26] have reported the peak splitting produced in splitless injection by solvent mixtures with higher methanol contents. The peak splitting disappeared when dichloromethane was used instead of methanol. The same behaviour was observed for fatty acid methyl esters (FAMEs) dissolved in methanol and injected on-column[27].

The nature of the solvent cannot be considered independently of the nature of the stationary phase. Besides its roughness, the hydrophilic or hydrophobic characteristics of the column wall surface will determine the spreading capacity for each type of solvent and, consequently, the liquid film stability. The polarities of the solvent and stationary phase are both important for liquid sample migration inside the column.

We found no published references to the influence of the nature of the stationary phase on the flooding effect. However, Sandra[28] and De Nijs[29] have mentioned the absence of peak splitting on immobilized Carbowax.

One of the most important factors influencing the flooding effect is the column temperature during the injection. We have shown[9–12] that by injecting at temperatures slightly higher than the boiling point of the solvent, broadening, distortion and splitting of chromatographic peaks generally disappear. Under such conditions, the liquid sample remains confined to the first 10–20 cm of the column inlet. Rapid evaporation of solvent drastically reduces the lifetime of liquid lenses, which hardly move out of the "initial flooded zone". We have also shown that this change in the liquid migration mechanism arises more or less suddenly in a "transition region" of 10–15°C

immediately above the boiling point of the solvent.

Very recently, other authors[30,31] have suggested the use of the same operating conditions. However, on-column injection at temperatures higher than the boiling point of the solvent requires adequate hardware[32–34].

EXPERIMENTAL AND DISCUSSION

The experiments performed are mainly simple chromatographic or physical measurements. Much important information has been obtained by using visual observations according to the method developed by Driessen and co-workers[35,36]. All these data have been used to: (i) ascertain the connection between solvent vaporization and liquid migration along the column, (ii) investigate the influence of solvent and nature of the stationary phase on peak broadening, distortion and splitting by the flooding effect, and (iii) study the variation of column separating power with the injection temperature for different types of solvents.

Solvent vaporization process

The experimental data have been used to calculate the amount of solvent (hexane, chloroform or methanol) transported outside the column by the carrier gas under the assumption that the solvent partial pressure corresponds to its saturation value:

$$P_{solv} = P^0_{solv}/P_{inlet} \tag{1}$$

The saturation pressure, P^0_{solv}, was estimated from the linear relationship log P^0_{solv} = f(1/T), obtained from the experimental data (see ref. 37, Tables D191, D192 and D197).

The number of moles of solvent transported outside the column, n_{transp}, was evaluated by using the ideal gas equation:

$$n_{transp} = P_{solv} V_{carr}(P_{inlet}, T_{col})/RT_{col} \tag{2}$$

The total volume of the carrier gas entering the column during the solvent evaporation, $V_{carr}(P_{inlet}, T_{col})$, was calculated on the basis of the corresponding volume, measured at the column outlet. The flow-rate variations were recorded with a laboratory-built device based on a Brooks electronic mass-flow controller. A small filter with active charcoal was inserted just behind the mass flowmeter inlet. The instrument response delay was taken into account.

The flow-rate varies during evaporation. The change of carrier gas viscosity is probably an important factor. However, calculations according to Wilke's approximation[38] of the viscosity of the mixture formed by the carrier gas with the solvent vapours led to values which did not justify the differences found between the different solvents.

The evaporation times were measured by using the technique proposed by Driessen and co-workers[35,36]. A 2-μl volume of solutions of pyrromethene pigment A (ref. 37, p. 316) in hexane, chloroform and methanol was injected by the on-column

TABLE I

COMPARISON OF THE AMOUNT (μMOLES) OF SOLVENT INJECTED AND TRANSPORTED BY THE CARRIER GAS DURING THE EVAPORATION TIME

It is assumed that the concentration in the gas phase corresponds to the saturation value.

Solvent	n_{inj}	n_{transp}	
		OV-1	Carbowax
Hexane	15.4	15.1	15.8
Chloroform	24.8	23.8	25.4
Methanol	49.4	47.5	47.7

technique and the fluorescence was observed by using the two-window gas chromatograph previously described[39]. The intensity of fluorescence changes significantly when the solvent is evaporated, permitting not only the evaluation of the evaporation time but also the visualization of liquid movement along the column.

The number of moles contained in the volume of liquid solvent injected, n_{inj}, was simply calculated on the basis of the densities reported in the literature (see ref. 37, Tables C327, C369 and C370) and the corresponding gram molecular weights, M_{solv}:

$$n_{inj} = \rho_{solv} V_{solv} / M_{solv} \qquad (3)$$

The calculated and experimental data are reported in Table I. The data show that the carrier gas which passes through the flooded zone is immediately saturated with solvent vapours and remains saturated up to complete evaporation of the solvent. The stationary phase apparently does not influence the evaporation process but only the liquid migration.

The visual observations demonstrate that the drying process continuously occurs at the rear side of the wet zone.

Influence of solvent polarity on the flooding effect

The FAMEs have very different peak shapes when analyzed on non-polar stationary phases in chloroform or methanol solution (Fig. 1). This suggests differences in liquid migration mechanism. In fact, visual observations demonstrate that at room temperature 2 μl of methanol travel in columns of non-polar immobilized stationary phases along 15–20 coils, producing a flooded zone of 5–7 m, while chloroform hardly reaches the third coil. Liquid chloroform moves according to the previously described mechanism[9-12]. Liquid lenses are formed in the initial flooded zone and migrate along the column. During their migration, the liquid lenses are continuously formed and deposited. The lenses are not only broken and reformed, they also coalesce into plugs and separate again in thin slices. We believe that gravitational forces play a rôle in this process.

For chloroform, as for other non-polar or slightly polar solvents, the apparent velocity of liquid advance is about 0.2–0.3 cm/sec. Methanol and other highly polar solvents travel in a different way. The initial flooded zone is broken into other liquid
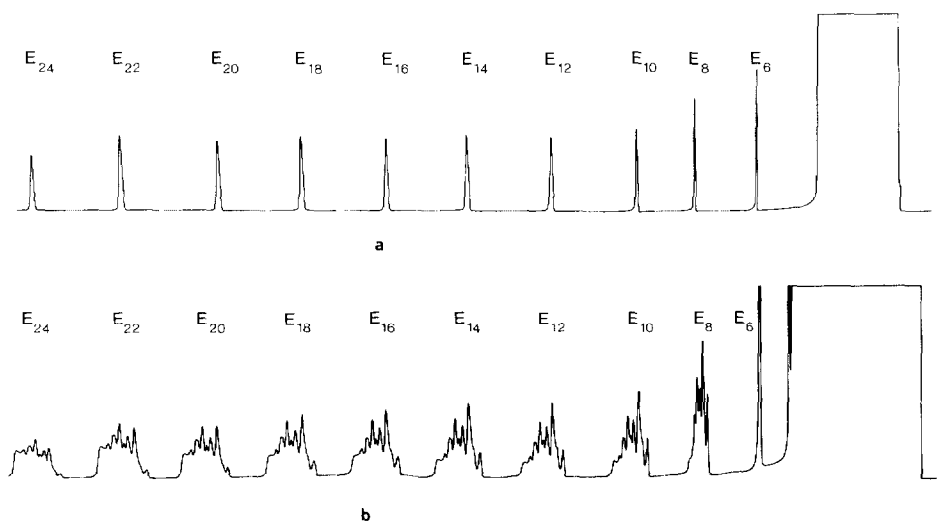
E_{24} $\quad$ E_{22} $\quad$ E_{20} $\quad$ E_{18} $\quad$ E_{16} $\quad$ E_{14} $\quad$ E_{12} $\quad$ E_{10} $\quad$ E_8 $\quad$ E_6

a

E_{24} $\quad$ E_{22} $\quad$ E_{20} $\quad$ E_{18} $\quad$ E_{16} $\quad$ E_{14} $\quad$ E_{12} $\quad$ E_{10} $\quad$ E_8 $\quad$ E_6

b

Fig. 1. Influence of solvent polarity of the flooding effect observed on a non-polar stationary phase. Chromatograms of FAMEs (5 ng/μl each), dissolved in chloroform (a) or methanol (b). A 2-μl volume of sample was injected at 30°C into a 25-m capillary column, 0.32 mm I.D., of immobilized OV-1 stationary phase (film thickness 0.15 μm). Temperature program: ballistic heating from 30°C to 80°C (using the full power of the heater) and then at 8°C/min from 80°C to 300°C. Carrier gas (hydrogen) velocity: 50 cm/sec. Strip chart recorder: speed, 10 cm/min. The subscripts of E indicate the carbon numbers of the fatty acids.

zones without necessarily producing lenses. These liquid zones have different lengths, ranging from a few mm to half a coil or even more. They are apparently stretched during their further migration, and can also coalesce or break apart.

The advance is apparently due to waves progressing at a velocity of about 2 cm/sec. During advance the fluorescence of the front makes it look like a flickering flame. It seems that inside the zone the substance is distributed in a strange manner, leaving the larger amount of liquid in the rear.

Sometimes the zone velocity decreases and lenses are formed, but they are quickly broken by the carrier gas.

As in the other cases, the drying process is continuous and begins with the rear of the last liquid zone. It does not take place as long as the initial zone is not dried.

We are unable to explain the differences in liquid migration mechanism, even where it is clear that this results from the high cohesion forces of the solvent and its low adhesion to the surface.

We attempted to find out whether the change in migration mechanism is gradual when the polarity of the solvent is gradually increased. FAMEs were dissolved in solvents with different proportions of methanol and chloroform and injected into a column with a non-polar stationary phase. Plotting the column separating power (expressed as separation number TZ) for different ester pairs against the percentage of chloroform in the mixture (%, v/v), we obtained an S-shaped curve, indicating a sudden change in liquid migration mechanism (Fig. 2). This arises at a molar fraction of ca. 0.5.

Another puzzling fact is that when non-polar or slightly polar solvents are used, the peaks are not always split but sometimes only broadened. For example,
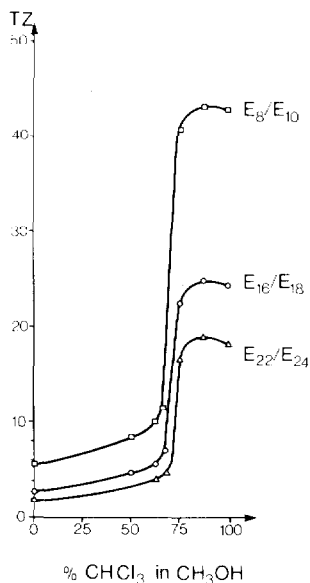
Fig. 2. Variation of column separating power with solvent polarity, due to the flooding effect on the non-polar stationary phases. A 2-μl volume of FAMEs (5 ng/μl each), dissolved in various mixtures of chloroform and methanol, was injected into a 25-m glass capillary column, 0.32 mm I.D., of OV-1 immobilized stationary phase (film thickness 0.15 μm). For operating conditions see Fig. 1.
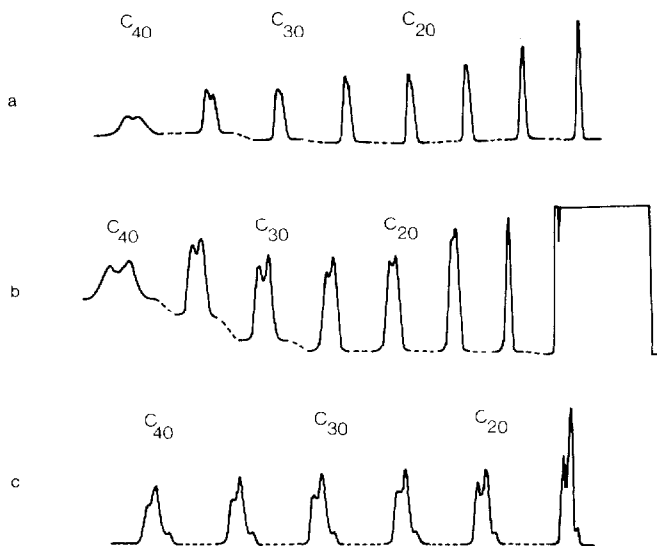

Fig. 3. Peak shapes of n-alkanes in non-polar or slightly polar solvents on non-polar stationary phases. A 2-μl volume of n-alkanes (5 ng/μl each), dissolved in pentane (a) or hexane (b), was injected into a 25-m glass capillary column, 0.32 mm I.D., of immobilized OV-1 stationary phase (film thickness 0.4 μm). Temperature program: a, injection at 25°C and then 15°C/min up to 340°C; b, injection at 60°C and then 15°C/min up to 340°C. c, A 2-μl volume of n-alkanes in chloroform (5 ng/μl each) injected into a 15-m fused-silica column, 0.20 mm I.D., of SE-52 immobilized stationary phase (film thickness 0.15 μm). Temperature program: injection at 30°C, ballistic heating up to 100°C and then 8°C/min up to 340°C.

FAMEs in chloroform showed peaks that were not split (Fig. 1), while *n*-alkanes dissolved in hexane, pentane or chloroform gave split peaks (Fig. 3).

The final flooded zone usually covers between one and two coils. The fluorescent and coloured substances seem more or less homogeneously distributed. In order to obtain information about the real distribution of the compounds in the flooded zone, we tried to decrease the influence of the smoothing process due to the band-broadening phenomena inside the column. A composite column was prepared, coated with a thicker film of stationary phase in its first five coils. This creates a band decompression (reversed retention gap) so that the final peak shapes better reflect the initial profile of the component. The chromatograms in Fig. 4 demonstrate that the FAMEs are distributed in an inhomogeneous manner in the final flooded zone, for both the hexane and chloroform solutions.

The FAME solution in hexane, chloroform and methanol (2 μl) was also injected into an immobilized Carbowax column. In all cases, we obtained peaks that were not split and a column separating power (*TZ*) 10–15% lower than the value obtained by injecting 0.2–0.3 μl.

By visual observation we found that all solvents migrated according to the "conventional mechanism" previously described[9–12], forming final flooded zones ranging between one and two coils. Their behaviour is similar to that of non-polar or slightly polar solvents on a non-polar stationary phase.

The column separating power increased by about 30% when small amounts of sample dissolved in non-polar solvents were injected into columns with non-polar
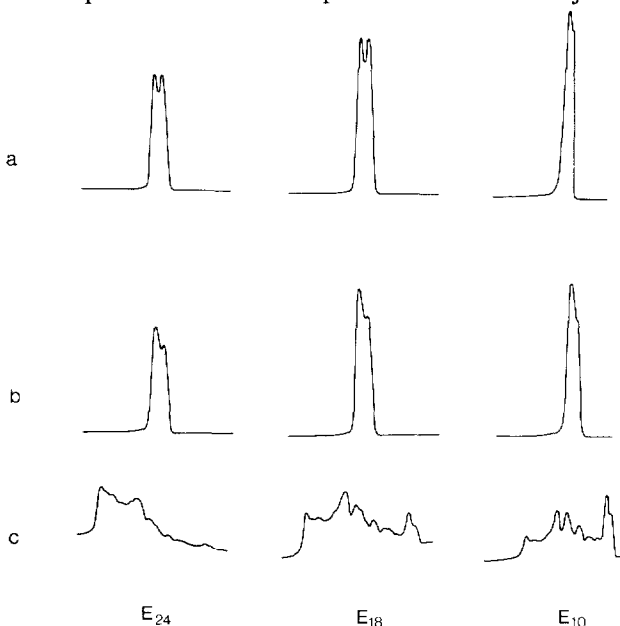

Fig. 4. Real concentration profile of FAMEs in the flooded zone. A 2-μl volume of FAMEs (5 ng/μl each), dissolved in hexane (a), chloroform (b) or methanol (c) was injected into a composite column, formed by a 1.8-m OV-1 fused-silica column, 0.32 mm I.D. (film thickness 0.45 μm), followed by a 25-m OV-1 fused-silica column, 0.32 mm I.D. (film thickness 0.15 μm). For other operating conditions, see Fig. 1. The capacity constant variation produces the peak decompression, showing the real concentration profiles of the components in the flooded zone.

phases. In such cases, the *TZ* values were similar to those obtainable by split injection. Under equivalent conditions an increase of only about 10% is observable on polar stationary phases. This suggests that, in this last case, the sample is less spread out over the final flooded zone. Further experiments are necessary in order to verify this.

Influence of column temperature during the injection

We have already shown[9-12] that, on non-polar stationary phases, on-column injections at column temperatures above the boiling point of the solvent produce important increases in column separating power. For non-polar or slightly polar solvents this gain is at least 30%, while for highly polar solvents it becomes enormous (about 10 times).

These observations are confirmed by the data presented in Fig. 5, where the *TZ* values of the pair formed by the methyl esters of *n*-docosanoic and *n*-tetracosanoic acids (E_{22}/E_{24}), dissolved in various mixtures of chloroform and methanol, have been plotted against column temperature during injection. The curves show that at temperatures above the boiling points of the solvent the differences between the non-polar or slightly polar solvents and the highly polar ones become negligible.
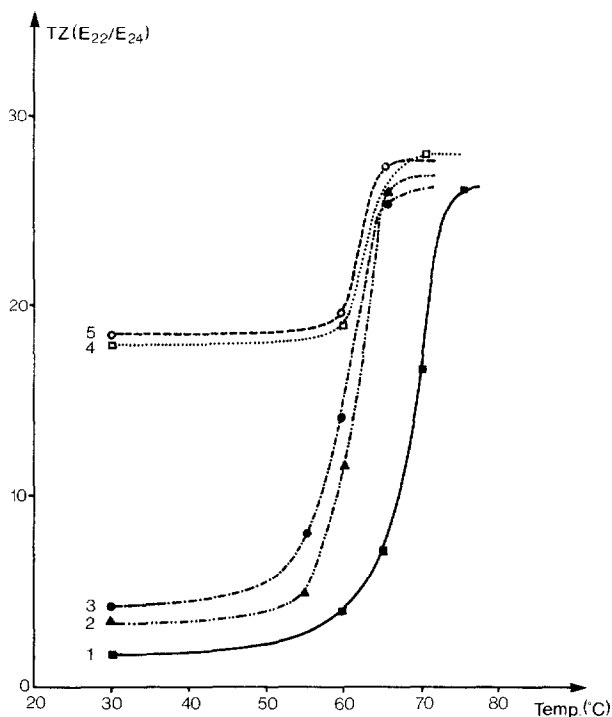
Fig. 5. Variation of column separating power with solvent polarity and column temperature, due to the flooding effect on non-polar stationary phases. A 2-μl volume of FAMEs (5 ng/μl each), dissolved in different mixtures of chloroform and methanol, was injected at various column temperatures below and above the boiling points of the solvents, into a 25-m glass capillary column, 0.32 mm I.D., of immobilized OV-1 stationary phase (film thickness 0.45 μm). Temperature program: from injection temperature, ballistic heating up to 80°C and then 8°C/min up to 300°C. Solvents: chloroform ($\square$); methanol–chloroform, 12.5:87.5 ($\bigcirc$), 37.5:62.5 ($\bullet$) and 50:50 ($\blacktriangle$); methanol ($\blacksquare$).

Visual observations show that under such conditions the initial and final flooded zones coincide. This is in accordance with the model proposed[9-12] to describe the liquid migration in the "transition zone".

On immobilized polar stationary phase the gain in column separating power obtained by increasing the column temperature above the solvent boiling point does not generally exceed 10–15%, both for polar and non-polar solvents. As the visual observations showed, in this range of temperatures the flooded zone length decreased on both polar and non-polar stationary phases. The above-mentioned results suggest again differences in sample spreading over the flooded zone.

CONCLUSIONS

Liquid migration does not influence solvent evaporation. The evaporation time strictly depends on the amount of solvent vapours transported at equilibrium by the carrier gas. It is not influenced by the nature of the stationary phase. All solvents on polar stationary phases and non-polar and slightly polar solvents on non-polar phases migrate according to the mechanism previously proposed[9-12].

Visual observations have shown that the flooded zone lengths are similar. The sample distribution inside the flooded zone is probably responsible for the better peak shapes and better separating power observed on polar stationary phases.

A different liquid migration mechanism was observed for highly polar solvents on non-polar stationary phases. Sample injection at temperatures slightly above the solvent boiling point drastically increased the column separating power.

ACKNOWLEDGEMENTS

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