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Reversed-phase liquid chromatography coupled on-line with capillary gas chromatography

I. Introduction of large volumes of aqueous mixtures through an on-column interface

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ABSTRACT

The feasibility of on-line reversed-phase liquid chromatography (LC)–capillary gas chromatography (GC) using an on-column interface was investigated. Large volumes of acetonitrile–water mixtures were introduced into a retention gap which was coated with a very thin film of Carbowax. The retention gap was tested regularly after repeated acetonitrile–water injections; its lifetime was several months. The maximum water content in the eluent must be close to that in the azeotropic mixture, otherwise water which is left in the gap after evaporation of the azeotropic mixture will distort the analysis. An acetonitrile–water mixture allows the introduction of 20–40 μl with an introduction speed of up to 100 μl/min by using the retention gap technique. This means that 1 mm I.D. LC columns can be used in on-line reversed-phase LC–capillary GC work.

INTRODUCTION

Capillary gas chromatography (GC) is the separation method of choice for the trace-level analysis of complex mixtures because good separation efficiencies can be obtained and sensitive detectors are available. Sample pretreatment techniques are highly important in capillary GC. There are several pretreatment techniques and the difference in volatility and polarity between solutes and solvents generally is the fundamental factor determining the method of choice. Liquid chromatography (LC) is one of these techniques and is a very powerful tool for preseparating complex samples. Coupled LC–GC is probably the most efficient method for determining trace compounds in a complex mixture, mainly because it involves direct transfer of relevant LC fractions into the GC system. Generally two types of interfaces are used for

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LC–GC, *viz.*, an on-column interface for relatively volatile components and a loop-type interface which is mostly applied for less volatile components. Details of these interfaces have been reported elsewhere [1–6].

Many papers have been published on LC–GC applications. Almost all these applications and other LC–GC work have involved normal-phase LC–GC [7]. As about 80% of all LC analyses are performed using reversed-phase systems, there is growing interest in the on-line coupling of reversed-phase LC with GC. The large-volume introduction of polar solvents is more difficult than that of apolar solvents because of the poor wettability of most retention gaps by polar solvents; this is especially true for water, which does not wet surfaces suitably deactivated for GC use. In addition, water forms a very large volume of vapour per unit of liquid and it is a very poor solvent for creating useful solvent effects [8]. Some reversed-phase LC–GC applications have been mainly reported using fully concurrent solvent evaporation, because then there is no need to form a sample film [9–11]. However, the transfer of aqueous eluents using fully concurrent solvent evaporation requires large temperature differences between the transfer step and the elution of the first sharp peak. This means that only very high-boiling solutes can be assayed.

Recently, Grob and Li [12] reported on the wettability of silylated retention gap surfaces by various mixtures of organic solvents and water. They found that deactivation with diphenyltetramethyldisilazane (DPTMDS) produced surfaces with good wettability characteristics. They did not test the wettability of Carbowax-deactivated retention gaps, because they were unable to bind Carbowax to the surface in such a way that it was not removed after a few injections [13]. However, our preliminary results indicated that a Carbowax-deactivated retention gap is appropriate for the introduction of acetonitrile–water mixtures, whereas the DPTMDS-deactivated retention gap did not function satisfactorily either for several model components [14] or for the drug idaverine in an acetonitrile–water mixture [15]. Cortes and co-workers [11,16] investigated the possibility of injections of up to 20 μl of acetonitrile–water mixtures using a non-deactivated fused-silica retention gap, and contrary to our results with raw fused silica [14], this approach worked satisfactorily. This indicates that the selection of an appropriate retention gap for the introduction of aqueous mixtures is still not unambiguous.

In this study, we extended the experiments with the Carbowax-coated retention gap and examined the practicability of large-volume injections (8–40 μl) of acetonitrile–water mixtures into a capillary GC column using the retention gap technique. The influence of the composition of the acetonitrile–water mixture on the peak shape and peak area of solutes and the lifetime of the retention gaps were studied. As it is our final aim to use reversed-phase LC coupled on-line with capillary GC, the eluent introduction speed and introduction volume into the GC system were varied in order to investigate the largest allowable dimensions of the LC column.

EXPERIMENTAL

Equipment

A Phoenix 20 syringe pump (Carlo Erba, Milan, Italy) was used for solvent delivery. A Rheodyne (Cotati, CA, U.S.A.) six-port switching valve was inserted at the outlet of the LC column to lead the eluent either to the UV detector (Jasco,

Tokyo, Japan) or to the gas chromatograph via a fused-silica interface (30 cm × 75 μm I.D.). Injections on to the LC column can be made by means of a Rheodyne injection valve. In this study, an LC column was not used, so the outlet of the Phoenix pump was connected directly to the Rheodyne switching valve. The GC system consisted of two Carlo Erba gas chromatographs, *viz.*, a 5300 Mega Series gas chromatograph equipped with a cold on-column autosampler (AS550, Carlo Erba) in which the retention gap was installed, and a Vega gas chromatograph equipped with a flame ionization detector for the analytical column. The gas chromatographs were connected with each other by a temperature-controlled interface.

The retention gap used was a fused-silica wide-bore precolumn coated with a very thin film of CPWax 52 CB (2–5 m × 0.53 mm I.D., film thickness 0.025 μm) from Chrompack (Middelburg, The Netherlands). The analytical column was either a 25 m × 0.32 mm I.D. CPWax 52 CB fused-silica column with a film thickness of 1.6 μm (Chrompack) or a 30 m × 0.32 mm I.D. DB-1 fused-silica column with a film thickness of 0.25 μm (J&W, Folsom, CA, U.S.A.).

The two-oven system allows us the temperatures of the retention gap and the analytical column to be programmed independently of each other. This means that solutes that elute only at very high temperatures (*e.g.*, idaverine, which elutes at 325°C) can also be determined in spite of the maximum operating temperature of a Carbowax retention gap being 225°C. The two-oven system can also be used to create a cold trapping effect at the entrance of the separation column.

Chemicals

The Grob test mixture from Alltech (Deerfield, IL, U.S.A.) contained 53.0 mg/l of 2,3-butanediol, 28.3 mg/l of *n*-decane, 35.5 mg/l of 1-octanol, 32.0 mg/l of 2,6-dimethylphenol, 40.0 mg/l of nonanal, 28.7 mg/l of *n*-undecane, 31.3 mg/l of 2,6-dimethylaniline, 38.0 mg/l of 2-ethylhexanecarboxylic acid, 42.3 mg/l of methyl decanecarboxylate, 31.3 mg/l of dicyclohexylamine, 41.9 mg/l of methyl undecanecarboxylate and 41.3 mg/l of methyl dodecanecarboxylate in dichloromethane. Acetonitrile was of high-performance liquid chromatographic grade (Baker, Deventer, The Netherlands). Idaverine was obtained from Duphar (Weesp, The Netherlands). All other chemicals were of analytical-reagent grade.

Variation of eluent composition

Acetonitrile–water mixtures containing 0.02 mg/ml each of naphthalene, biphenyl, phenanthrene and acetanilide were introduced into the GC system by the Phoenix LC pump, which was connected directly with the Rheodyne switching valve. The acetonitrile–water ratio was varied from 84:16 to 50:50. The eluent flow-rate (introduction speed) was 25 μl/min and the injection time 45 s. The analytical column was the CPWax 52 CB column (25 m × 0.32 mm I.D., $d_f = 1.6$ μm). The inlet pressure was 100 kPa (helium) and the inlet temperature was 70°C. After 45 s the temperature was raised to 85°C and, after elution of the solvent peak, further to 225°C at 30°C/min. The retention gap (see above) had a length of 2 m.

The above experiment was repeated with the 30 m × 0.32 mm I.D. DB-1 column ($d_f = 0.25$ μm), now using the two-oven system. The temperature programme of the Mega gas chromatograph (T_1) was 80°C for 4 min, increased to 200°C at 30°C/min, and that of the Vega oven (T_2) was 60°C for 12 min, increased to 200°C at

30°C/min. The temperature of the oven interface was 200°C. The injection time was 10 or 20 s and the eluent flow-rate was 25 μl/min. The retention gap was the same as in the earlier experiments.

Retention gap test

The retention gaps were tested using the Grob test mixture containing compounds which vary widely in polarity and volatility. The test mixture was diluted 10-fold in *n*-hexane and 1 μl was injected on-column onto the retention gap. The analytical column used was the 30 m × 0.32 mm I.D. DB-1 column ($d_f = 0.25$ μm). Helium was used as the carrier gas with a column pressure of 95 kPa. The temperature was programmed from 40 to 120°C at 1°C/min.

Variation of introduction speed and introduction volume

A solution of 10 μg/ml of idaverine in acetonitrile–water (90:10) containing 0.1% of triethylamine was introduced into the GC system at speeds varying from 20 to 100 μl/min. The injection volumes were varied from 20 to 40 μl. The inlet pressure was 150 kPa (helium). During the introduction of the idaverine solution, T_1 was 90°C and T_2 220°C. After solvent evaporation, T_1 was immediately raised to 220°C at 30°C/min, but T_2 was kept at 220°C for a further 15 min to await complete reconcentration of idaverine at the inlet of the analytical column; it was then raised to 325°C at 30°C/min. The analytical column was a 30 m × 0.32 mm I.D. DB-1 column ($d_f = 0.25$ μm). The retention gap had a length of 5 m.

RESULTS AND DISCUSSION

Variation of eluent composition

Acetonitrile and water in the volume-to-volume ratio 84:16 form an azeotropic mixture, which has a boiling point of 76°C at atmospheric pressure. Introduction of water containing solvent mixtures in a retention gap seems to be feasible for azeotropic mixtures and for mixtures containing less water, because with such mixtures no water will remain in the retention gap after evaporation [12].

In a first series of experiments, using a thick-film CPWax 52 CB column and 20-μl injections of the test mixture, we therefore further investigated whether acetonitrile–water mixtures containing over 16% of water can also be used and if so, under what conditions. As an example, two chromatograms are shown in Fig. 1, (A) for the azeotropic mixture, (B) and for acetonitrile–water (50:50) as solvent mixture. Obviously, the solutes in the latter chromatogram still have a good peak shape, but the peaks are very small compared with those in Fig. 1A. Peak areas at different eluent compositions are given in Table I. As can be seen from these data, up to a water content of 20% the peak areas remain virtually the same. At 30% water the peak areas start to decrease and at 40 and 50% water they suddenly collapse. The amount of water that is left behind in the retention gap after evaporation of the azeotropic mixture can be calculated and is given in the last column of Table I. Obviously, if only up to 1 μl of water remains in the gap, there is no significant difference between the peak shapes and areas of the compounds at the various eluent compositions. Peak areas start to decrease if *ca.* 2–3 μl of water remain behind in the retention gap, and higher values cannot be tolerated at all.

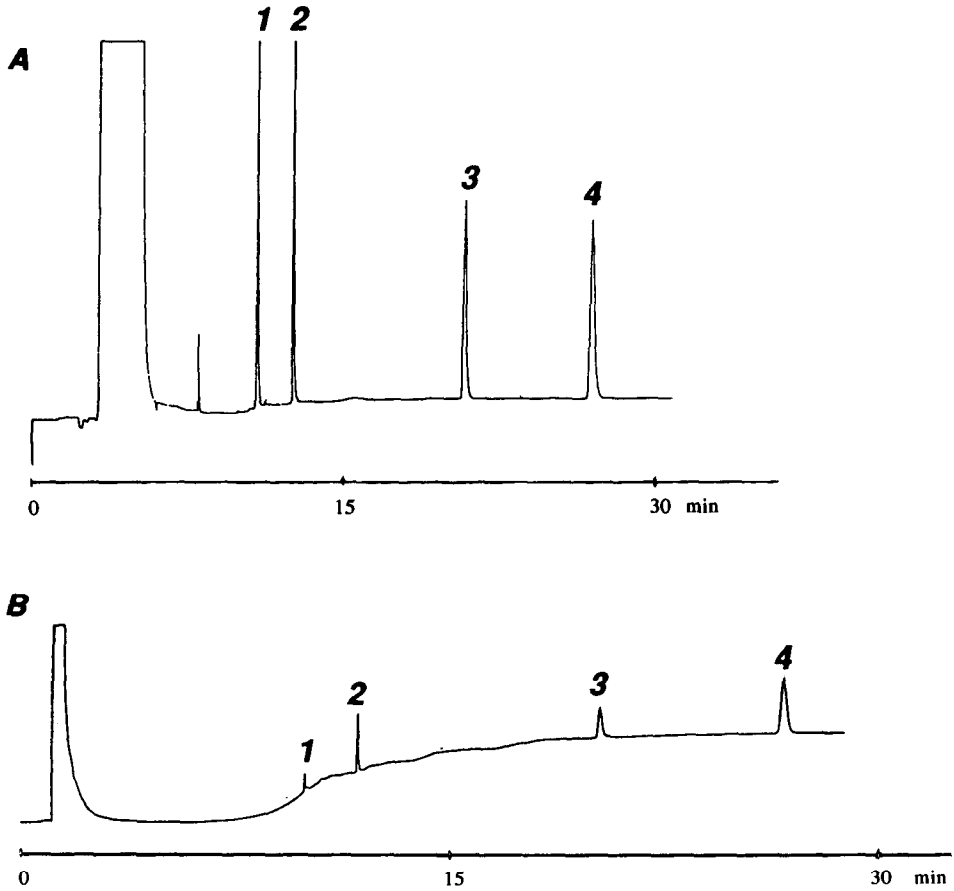


Fig. 1. Gas chromatograms of a 20-μl injection of 0.02 mg/ml solution each of (1) naphthalene, (2) biphenyl, (3) acetanilide and (4) phenanthrene in (A) acetonitrile–water (84:16) and (B) in acetonitrile–water (50:50) using a 25 m × 0.32 mm I.D. CPWax 52 CB analytical column ($d_f = 1.6$ μm) and a 2 m × 0.53 mm I.D. CPWax 52 CB retention gap ($d_f = 0.025$ μm). $T = 70$°C (45 s), then increased at 30°C/min to 220°C.

TABLE I

PEAK AREAS OF VARIOUS COMPOUNDS AFTER 20-μl INJECTIONS OF TEST MIXTURES WITH DIFFERENT ELUENT COMPOSITIONS INTO THE GC SYSTEM AND THE AMOUNT OF WATER LEFT BEHIND IN THE RETENTION GAP AFTER EVAPORATION OF THE AZEOTROPIC MIXTURE

Conditions: retention gap 2 m × 0.53 mm I.D. CPWax 52 CB ($d_f = 0.025$ μm); analytical column 25 m × 0.32 mm I.D. CPWax 52 CB (d_f = 1.6 μm).

Acetonitrile: water ratio	Peak areas (× 10 μV) ($n = 2$)				Water left in gap (μl)
	Naphthalene	Biphenyl	Acetanilide	Phenanthrene	
84:16	12	10	7	10	-
80:20	13	10	8	10	1
70:30	10	8	6	7	3
60:40	1	1	0.8	0.3	6
50:50	0.06	0.2	0.3	0.5	8

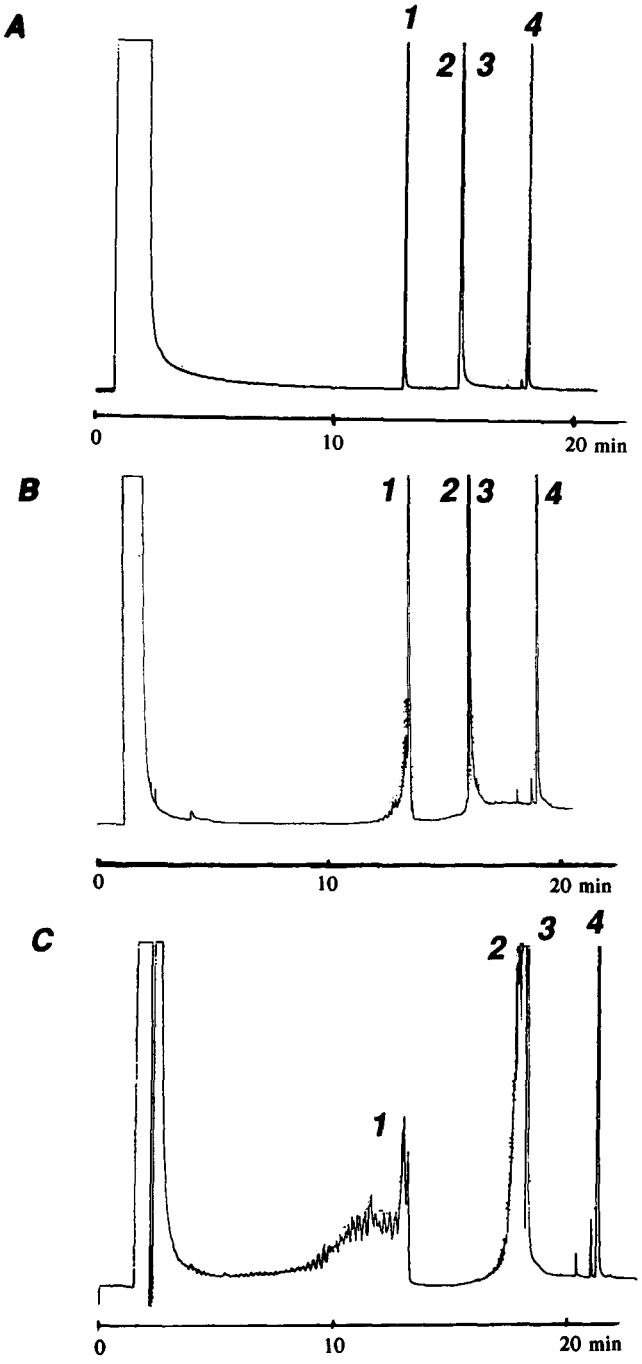


Fig. 2. Gas chromatograms of (A) an 8-μl injection of a 0.02 mg/ml solution each of (1) naphthalene, (2) biphenyl, (3) acetanilide and (4) phenanthrene in acetonitrile–water (84:16), (B) a 4-μl injection in acetonitrile–water (60:40) and (C) an 8-μl injection in acetonitrile–water (60:40) using a 30 m × 0.32 mm I.D. DB-1 column ($d_f = 0.25$ μm) and 2 m × 0.53 mm I.D. CPWax 52 CB retention gap ($d_f = 0.025$ μm). $T_1 = 80$°C (4 min), then increased at 30°C/min to 200°C; $T_2 = 65$°C (12 min), then increased at 30°C/min to 200°C.

The above experiment was repeated using another column, *viz.*, a 30 m × 0.32 mm I.D. DB-1 ($d_f = 0.25$ μm) column using the two-oven system. Fig. 2 shows some relevant data. In this instance a cold trapping effect was created at the entrance of the analytical column by holding the Vega oven at 65°C, while raising the temperature of the Mega oven to 200°C, which was necessary to reconcentrate acetanilide. Using the azeotropic mixture (8 μl), all peaks had a perfect shape, as is shown in Fig. 2A. A 4-μl injection of an eluent containing 40% water led to deformation of the napthalene peak (see Fig. 2B), while the other peaks were still of good shape. After the evaporation of the azeotrope, about 1 μl of pure water remains in the gap. An 8-μl injection of the same mixture led to deformation of the first three peaks (see Fig. 2C). In this instance, about 2 μl of water remain in the retention gap after evaporation, and this obviously is enough to distort the solvent trapping of the first three peaks completely. Probably owing to the cold trapping effect and the difference in film thickness, the heavier compound (phenanthrene) was reconcentrated after evaporation of the remaining water, giving a peak of perfect shape.

The combined results indicate that if more than *ca.* 1 μl of water is left in the retention gap after the azeotropic solvent mixture has evaporated, the peaks start to distort or areas to collapse. With a thick-film CPWax 52 CB column, the phase soaking effect due to the combination of the polar stationary phase and the polar eluent and also the large difference in retention power between the retention gap and the thick-film column probably contribute to an extra reconcentration of the components [4]. If a few microliters of water are left behind in the retention gap, part of the solute is reconcentrated at the inlet of the column and the remainder is lost during the evaporation of the water, which results in well shaped but too small peaks, whereas with the DB-1 column, where the phase soaking effect is missing and the increase in retention power difference is smaller, complete distortion of the peaks occurs. In this context it is interesting that Cortes and co-workers published two papers on direct large-volume injections of aqueous solutions into a GC system [11,16]. Using a non-deactivated fused-silica gap with a length of 20 m they succeeded in introducing 20 μl of water, still obtaining sharp peaks for the late-eluting components. However, they did not report quantitative results and it is obvious from the published data that the relative peak heights of the components vary considerably between the several chromatograms. However, the major drawback of non-deactivated fused silica is that polar components will be adsorbed by this material so that only apolar and slightly polar components can be analysed.

Grob and Li [12] observed that with a 50-μl on-column injection of water–*n*-propanol (30:70) using an OV-17 deactivated fused-silica retention gap, early-eluting peaks were deformed whereas the later-eluting peaks were of perfect size and shape. One can easily calculate that after the evaporation of the azeotrope water–*n*-propanol (28:72), 1.4 μl of water will remain in the gap and contribute to peak distortion and loss of analytes. On introducing 50 μl of water–*n*-propanol (35:65), which means that 4.8 μl of water will remain in the gap after azeotropic evaporation, they found all peaks to be distorted. Our results essentially agree with their findings. They indicate that the maximum amount of water that can be left behind in the gap after azeotropic evaporation is of the order of 1 μl, the exact amount depending on the retention of the solute and the nature and thickness of the stationary phase of the column.

Lifetime of the Carbowax retention gap

Grob *et al.* [13] stated that water at elevated temperatures (100°C) rapidly deteriorates the deactivation of all kinds of retention gaps, including Carbowax-deactivated gaps. We therefore regularly tested the CPWax 52 CB retention gaps after repeated large-volume injections (8–40 μl) of acetonitrile–water mixtures with a varying water content. In Fig. 3 several chromatograms are shown for these retention gaps using the Grob test mixture. The peak shape and height of the various solutes give information about the quality of the retention gap [17]. In the chromatogram obtained with a new Carbowax-coated retention gap, no deformation or disappearance of peaks was observed, as is shown in Fig. 3A, except for the dicyclohexylamine

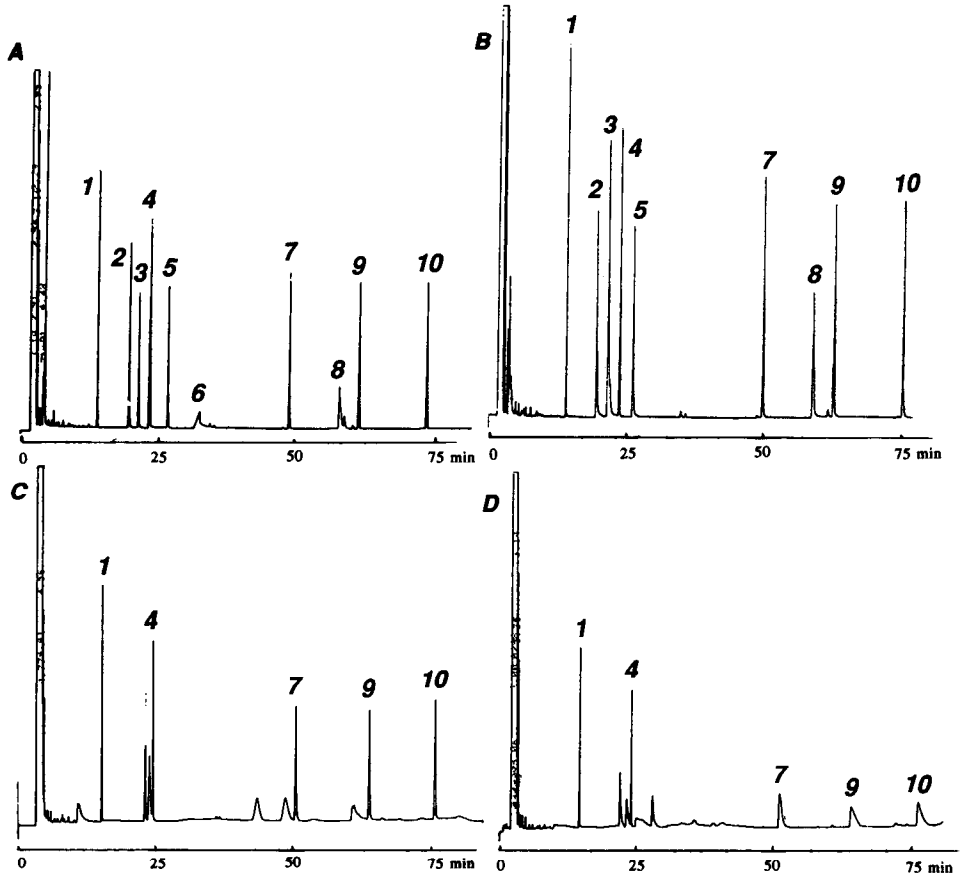


Fig. 3. Test chromatograms of several retention gaps using the Grob test mixture. (A) New, unused 2 m × 0.53 mm I.D. CPWax 52 CB retention gap (d_f = 0.025 μm); (B) retention gap A after about 125 acetonitrile–water large-volume injections; (C) 1 m × 0.53 mm I.D. CPWax 52 CB retention gap (d_f = 0.05 μm) after 1.5 year of use; (D) 1 m × 0.53 mm I.D. non-deactivated fused silica; analytical column: 30 m × 0.32 mm I.D. DB-1 (d_f = 0.25 μm). Conditions: T = 40°C, then increased at 1°C/min to 120°C; carrier gas, helium (100 kPa); injection, 1 μl on-column. 1 = *n*-Decane; 2 = 1-octanol; 3 = 2,6-dimethylphenol; 4 = *n*-undecane; 5 = 2,6-dimethylaniline; 6 = 2-ethylhexanecarboxylic acid; 7 = methyl decanecarboxylate; 8 = dicyclohexylamine; 9 = methyl undecanecarboxylate; 10 = methyl dodecanecarboxylate.

peak and 2-ethylhexanecarboxylic acid peak. We used this retention gap during the experiments described in the previous section and for other large-volume experiments with acetonitrile–water solutions. After about 125 large-volume injections, this gap was tested again and still no peak deformation was observed, although it is certain that on various occasions pure water had been in contact with the deactivation of the gap. Even the peaks of the polar compounds still were of good shape (See Fig. 3B); the shape of the dicyclohexylamine peak was improved but 2-ethylhexanecarboxylic acid disappeared completely. This might be explained by the fact that the water introduced probably removed the traces of acid from the new, unused carbowax retention gap, resulting in a less acidic deactivation.

In Fig. 3C, a chromatogram is shown for another CPWax 52 CB gap which was used for more than 1.5 years for several large-volume injections of polar and aqueous solvent mixtures. Most of the polar compounds have disappeared, which means that the deactivation of the retention gap becomes affected, but the apolar compounds still give well shaped peaks. Grob *et al.* [13] found that even after a few water injections the Carbowax was already completely removed from the gaps, leaving a fused silica which was more active than raw fused silica and which adsorbed even the apolar peaks. To verify whether this was also true for our retention gap, for comparison we ran a chromatogram for a raw fused-silica retention gap (see Fig. 3D). Contrary to what is seen in Fig. 3C, the methyl ester peaks are deformed in Fig. 3D. This means that the raw fused-silica column is more active than the Carbowax retention gap which was used for 1.5 years and that even after frequent use the Carbowax deactivation of the gap is not completely lost.

In summary, the experimental data indicate that a very thin-film CPWax 52 CB-coated retention gap can be used for the introduction of aqueous mixtures for several months, without serious determination of the deactivation, even if pure water has remained in the gap.

Introduction speed and introduction volume

Using an on-column interface in on-line LC–GC, the eluent flow-rate of the LC column equals the sample introduction speed in GC. Therefore, the maximum sample

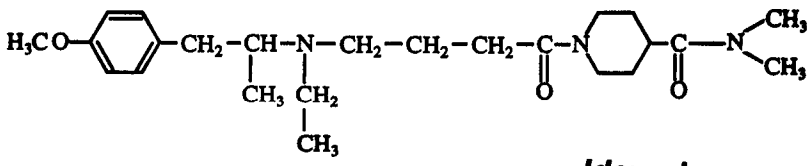
TABLE II

PEAK AREAS OF IDAVERINE AFTER 20-μl INTRODUCTION OF A 10 μg/ml IDAVERINE SOLUTION IN ACETONITRILE–WATER (90:10) + 0.1% TRIETHYLAMINE USING VARIOUS INTRODUCTION SPEEDS

Conditions: analytical column 30 m × 0.32 mm I.D. DB-1 (d_f = 0.25 μm); retention gap 5 m × 0.53 mm I.D. CP Wax 52 CB (d_f = 0.025 μm); average area, 4.1 × 10^6.

Introduction speed (μl/min)	Peak area (× 10^6 counts)[a]
20	4.0
40	3.9
60	4.0
80	4.2
100	4.3

[a] S.D. = 0.3 × 10^6; R.S.D. = 6.8%.



Idaverine

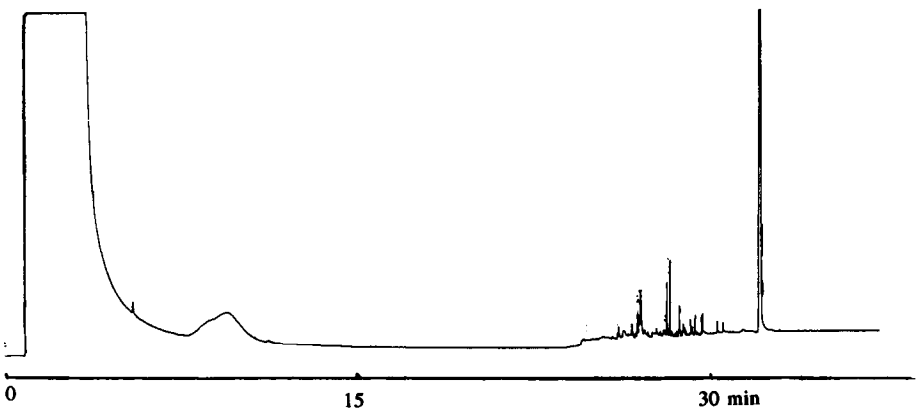


Fig. 4. Gas chromatograms of 10 μg/ml idaverine solution in acetonitrile–water (90:10), introduced into a CPWax 52 CB retention gap ($d_f = 0.025$ μm) (A) with a speed of 100 μl/min and an introduction volume of 20 μl and (B) with a speed of 40 μl/min and an introduction volume of 40 μl. Analytical column 30 m × 0.32 mm I.D. DB-1 ($d_f = 0.25$ μm); retention gap, 5 m × 0.53 mm I.D. CPWax 52 CB ($d_f = 0.025$ μm); $T_1 = 90$°C (during solvent evaporation), then increased at 30°C/min to 220°C; $T_2 = 220$°C (during solvent evaporation + 15 min), then increased at 30°C/min to 325°C; $T_{interface} = 220$°C.

introduction speed into the GC column determines the maximum dimensions of the LC column. In trace analysis low detection limits are required; e.g., drugs often have to be determined in plasma at a level of 0.1–1 μg/l. This means that for on-line LC–GC the LC column must have sufficient sample capacity; a large inside diameter is desirable. We therefore determined the maximum introduction speed of an acetonitrile–water mixture into the GC retention gap, using the drug idaverine as a test component. The results of the experiments are shown in Table II, where the peak areas of a 20-μl injection of a solution of 10 μg/ml of idaverine in acetonitrile–water (90:10) containing 0.1% triethylamine are given for introduction speeds varying from 20 to 100 μl/min. Triethylamine was added to the solution to prevent adsorption on the LC column. The relative standard deviation (R.S.D.) is small, which means that up to 100 μl/min no problems are observed. The average peak area of $(4.1 \pm 0.3) \cdot 10^6$ counts is almost equal to the $4.3 \cdot 10^6$ counts obtained on direct on-column injection of 1 μl of a solution of 200 μg/ml of idaverine in methanol. Obviously, no significant losses of idaverine occur during the large-volume introduction of an acetonitrile–water mixture. During the above work, we observed that, using an inlet pressure of 150 kPa and an introduction temperature of 90°C, sometimes backflow into the injector occurred, which caused long tailing solvent peaks. This problem was solved by increasing the inlet pressure to 200 kPa. A chromatogram of a 20-μl injection of an idaverine solution, introduced at a speed of 100 μl/min, is shown in Fig. 4A. As regards the eluent flow-rate, the above experiments indicate that commercially available 1 mm I.D. microbore columns, which require a flow-rate of about 50 μl, can be used for on-line (reversed-phase) LC–GC using an on-column interface.

However, an increase in the inside diameter of the LC column also results in larger peak volumes and consequently in larger fraction volumes, which have to be transferred to the gas chromatograph (*ca.* 5 μl for a 0.33 mm I.D. LC column *vs. ca.* 50 μl for a 1 mm I.D. LC column). Therefore, in Fig. 4B a chromatogram is given for a larger injection volume, *i.e.*, 40 μl, of the 10 μg/ml idaverine solution using an introduction speed of 40 μl/min. The retention gap used in this instance is a 5 m × 0.53 mm I.D. CPWax 52 CB ($d_f = 0.025$ μm). Both the idaverine and the solvent peak have a similar shape to that obtained on introduction of 20 μl.

The length of the flooded zone in the retention gap appears to be an indication of the wettability of the gap by the solvent mixture [12]. This length can be calculated from Fig. 4B. As the solvent peak of the 40-μl injection has a width of *ca.* 2 min, the 40 μl must have evaporated at a rate of *ca.* 20 μl/min. This indicates that, using an eluent introduction speed of 40 μl/min, *ca.* 20 μl flooded the retention gap, which means that the flooded zone was *ca.* 25 cm per 1-μl injection. This corresponds well with the flooded zone of 38 cm/μl found for acetonitrile–water (90:10) using a 0.32 mm I.D. DTMDS retention gap at 25°C [12]. Although in the present instance solvent effects do not play any role because idaverine is a high-boiling compound, it can be concluded that the retention gap has a good wettability for the acetonitrile–water mixture.

CONCLUSIONS

The aim of this study was to investigate the large volume introduction of aqueous solvent mixtures into a retention gap in order to couple reversed-phase LC with

GC using an on-column interface. Retention gaps coated with a very thin film of CPWax 52 CB (0.025 μm) are suitable for this purpose. The lifetime of these retention gaps is several months, in spite of several exposures to acetonitrile–water mixtures of varying compositions. This means that reversed-phase LC can be coupled to capillary GC. The following aspects should, however, be considered. The maximum amount of water in an aqueous–organic LC eluent that can be tolerated is limited. No problem will be encountered if an acetonitrile–water mixture has an azeotropic composition, *i.e.*, 84:16, (v/v), or contain less water. In principle, solvent mixtures containing a higher percentage of water can also be introduced into the retention gap. However, as the performance of the chromatographic system rapidly deteriorates if more than 1 μl of water remains in the retention gap after azeotropic evaporation, the volume that can be introduced into the system will be restricted. The maximum introduction speed into the described GC system of an acetonitrile–water mixture (90:10) is at least 100 μl/min. The maximum introduction volume of such a mixture under the described conditions at a flow-rate of 40 μl/min is *ca.* 40 μl if a 5 m $\times$ 0.53 mm I.D. CPWax 52 CB retention gap is used. This volume can be increased by lengthening the retention gap or by the use of an early vapour exit [18,19]. These results imply that micro-bore LC columns with inside diameters of up to 1 mm can now be used in reversed-phase LC–GC. Further research in this field will be concentrated on applications of the results obtained here.

REFERENCES

1 K. Grob, Jr., B. Schilling, C. Walder, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 95.
2 K. Grob, Jr., and J. M. Stoll, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 9 (1986) 518.
3 K. Grob, Jr., *On-line coupled LC–GC*, Hüthig, Heidelberg, in press.
4 K. Grob, Jr., *On-Column Injection in Capillary GC*, Hüthig, Heidelberg, 1987.
5 K. Grob, Jr., *J. Chromatogr.*, 279 (1983) 225.
6 K. Grob, Jr., and B. Schilling, *J. Chromatogr.*, 391 (1987) 3.
7 I. L. Davies, K. E. Markides, M. L. Lee, M. W. Raynor and K. D. Bartle, *J. High Resolut. Chromatogr.*, 12 (1989) 193.
8 K. Grob, Jr., and Z. Li, *J. Chromatogr.*, 473 (1989) 381.
9 D. Duquet, C. Dewaele, M. Verzele and S. McKinley, *J. High Resolut. Chromatogr. Chromatogr. Commun.* 11 (1988) 824.
10 K. Grob, Jr., and Z. Li, *J. Chromatogr.*, 473 (1989) 423.
11 H. J. Cortes, C. D. Pfeiffer, G. L. Jewett and B. E. Richter, *J. Microcolumn Sep.*, 1 (1989) 28.
12 K. Grob, Jr., and Z. Li, *J. Chromatogr.*, 473 (1989) 391.
13 K. Grob, Jr., H. P. Neukom and Z. Li, *J. Chromatogr.*, 473 (1989) 401.
14 A. V. Pouwelse, D. de Jong, and J. H. M. van den Berg, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 607.
15 A. V. Pouwelse, D. de Jong, N. G. F. M. Lammers and J. H. M. van den Berg, paper presented at the Twelfth International Symposium on Column Liquid Chromatography, HPLC '88, Washington, DC, June 19–24, 1988.
16 B. B. Gerhart and H. J. Cortes, *J. Chromatogr.*, 503 (1990) 377.
17 K. Grob, Jr., *J. Chromatogr.*, 156 (1978) 1.
18 H. G. Schmarr, A. Mosandl and K. Grob, *J. High Resolut. Chromatogr.*, 12 (1989) 721.
19 E. Dolecka, J. J. Vreuls, F. A. Maris, G. J. de Jong and U. A. Th. Brinkman, *J. High Resolut. Chromatogr.*, 13 (1990) 405.