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## INTRODUCTION OF WATER AND WATER-CONTAINING SOLVENT MIXTURES IN CAPILLARY GAS CHROMATOGRAPHY

### III. WATER-RESISTANT DEACTIVATION OF UNCOATED PRE-COLUMNS?

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#### SUMMARY

Uncoated capillaries, deactivated by silylation or by layers of non-extractable Carbowax or phenylsilicones, rapidly become active when water or (a far weaker effect) alcohols such as methanol are introduced. Apparently, the effect is far more pronounced for condensed water than for water vapour, as analogous separation columns hardly suffer under the action of water vapour. The resulting high adsorptivity of the precolumns is hardly noticed as long as the sample matrix contains water by itself. A thin layer of OV-17, "baked" on to raw fused silica, produced the best, but still unsatisfactorily, water-resistant deactivation.

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#### INTRODUCTION

In Parts 1 and 2<sup>1,2</sup> we described the possibilities and limitations of introducing large volumes of water or water-containing solvent mixtures, concentrating on retention gap techniques. We did not find any precolumn surface suitable for gas chromatography (GC) that was water-wettable. On the other hand, admixture of organic solvents greatly improves the wetting properties. For instance, the addition of 30% of 1-propanol to water renders a phenyldimethylsilylated surface wettable. However, for retention gap techniques (with or without partially concurrent solvent evaporation), the problem still remains that organic solvents tend to evaporate before the water, leaving behind the non-wetting water.

Introduction of water or water-containing solvent mixtures presupposes the availability of uncoated precolumns (retention gaps) that are deactivated in such a way that deactivation resists water and alcohols. Such precolumns are required, regardless of whether retention gap techniques or concurrent solvent evaporation (with or without co-solvent trapping) are applied.

Experimentally, it was found that after a limited number of large transfers of

water or water-containing mixtures, the adsorptivity of the GC system was strongly enhanced; the peaks started to tail, were strongly broadened or even disappeared. Typically, peak deformation became more pronounced towards the end of the analysis (e.g., at increased temperature during long temperature programmes). Presumably, this is due to temporary deactivation of an adsorptive surface by adsorbed water, which loses its efficiency as more adsorbed water evaporates. Such activity was primarily observed when glass capillary precolumns were used. Fused-silica precolumns also became active, but not to the same extent.

This paper reports the alcohol and water resistance obtained with the deactivation procedures available. The result was disappointing: water destroyed all deactivation.

## EXPERIMENTAL

### *Deactivation of capillaries*

The following procedures were used to deactivate 10 m × 0.32 mm I.D. glass and fused-silica capillary tubes.

*Silylation.* After leaching (glass) or hydrothermal treatment (fused silica)<sup>3</sup>, the capillaries were silylated with diphenyltetramethyldisilazane (DPTMDS) at 400°C (glass) or 370°C (fused silica). After silylation, the capillaries were rinsed with toluene, methanol and diethyl ether.

*Carbowax deactivation by "baking".* Raw fused silica was rinsed with 1% of Carbowax 20M in dichloromethane, flushed with carrier gas and heated at 280°C for 2 h. The capillaries were rinsed with ca. 2 ml of dichloromethane before testing.

*Carbowax deactivation by "bonding".* Capillaries were dynamically coated with a 0.3% solution of Superox 20M in dichloromethane, containing 10% (referred to the Superox) of dicumyl peroxide and 20% of vinyltriethoxysilane. They were flushed with nitrogen (removing dichloromethane and air) and sealed with a flame, then heated at 210°C for 2 h. The capillaries were again rinsed with dichloromethane before testing.

*Coating with OV-17.* Raw fused silica was dynamically coated with a 0.3% solution of OV-17 in dichloromethane at a speed of 2 cm/s, using a damping column at the exit. It was heated at 360°C for 2 h, then thoroughly rinsed with dichloromethane.

*Coating with OV-61-OH.* Leached glass or raw fused silica was dynamically coated with a 1% solution of OV-61-OH (33% phenyl) in dichloromethane (see above) and heated at 320°C for 30 min. Then the capillary was rinsed with azo-*tert.*-butane vapour in nitrogen, sealed and heated at 200°C for 2 h. Before testing, the capillary was rinsed with dichloromethane.

### *Testing of the deactivated capillaries*

Testing of the deactivated capillaries was carried out by the retention gap test, described previously<sup>4</sup>. Capillaries were installed in a conventional split/splitless injector and a flame ionization detector was used. Before testing, the capillaries were heated at 250°C for 10 min. Tests were carried out isothermally at 70°C; the carrier gas inlet pressure was 0.15 bar (hydrogen). Gas hold-up times were determined by injecting 1 μl of fuel gas (methane). Then, 5–20 μl of the headspace gas from the test components (taken from the bottle) were injected by splitting (about 10:1), analysing at low attenuation to avoid excessive overloading. Retention times of reasonably shaped

peaks were measured with a stop-watch in order to calculate the adjusted capacity ratios ( $k'$ ), which were then converted to "apparent film thicknesses", calibrated for an apolar stationary phase.

Test results were interpreted in terms of retention power and adsorptivity. Adsorptivity was deduced from peak shapes, but also from retention powers for polar compounds.

#### *Treatment with methanol and water*

To simulate the conditions during solvent evaporation in a reproducible way, capillaries to be tested for stability of the deactivation were filled to 90% with methanol, closed with a press-fit cap and heated at 90°C for 30 min, then rinsed and tested. Subsequently, the capillaries were filled to 90% with water, heated at 100°C for 30 min, rinsed and tested again. Finally, they were refilled with water and heated at 140°C for 30 min, followed by testing.

#### *Resilylation*

Silylated and silicone-coated capillaries were resilylated, after turning active due to methanol and water, using a technique described by Grob and Grob<sup>5</sup> for coated columns. An amount of 20–30  $\mu$ l of a 1:1 mixture of DPTMDS and *n*-pentane was passed through the capillary at *ca.* 3 cm/s with nitrogen. Then, the capillary was installed in an injector, leaving the column exit hanging freely. The capillary was flushed with carrier gas for 5 min, the inlet pressure was reduced to 0.05 bar and the oven temperature was programmed at 3°C/min from 150 to 330°C, keeping it at the final temperature for 2 h. Finally, the capillary was rinsed with dichloromethane before being tested.

## RESULTS AND DISCUSSION

#### *Glass versus fused silica*

Glass capillaries, deactivated by Carbowax or silylation, exhibited extremely high adsorptivity after water treatment, substantially exceeding that observed with fused-silica capillaries. This can easily be explained by the far higher concentration of silanol groups on the glass than on fused-silica surfaces. Deactivation by thin layers of OV-17 was less efficient on glass than on fused silica to begin with. For this reason, the results reported are restricted to fused-silica capillaries.

#### *Carbowax-deactivated fused silica*

The test results for a fused-silica capillary, raw as obtained (Polymicro Technologies, Phoenix, AZ, U.S.A.) and after deactivation with Carbowax by the "baking" method, are shown in Fig. 1. In the top row, test results for the raw fused silica show not only that the *n*-tetradecane (C-14) peak (upper left corner) is well shaped, but also that the capillary has a surprisingly high retention power (see Table I). The same is true for ethylnaphthalene. Methyl esters and alcohols were not eluted as detectable peaks, even when large amounts were injected, thus confirming the strong adsorptivity of the raw fused silica.

The second row of chromatograms was obtained with the freshly Carbowax-deactivated capillary. All test components were eluted as well shaped peaks. The

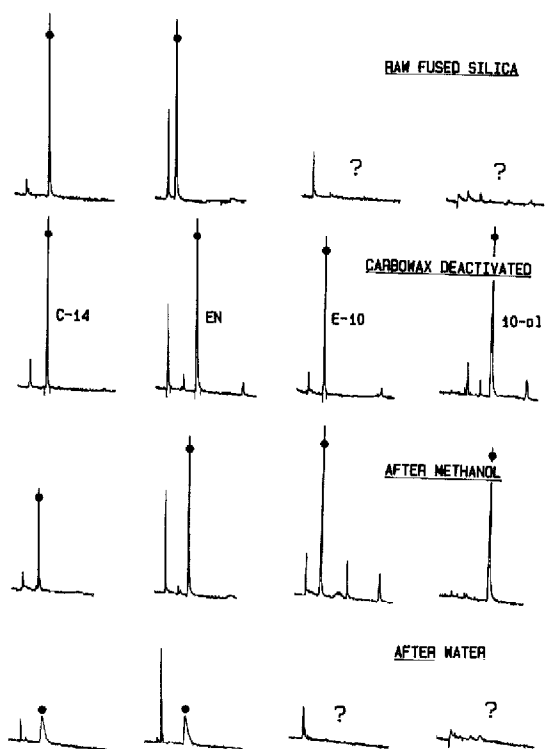


Fig. 1. Test results for raw fused silica, for the same capillary after Carbowax deactivation and after treatment of the latter with methanol at 90°C for 30 min and with water at 140°C for 30 min. Split injections of headspace gas of *n*-tetradecane (C-14), ethylnaphthalene (EN), methyl decanoate (E-10) and 1-decanol (10-o1). Corresponding peaks are marked by a spot; a question mark indicates that no peak is observed. Methanol hardly affects the deactivation, whereas water completely ruins it.

retention powers were moderate (except for the most polar test compound, showing the high polarity of the deactivation), but are acceptable for precolumns of up to intermediate length (requiring a moderate reconcentration efficiency). It is interesting that the retention power for the alkane was below that of the raw fused silica.

Methanol (after heating at 90°C) removed some of the Carbowax (as concluded from the slightly decreased retention powers) but did not seriously affect the deactivation (peak shapes are hardly affected). Water, however, left behind a fused-silica surface that was more active than the raw fused silica. Apparently, water even attacks the silica, opening some strained siloxane bonds and forming (adsorptive) silanol groups.

The results obtained with the "bonded" Carbowax were even slightly worse. The Carbowax film was relatively thick (see retention powers). Methanol removed more than half of it (see retention powers in Table I), and the remainder was completely ruined by the water. Thus "bonded" Carbowax, non-extractable by solvents including methanol and water at ambient temperature, becomes completely extractable after some hydrolysis.

On the other hand, we noted that separation columns coated with Carbowax-

TABLE I

RETENTION POWERS IN TERMS OF APPARENT FILM THICKNESSES OF AN APOLAR STATIONARY PHASE FOR DIFFERENTLY DEACTIVATED PRECOLUMNS WITH AND WITHOUT TREATMENT WITH METHANOL AND WATER

Deactivation	Further treatment	Retention power (nm) <sup>a</sup>					
		C-14	EN	E-8	E-10	8-ol	10-ol
Raw FS	—	5.2	3.1	—	—	—	—
"Phesil"	Fresh	1.4	0.5	—	1.4	—	3.6
	Methanol, 90°C	1.4	0.6	—	1.9	—	3.3
	Water, 140°C	1.8	0.9	—	—	—	—
CW "baked"	Fresh	3.4	7.5	—	7.4	—	22.7
	Methanol, 90°C	3.6	6.6	—	5.4	—	20.5
	Water, 140°C	5.4	8.3	—	—	—	—
CW "bonded"	Fresh	20.1	9.4	—	—	—	—
	Methanol, 90°C	7.9	4.6	—	—	—	—
	Water, 140°C	—	—	—	—	—	—
1% OV-17	Fresh	6.1	13.7	35.3	—	30.5	—
	Methanol, 90°C	5.9	16.3	33.8	—	41.8	—
	Water, 100°C	6.5	17.9	46.9	—	68.2	—
	Water, 140°C	5.4	16.5	66.6	—	83.2	—
	Resilylated	5.2	13.4	21.3	—	21.8	—
0.3% OV-17	Fresh	4.2	9.1	20.9	—	19.7	—
	Methanol, 90°C	4.2	8.6	21.6	—	36.6	—
	Water, 140°C	3.6	6.5	16.3	—	20.9	—
1% OV-61-OH	Fresh	5.4	13.2	24.8	—	34.1	—
	Methanol, 90°C	5.4	13.9	28.2	—	51.4	—
	Water, 100°C	4.7	12.6	36.9	—	53.6	—
	Water, 140°C	4.7	13.4	51.6	—	—	—
	Resilylated	6.1	13.8	22.5	—	36.4	—

<sup>a</sup> C-14 = *n*-tetradecane; EN = ethylnaphthalene; E-8 = methyl octanoate; E-10 = methyl decanoate; 8-ol = 1-octanol; 10-ol = 1-decanol.

type stationary phases resisted water well; column bleeding did not significantly increase even after the passage of very large amounts of water. This suggests that condensed water (within the flooded precolumn) does far more harm to Carbowax than does water vapour (in the separation column).

#### *Silylated fused silica*

Fig. 2 shows test results obtained with a fused-silica capillary, deactivated by phenyldimethylsilylation. All test solutes form well shaped peaks. The retention power is low (Table I). After heating the column with methanol at 90°C for 30 min, the capillary still had nearly the same inertness; the retention power also did not change. However, water strongly increased the adsorptivity. The retention powers measured for polar solutes increased owing to increased interaction with adsorptive sites of the surface. Alcohols hardly formed peaks any more.

From the test results, and from the fact that resilylation of the capillary restored inertness, we concluded that water hydrolyses the silyl ethers, in other words, the capillary surface silylates water!

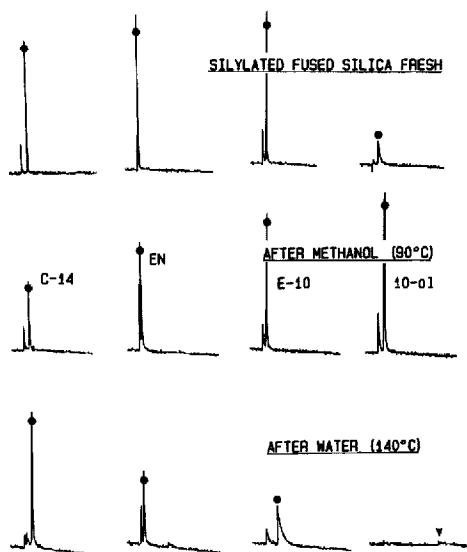


Fig. 2. Test results for a phenyldimethylsilylated fused-silica capillary (DPTMDS treatment), analogous to Fig. 1. Methanol hardly affects the inertness of the surface, whereas water ruins the deactivation.

### Thin silicone films

Repeated testing revealed that silicone-coated glass capillary separation columns are hardly affected by water vapour; after passage of the vapour from several millilitres of water at up to 160°C, the 1-octanol peak of the standard test mixture still produced 40–60% of the possible peak height, and the methyl ester peaks had not deteriorated at all. This suggested coating of the precolumn with a thin layer of a stationary phase to obtain the same performance. Provided that short pieces (up to about 10 m × 0.32 mm I.D.) of precolumn are used, *e.g.*, for concurrent eluent evaporation, a thin layer of stationary phase produces a retention power sufficiently low to provide the reconcentration power required for shortening the bands broadened in space<sup>6</sup>.

Simple coating with conventional silicone stationary phases results in films that are virtually completely extractable with organic solvents. However, as shown by Schomburg *et al.*<sup>7</sup>, after heating at high temperatures (350–400°C) part of the silicone material becomes non-extractable. The method was used for deactivating capillaries before coating them with silicone stationary phases. We expected two benefits from coating with a silicone polymer. First, the capillary surface is shielded by a layer through which the water is unable to penetrate. This should prevent leaching of the silica and the formation of new silanol groups on the support surface. Second, deactivation should no longer be as easy to destroy. “Baking” silicones on active silica surfaces causes some degradation of the silicone, producing fragments that react with the available silanol groups of the support. Hence, silanols are derivatized by rather large molecules, and even if water hydrolyses such bonds again, these macromolecules, insoluble in water, are no longer removed by water. In contrast, simple heating should restore the reaction and cause the free silanol group to disappear again.

The choice of the silicone stationary phase was determined by three arguments: first, the stationary phase surface should be optimally wettable, calling for a high critical surface energy; second, water and alcohols should not penetrate into the stationary phase in order to obtain the desired protection of the support surface; and third, the stationary phase should be chemically stable. Phenyl substituents efficiently increase the surface tension but keep the solubility of polar solvents low. Cyano functions are unstable, as they tend to become hydrolysed.

The experimental results were conflicting. The deactivation achieved by a well bonded layer of OV-61-OH was hardly more water-resistant than silylation. In contrast to the silylated and the Carbowax-deactivated capillaries, methanol also caused some damage. Resilylation restored the original retention power, indicating that no silicone polymer was lost. Thus, methanol and water must have introduced highly adsorptive functional groups into the silicone (silanols?) rather than destroying the deactivation.

On the other hand, capillaries deactivated by a layer of conventional OV-17, "baked" to the surface, produced better results than all other deactivations (Fig. 3). The capillary became slightly more active on treatment with methanol but, in the case shown, water restored the original inertness rather than further degrading the deactivation.

As shown in Table I, deactivations by 1% and 0.3% solutions of OV-17 resulted in retention powers that were less different than might be expected (owing to the reduced viscosity of the more dilute solution, the amount of stationary phase deposited on the capillary wall by dynamic coating differed by a factor exceeding 3). This effect corresponds to what was previously found for Carbowax deactivations. A certain amount of stationary phase is bonded to the surface, while any extra amount remains extractable.

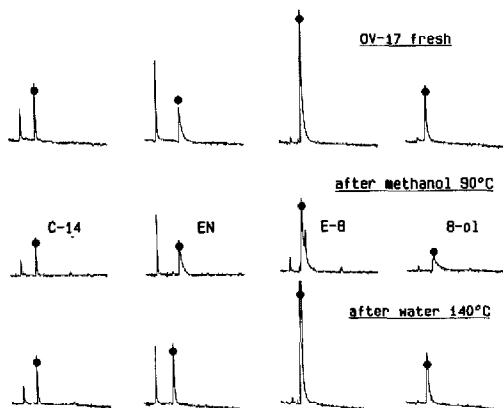


Fig. 3. Test results for a fused-silica capillary, deactivated by a thin layer of OV-17 (50% phenyl). As the retention power is relatively high, methyl octanoate (E-8) and 1-octanol (8-ol) were used as test components instead of E-10 and 10-ol. Methanol slightly affected the deactivation, whereas water almost restored the test result.

### Resilylation

The silylated and silicone-coated capillaries were resilylated after becoming active with water. Resilylation is rapid and simple, and the re-deactivation is nearly complete. It confirms that water generates activity by introducing hydroxyl groups into the silica backbone or the silicone polymer of the stationary phase layer.

### Consequences for practical GC

The above tests with the uncoated capillaries allow a rapid evaluation of precolumns, but there is no direct relationship between such test results and real GC analysis. This gap was filled by testing the precolumns as part of a separation system.

Precolumns (10–15 m × 0.32 mm I.D.) were joined with an excellent 15 m × 0.32 mm I.D. glass capillary, coated with PS-255 (a methylsilicone) of 0.3 μm film thickness (film thickness is an important parameter, as thicker films better reconcentrate bands broadened in the precolumn). The column quality test II<sup>8</sup> was injected by the on-column technique, using conditions standardized for the 15-m separation column.

The top test chromatogram in Fig. 4 was obtained with the Carbowax-deactivated precolumn shown in Fig. 1, after treatment of the latter with water (precolumn test, see bottom row in Fig. 1). Only alkanes produce reasonable peaks, all

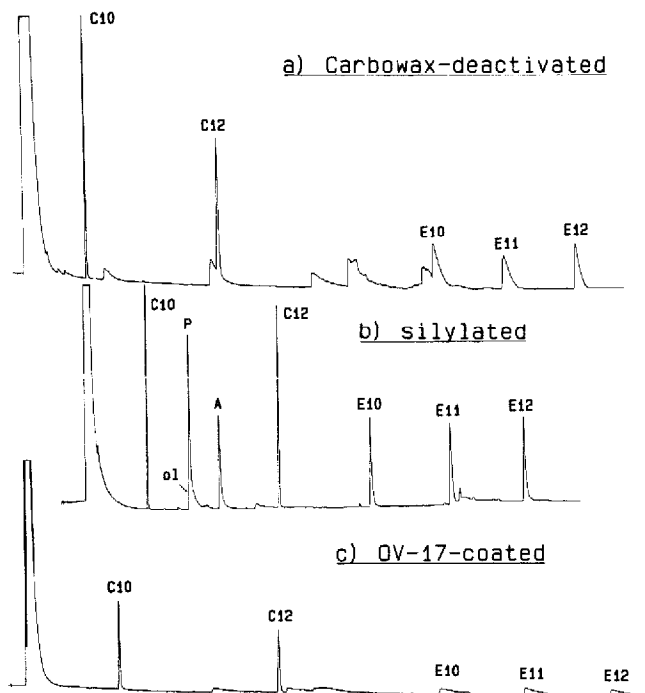


Fig. 4. Test chromatograms involving three precolumns, treated with water at elevated temperatures: (a) Carbowax deactivated; (b) silylated; (c) OV-17-coated. The 10–15 m × 0.32 mm I.D. precolumns were connected to an excellent apolar separation column; standard column quality test II. Peaks: C10, C12 = *n*-alkanes; E10–E12 = methyl esters; ol = 1-octanol; P = 2,6-dimethylphenol; A = 2,6-dimethylaniline. For details, see text.



other components being badly adsorbed. Not only are their peaks distorted, but also is their elution strongly retarded, and the elution pattern can no longer be recognized. The test chromatogram in the centre shows the effect of an active, initially silylated fused-silica capillary that was treated with water. The test on the uncoated capillary alone was similar to that shown in the bottom row of Fig. 2. The alcohol peak (ol) nearly disappeared, reflecting the strong adsorptivity also found by the test in Fig. 2. The peaks of the methyl esters are broadened and tail; their height should be similar to that for *n*-dodecane (C-12).

The last chromatogram in Fig. 4 gives a picture of a completely ruined precolumn, and one would not expect to obtain any reasonable peak, except for alkanes, when such a precolumn is used. However, this impression is wrong. This same fused-silica precolumn of 15 m length was previously used for injecting 10–60- $\mu$ l volumes of 1-propanol containing water at various concentrations. The last chromatograms for these experiments were shown in Part II<sup>2</sup>, where the methyl ester peaks (C<sub>8</sub>–C<sub>24</sub>) did not show any tailing. This confirms what we have observed in many other instances: the high activity of precolumns observed after the introduction of water affects GC analyses very differently, depending on whether water is still part of the sample matrix or not. Water appears to be very efficient in temporarily deactivating capillary surfaces.

It must be added that this last precolumn was deactivated by a thin layer of OV-17, which indicates that even the deactivation procedure showing the best test results does not yield an inertness that resists water over extended periods in practical work.

## CONCLUSIONS

We were not previously aware of the detrimental effect of condensed water on uncoated precolumns. It must be assumed that the low water content of many every-day samples also rapidly destroys the deactivation of uncoated precolumns.

At present, uncoated precolumns with water-resistant deactivation are not available. This certainly is a need that should be filled in the future, but we have no idea how it could be achieved. Silicones and polyglycols appear to be hydrolysed and silica surfaces are leached. What other inert, thermostable and reasonably wettable surface coatings are available in GC? Nevertheless, the lack of water-resistant deactivation of capillary precolumns does not prevent work involving the introduction of water, such as in on-line coupled reversed-phase LC–GC. During all of the experiments carried out, the adsorptivity of the precolumn did not become a limiting factor, provided that we continued to introduce water-containing solvent mixtures. It did, however, preclude the use of glass capillary precolumns.

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