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

### II. WETTABILITY OF PRECOLUMNS BY MIXTURES OF ORGANIC SOLVENTS AND WATER; RETENTION GAP TECHNIQUES

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#### SUMMARY

The wettability of phenyl- and cyanosilylated precolumn surfaces by mixtures of organic solvents and water (*e.g.*, typical reversed-phase eluents) was tested. Depending on the organic solvent, mixtures with high water concentrations, such as 1-propanol containing up to 70% water, still wet such standard precolumn surfaces. However, when retention gap techniques were applied, another requirement proved to be more limiting: water must evaporate at least as rapidly as the organic solvent, otherwise water is left behind the evaporating solvent mixture and, as water does not wet these surfaces, it floods the capillary. Hence high-boiling solvents are required; azeotropically boiling mixtures facilitate the work. It is shown that 1-propanol with up to 28% water allows the introduction of large volumes by the retention gap technique.

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#### INTRODUCTION

In Part I<sup>1</sup> we described experiments that failed to find capillary precolumns of glass or fused silica that are both water-wettable and sufficiently well deactivated to suit gas chromatography (GC). Interactions of water with the support surface required to spread the water on such a surface are so great that interactions with the solutes (adsorptivity) get out of control. This is not surprising when one considers the large gap between the extremely high surface tension of water and the substantially lower critical surface energies obtainable through deactivation of glass or fused-silica surfaces.

#### *Reversed-phase eluents for liquid chromatography*

In reversed-phase liquid chromatography (LC) coupled on-line to GC, the eluent seldom consists of water. However, the surface tensions of mixtures of organic solvents and water tend to be far below those of water, rendering wettability easier.

In fact, one of the earliest applications of LC-GC involving complete transfer of the LC fraction, published in 1985 by Cortes *et al.*<sup>2</sup>, involved reversed-phase LC with acetonitrile as eluent (although probably carried out under conditions causing concurrent eluent evaporation, where wettability is less important). Recently, Duquet *et al.*<sup>3</sup> injected 1- $\mu$ l volumes of methanol-water mixtures containing up to 50% water into 2 m  $\times$  0.25 mm I.D. GC precolumns. These precolumns were deactivated with hexamethyldisilazane (HMDS), aminopropylsilane and cyanopropylsilane. Unfortunately, no wettabilities were tested, and the tests on peak symmetry shown hardly serve as an indication of wettability.

This paper reports the wettabilities of silylated precolumn surfaces with various mixtures of organic solvents and water, of interest for reversed-phase LC-GC. Carbowax-deactivated precolumns were not considered because we were unable to bond Carbowax to the surface in such a way that it was not removed after a few injections<sup>1</sup>. Among the silylated surfaces, trimethylsilyl deactivation was not considered because of the known poor wettability<sup>4</sup>. Similarly, diphenylmethylsilylation was disregarded, because the resulting surfaces were found to be clearly less efficiently deactivated than phenyldimethylsilylated surfaces without offering noticeably better wettability.

## EXPERIMENTAL

### *Deactivation of capillaries*

Glass capillaries of 0.32 mm I.D. were leached and statically dried at 280°C according to Grob<sup>5</sup>. Silylation occurred with three reagents: (i) with a 1:1 mixture of hexamethyldisilazane (HMDS) and diphenyltetramethyldisilazane (DPTMDS) ("mesil/phesil"), (ii) with DPTMDS diluted with an equal volume of pentane ("phesil"), both heated overnight at 400°C, and (iii) with bis(cyanopropyl)tetramethyldisiloxane at 380°C ("cyanosil"), according to Blum<sup>6</sup>.

### *Capillary rise experiments*

For comparability purposes, the heights of rise of eluent mixtures of interest were measured. Approximately a third of a coil of a glass capillary was dipped into the liquid of interest, and the height of the meniscus above the surface of the liquid was measured.

### *Wettability tests*

In 10 m  $\times$  0.32 mm I.D. sections of the three types of capillaries tested, lengths of flooded zones were determined as described<sup>7</sup>. A whitish glass capillary was attached to them, and increasing volumes of liquid were introduced by on-column injection until some liquid penetrated into the whitish capillary. The capillary length (10 m) was divided by the volume of liquid retained to yield flooded zones in terms of cm/ $\mu$ l. For determinations at elevated temperatures, the GC oven door was replaced by a pane of Pyrex glass.

### *GC experiments*

The chromatographic experiments reported below were carried out with a 19 m  $\times$  0.32 mm I.D. glass capillary column coated with PS-255 (a methylsilicone) of 0.6

$\mu\text{m}$  film thickness. It was coupled to a 15 m  $\times$  0.32 mm I.D. fused-silica precolumn, deactivated by dynamic coating with a 0.3% solution of OV-17 in dichloromethane, followed by heating at 320°C for 2 h and thorough rinsing with dichloromethane (see Part III<sup>8</sup>).

On-column injections of 50- $\mu\text{l}$  volumes were carried out at an oven temperature of 100°C and an inlet pressure of 1.5 bar (hydrogen). After 3 min, the inlet pressure was reduced to 0.8 bar. This increase of the inlet pressure during injection helped to carry the sample liquid from the syringe needle further into the precolumn and to spread the sample plug at a suitable speed. High inlet pressures are preferable because of the high friction of water-containing solvents on wetted surfaces. In addition, the increased inlet pressure reduced the solvent evaporation time to about 3.5 min. When the pen of the recorder returned from the solvent peak, the oven temperature was programmed at 6°C/min to 270°C.

## RESULTS AND DISCUSSION

### *Capillary rise experiments*

Interaction forces between differently deactivated 0.32 mm I.D. (glass) capillaries and aqueous solvent mixtures were compared by the capillary rise method. Heights of rise determined at ambient temperature are listed in Table I. The listed heights of rise indicate that the critical energy of the mesil/phesil surface is noticeably below that of the phesil surface but that there is no significant difference between the phesil and cyanosil surfaces. In this situation, we prefer the phesil surface because of the chemical inertness of a phenyl group compared with the cyano group, which is rapidly hydrolysed.

### *Lengths of flooded zones/wettabilities*

Lengths of flooded zones per microlitre of liquid in 0.32 mm I.D. capillaries are listed in Table II. Pragmatically, lengths up to *ca.* 30 cm/ $\mu\text{l}$  are interpreted as good wettability. Longer flooded zones indicate critical wettability, where the lengths of flooded zones may depend on small details of the capillary deactivation procedure and other unknown factors. Finally, lengths exceeding 1 m indicate a lack of wettability.

As expected, admixture of different organic solvents to water improves the wettability to different extents. Mixtures with acetonitrile are poorly suited to obtaining good wettability, as 10% of water in acetonitrile already causes the flooded zone to be elongated. Among the alcohols, the propanols produce the shortest flooded zones with the highest water concentrations. 1-Propanol containing up to 70% water wets phesil and cyanosil surfaces. Dimethylformamide and dioxane, of interest because of their high boiling points (see below), have poor wettability characteristics.

As observed previously<sup>9</sup>, flooded zones are shortened when the oven temperature is increased. For 1-propanol containing up to 50% water, flooded zones of 13–15 cm/ $\mu\text{l}$  were determined at 100°C. We still do not fully understand this extreme shortening of flooded zones. The surface tension of liquids decreases with increasing temperature<sup>1</sup>. However, the height of rise of liquids did not increase with elevated temperature (in some instances it even slightly decreased), indicating more or less temperature-independent wettability (*i.e.*, there must be a decrease in critical energy of the surface to be wetted, corresponding to the decrease in surface tension).

TABLE I

HEIGHTS OF RISE OF SOME SELECTED REVERSED-PHASE ELUENTS IN GLASS CAPILLARIES DEACTIVATED WITH HEXAMETHYLDISILAZANE-DIPHENYLTETRAMETHYLDISILAZANE (1:1) (MESIL/PHEsil), DIPHENYLTETRAMETHYLDISILAZANE (PHEsil) AND DICYANOPROPYLTETRAMETHYLDISILOXANE (CYANOSIL)

Organic solvent	Water (%)	Height of rise (mm)		
		Mesil/phe <sub>sil</sub>	Phe <sub>sil</sub>	Cyanosil
Methanol	0	33	39	38
	10	32	38	
	20	23	37	
	30	19	30	
	50	8	25	
Ethanol	0	33	37	36
	10	31	36	35
	30	28	35	35
	50	22	32	32
1-Propanol	0	35	38	36
	10	34	36	35
	20	32	35	35
	50	24	34	34
	70	20	33	32
2-Propanol	80	16	29	29
	0	35	37	35
	10	32	36	35
	20	29	34	34
	50	27	33	33
Dioxane	70	23	32	33
	0	27	36	38
	10	26	36	38
	20	25	34	34
Dimethylformamide	30	20	32	33
	0	29	37	38
	10	17	37	35
	20		35	34

### *Sequence of solvent evaporation*

The lengths of flooded zones listed above were determined before substantial proportions of the solvents evaporated. However, when uncoated precolumns are applied in the retention gap technique (on-column injection of large volumes, LC-GC involving the retention gap technique), formation of a film on the capillary wall by the solvent mixture is not the only condition to be fulfilled.

Most of the commonly used organic solvents evaporate more rapidly than water, which is due to the extraordinarily large volume of vapour created per unit volume of condensed water and also its relatively high boiling point. This causes a serious problem. The solvents do not evaporate evenly throughout the flooded zone, but from the rear towards the front of the sample film. The more rapidly evaporating organic solvent, responsible for the wettability of the surface, withdraws more rapidly from the rear towards the front of the flooded zone than water, leaving pure water behind, which no longer wets the capillary surface (Fig. 1).

TABLE II  
 LENGTHS OF FLOODED ZONES IN 0.32 mm I.D. CAPILLARIES FOR SOLVENTS AND  
 SOLVENT MIXTURES USEFUL IN REVERSED-PHASE LC

<i>Organic solvent</i>	<i>Water (%)</i>	<i>Length of flooded zone (cm/<math>\mu</math>l)</i>			
		<i>Phesil</i>		<i>Cyanosil</i>	
		<i>25°C</i>	<i>60°C</i>	<i>25°C</i>	<i>60°C</i>
Acetonitrile	0	29		32	
	10	38			
	20	48			
	30	68			
	40	> 100			
Methanol	0	29		28	
	10	36			
	20	39			
	30	47			
	50	> 100			
Ethanol	0	26			
	10	27			
	20	29			
	30	35			
	50	48			
1-Propanol	0	28		25	
	10	33	23		17
	30	31	24		22
	50	28	23		18
	70	28	25		20
2-Propanol	80	> 100			
	0	25		23	
	10	29	18		16
	30	26	19		18
	50	23	18		25
Tetrahydrofuran	70	> 100	> 100		> 100
	0	27			
	20	28			
	30	33			
	50	> 100			
Dioxane	0	28			
	10	28			
	20	28			
	30	> 100			
Dimethylformamide	0		33		
	10		> 100		
Diethylene glycol monomethyl ether	0		30		
	10		28		
	30		26		
	50		> 100		

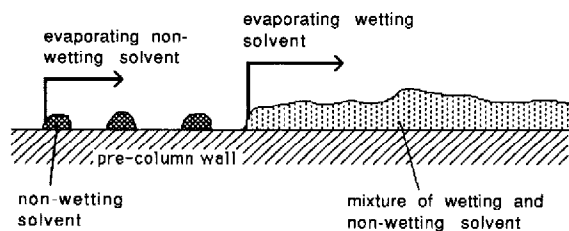


Fig. 1. Evaporation of a solvent mixture composed of a wetting organic solvent and a non-wetting solvent (such as water) on the surface of the capillary precolumn under conditions typical of retention gap techniques. Most organic solvents evaporate more rapidly than water, leaving the water behind, which no longer wets the capillary wall. Eventually, the resulting water droplets accumulate and penetrate further into the capillary column.

### *Accumulated water floods the column*

First, this water forms small droplets, and the internal wall of the glass capillary turns whitish, like the fogging of a window. The stability of such droplets is delicate. In some instances, the droplets remain stationary until the water has evaporated. More often, however, one or a few water droplets start to move, driven by the carrier gas. They take other droplets with them, starting a breakthrough of water akin to an avalanche. Finally, the droplets become a plug, closing the capillary bore. This plug moves at the speed of the carrier gas, collecting all the water droplets encountered ahead. In fact, such a plug penetrates into the capillary column, like the droplets gliding down a window when it rains. If the organic solvent has been fully evaporated previously, the plug of water may not stop before having passed through the whole separation column. The whitish aspect of the capillary disappears, indicating almost complete removal of the water beyond the point where the "avalanche" started.

There is hardly any way to control whether or not the water droplets remain stable on the capillary wall until the water has evaporated. Large droplets clearly rupture more often than small droplets. As the size of the water droplets depends primarily on the concentration of the water in the solvent mixture, the "disaster" happens more regularly when high water concentrations are used. From visual observation, we have the impression that a 30% concentration of water in a much more rapidly evaporating organic solvent almost always causes a breakthrough. Temperature and gas flow-rates are other factors that influence the stability of the water droplets.

However, even if the water does not flood the separation column, there remains a problem concerning solvent trapping. After complete evaporation of the well retaining (strongly trapping) organic solvent, volatile solute material is partially released, as water is a poor solvent for trapping solutes of up to intermediate polarity. Resulting peaks are deformed, often starting with shoulders.

### *Effect of flooding water*

The effect of the moving water has not been fully investigated yet. It was observed visually in glass capillaries (using a glass pane as an oven door) that it depends on the moment when the rupture occurs whether or not the water will run into the separation column. A droplet starting to move at an early stage is stopped by the solvent mixture forming a film ahead, the wetting liquid acting as a barrier. On the

other hand, a water droplet starting to move after all organic solvent has evaporated is hardly stopped anymore. However, even in this case, the "disaster" is not obvious. Water running into the separation column spreads all dissolved solute material throughout the flooded zone, possibly including the whole separation column. However, most components chromatographed by GC are hardly soluble in water at all, and one should expect that such substances rather tend to remain on the precolumn surface or dissolved in the stationary phase of the first part of the separation column. As long as there is no excessive spreading far into the separation column, there should be no peak distortion. Such uncertainties can only be clarified experimentally, although, as explained above, no simple, generally valid answer can be expected.

#### *Water-1-propanol*

1-Propanol and water form an azeotropic mixture containing 28% water. When 1-propanol-water mixtures are distilled at up to 1 bar, this composition was found to remain unchanged. This means that we can inject 1-propanol containing up to 28% of water without the risk that water will flood the column after evaporation of the wetting solvent. In fact, chromatographic experiments confirm this.

The chromatogram in Fig. 2 was obtained by injecting 50  $\mu$ l of a solution of methyl esters in 1-propanol containing 25% water. All the peaks are well shaped, indicating successful reconcentration at the head of the separation column. A problem that is not shown here concerns the high polarity of the solvent mixture. Highly volatile components are only partially trapped and are eluted as peaks, deformed into "stools" or "chairs" (partial solvent trapping<sup>10</sup>).

On injecting the same sample in a solvent mixture containing more than 28% water, two kinds of deficiencies were observed, the reproducibility of which was limited. A solvent mixture containing 30% water caused the early peaks to be deformed (Fig. 3), while the later peaks were of perfect size and shape. The perfect shape of the peaks at elevated elution temperatures indicates that the very small

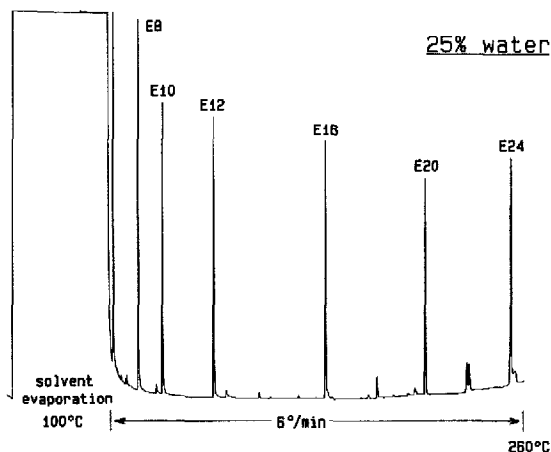


Fig. 2. Results of a 50- $\mu$ l on-column injection of  $C_8$ - $C_{24}$  methyl esters (E8-E24) in 1-propanol containing 25% of deionized water. Wettability of a standard "phasil" precolumn is no problem, and solvent trapping causes the early eluted ester peaks to be of almost perfect shape. For experimental details, see Experimental.

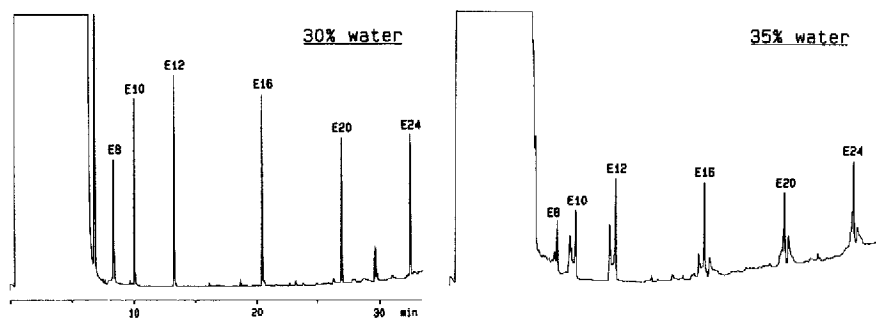


Fig. 3. Result of 50- $\mu$ l on-column injections, as in Fig. 2, but with 30 and 35% water in 1-propanol. Now 1-propanol evaporates more rapidly than water, leaving behind water, which does not wet the precolumn surface.

amount of water left behind by the evaporating 1-propanol remained stationary without spreading the solute material. The broadening of the early eluted peaks, with the characteristics typical of "band broadening in time"<sup>11</sup>, is due to water being a poor solvent for solvent trapping. As shown in Fig. 2, the methyl esters were fully trapped (completely retained) by the 1-propanol-water mixture. However, at the end of solvent evaporation, when all 1-propanol had left the inlet, it took some time to evaporate the remaining water. During this time, some of the methyl esters escaped.

When the test sample containing 35% water was injected, all methyl ester peaks were greatly distorted (not reproducibly in detail). This typical phenomenon of "band broadening in space" shows spreading of the solute material far into the separation column by the flooding effect. It indicates not only that the water droplets ruptured, but also that the water was able to carry along the sparingly soluble methyl esters. Peak distortion of solutes eluted at high temperatures was reduced or even disappeared when the column temperature was increased nearly to the boiling point of the solvent at the inlet pressure (experiments with 1-propanol containing 40% water). Such high oven temperatures produce concurrent eluent evaporation at the front end of the flooded zone, preventing the water from penetrating into the separation column. However, such techniques are difficult to carry out by conventional on-column injection (danger of back-flow), and peaks of volatile components are nevertheless severely distorted. Rather than using an on-column injector for sample introduction, such techniques should be carried out with a system corresponding to the loop-type LC-GC interface.

#### *High-boiling organic solvents*

Application of retention gap techniques to samples or LC eluents containing water appears to be restricted to mixtures with organic solvents that allow the water to evaporate first. This severely restricts the choice of organic solvents, almost ruling out all the more volatile, most commonly used solvents, such as methanol, ethanol and tetrahydrofuran. Some interesting azeotropic mixtures of higher boiling organic solvents with water are listed in Table III.

To evaporate water-rich organic solvents, organic solvents with high boiling



TABLE III  
SOME AZEOTROPIC MIXTURES WITH WATER

<i>Organic solvent</i>	<i>B.p.</i> (°C)	<i>Water in</i> <i>mixture</i> (%)	<i>Azeotropic</i> <i>boiling point</i> (°C)
Ethanol	78	4	78
Acetonitrile	80	16	76
2-Propanol	82	13	80
1-Propanol	97	28	87
Pyridine	115	41	94
Butyloxyethanol	171	72	98
Ethylene glycol diacetate	191	85	94

points are required. Moreover, these solvents must be water-miscible and the aqueous mixture must wet the precolumn surfaces, which rules out solvents such as dimethylformamide (Table II).

## CONCLUSIONS

Wettability tests have demonstrated that deactivation with DPTMDS, commonly used for capillary precolumns, produces surfaces of surprisingly good wettability. Not only do almost all organic solvents wet such phenylsilylated surfaces, but many of them still wet them when they contain considerable concentrations of water. The most commonly applied LC eluents, methanol and acetonitrile, are not those which wet at the highest water concentrations. Of the organic solvents tested, the best solvent in this respect is 1-propanol, still fully wetting the "phesil" surface when containing 70% water.

Wettability of precolumn surfaces is required for several techniques, including concurrent solvent evaporation with co-solvent trapping<sup>12</sup>, and even for concurrent solvent evaporation it is of advantage (although not a prerequisite). Here, we have considered only retention gap techniques, for which wettability by the solvent mixture is just one requirement. The more severe restriction is due to the requirement that the water must evaporate more rapidly than the organic solvent. This limits the maximum water concentration in 1-propanol to 28% and in acetonitrile to 16%. For introduction of higher proportions of water by the retention gap technique, higher boiling solvents (or co-solvents) must be used.

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