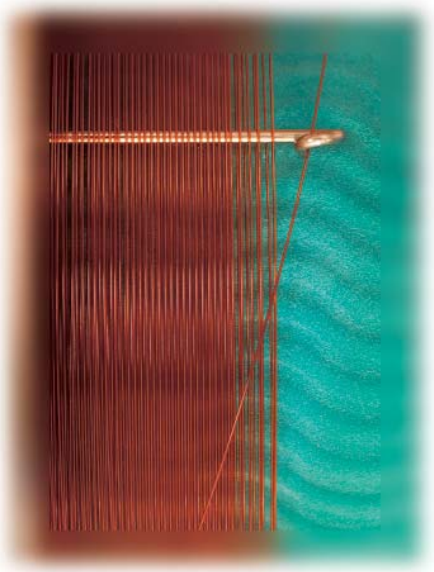
Water Injections in GC — How Wet Can You Get?



In gas chromatography, injecting aqueous samples can be a necessity or a convenient way of introducing a sample onto a column. In addition to the well-known problems in the injector (such as backflash caused by the large expansion volume of water), concerns about degradation of the stationary phase, in particular polyethylene glycol (wax-type) phases, often arise because water can react with the phase polymer. Frequently, chromatographers report that water injections have damaged their columns. In this article, the author reports results from a systematic study of the effect of water injections on column performance with a number of common stationary phases, including polyethylene glycol and dimethylpolysiloxane.

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Injecting aqueous samples for gas chromatography (GC) analyses is a topic of great interest. In some cases, such as purge-and-trap, injecting water into a GC column is unavoidable. In other cases, it might be more convenient to inject water directly rather than to perform a cumbersome solvent-extraction procedure before injection. From a GC point of view, water is a less-than-ideal solvent. The problems associated with water include large vapor expansion volume, poor wettability and solubility in many stationary phases, detector problems, and perceived chemical damage to the stationary phase.

Table I: Vapor expansion volumes of various solvents*

Solvent	Approximate Vapor Volume (μL)
Isooctane	110
n-Hexane	140
Toluene	170
Ethyl acetate	185
Acetone	245
Methylene chloride	285
Carbon disulfide	300
Acetonitrile	350
Methanol	450
Water	1010

*Injection volume = 1 μL; injector temperature = 250 °C; pressure = 20 psi. Readers can download the flow calculator from Agilent Technologies' web site (http://www.chem.agilent.com/cag/servsup/usersoft/main.html#flowcalc205). This program allows users to calculate the expansion volume of many solvents under specified conditions.

The potential problems with water start in the injector in which samples are vaporized so the analytes can be swept onto the column by the carrier gas. During vaporization in the injector, a sample expands to many times its original liquid volume (see Table I). Of the commonly used solvents, water has the largest vapor expansion volume by far; therefore, the vapor volume of water (assuming a 1-μL injection) easily can exceed the physical volume of the injection liner (typically 200–900 μL). The vapor then could expand outside the liner. This phenomenon is called *backflash,* and it can be a problem during subsequent injections.

After chromatographers have dealt with backflash, water still poses more challenges. Water has a very high surface energy. By contrast, most capillary-column stationary phases have low surface energies. This difference in surface energy causes the poor wetting properties of water. Grob and co-

Table II: Pre- and postinjection retention factor, retention index, resolution, theoretical plate, and bleed values for various stationary phases

	Retention Factor		Retention Index 1		Retention Index 2		Resolution		Theoretical Plates		Bleed (pA)	
Column	Before Injection	After Injection	Before Injection	After Injection	Before Injection	After Injection	Before Injection	After Injection	Before Injection	After Injection	Before Injection	After Injection
100% Dimethylpolysiloxane*	14.6	14.5	1349.88	1350.02	1427.77	1428.16	—	—	1448	1474	12.8	11.2
Polyethylene glycol*	12.6	12.6	1149.54	1149.73	1163.44	1163.71	—	—	1277	1281	44.8	32.1
50% Cyanopropylphenyl polysiloxane*	11.5	11.4	1622.30	1621.26	1711.51	1711.03	—	—	1101	1110	34.5	39.3
Derivatized cyclodextrin†	7.8	7.6	1306.30	1306.01	—	—	1.9	1.3	2631	2025	28.4	15.1
Divinylbenzene–ethyleneglycol dimethacrylate‡	5.2	5.3	538.0	540.0	—	—	—	—	950	982	74.2	35.6

*30 m × 0.53 mm, 1.0-µm d_f.
†30 m × 0.32 mm, 0.25-µm d_f.
‡30 m × 0.53 mm, 20-µm d_f.

workers (1) found that no surfaces were both wettable by water and sufficiently deactivated to provide the necessary inertness for good chromatography. (Although Grob and his group specifically investigated retention gaps [guard columns], their results can be extrapolated to regular columns.) As a consequence of this poor wettability and water's high boiling point, some of the water will pass through a column as a liquid. Solutes with a high solubility in water could exhibit band broadening and peak splitting in severe cases. In on-column injections, nonvolatile compounds such as salts can be carried far into columns by the liquid water plug and increase the potential for severe column contamination.

Chromatographers often use retention gaps to focus samples at the front of columns, particularly in splitless injections. This process involves evaporating the solvent and analytes from the back end of the flooded zone (2), which is formed inside the retention gap when the carrier gas forces liquid into a thin layer on the capillary wall. Because retention gaps typically have a nonpolar surface (deactivation coating), water could fail to exhibit the normal solvent effect and, in fact, might act aggressively toward the deactivation layer (3). One approach to overcoming this problem is to coat the retention gap with a thin layer of a more polar polymer (4).

In 1981, Schomburg and co-workers (5) published results indicating that using water as a solvent is acceptable if the column is prepared properly. Jenkins (6) obtained similar results that showed the initial oven temperature is a significant factor. Oven temperatures equal to or greater than the boiling point of water (100 °C) produced better chromatograms (less tailing) than did lower temperatures.

Water also can cause problems with detectors. Water sometimes extinguishes the flames of flame ionization detectors, especially when the detector gas flows are set improperly. Electron-capture detectors also are quite sensitive to water vapors, and the presence of water lowers their sensitivity.

This article describes the results of a systematic study to determine damage to stationary phases in capillary GC columns caused by water. The main purpose of this study was to establish if, and by how much, water injections change the chromatographic performance of a column. The quantitative data could then establish guidelines for safe water injections.

Experimental

I chose the stationary phases to cover a full range of polarities and chemical composition. They included 100% dimethylpolysiloxane (DB-1), 50% cyanopropylphenyl methylpolysiloxane (DB-225), polyethylene glycol (DB-Wax), 30% heptakis (2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-β-cyclodextrin (CycloSil B), and divinylbenzene–ethyleneglycol dimethacrylate (HP-PLOT U) (all from Agilent Technologies, Folsom, California, USA). This selection covered a wide range of functional groups and allowed extrapolation to other phases.

For analysis, I used an Agilent 6890 gas chromatograph with an autoinjector. The injector temperature was 250 °C, and the injection volume was 1 µL. I chose a low split ratio of 5:1 to place as much water onto the column as possible and to avoid the notorious backflash problem. I used a flame ionization detector with a temperature of 300 °C. The column oven temperatures were 60 °C and 130 °C for isothermal analysis. The carrier gas was helium with an average linear velocity of 45 cm/s.

Table III: Skew numbers for polyethylene glycol* before and after injection

Compound	Before Injection	After Injection
Chlorophenol	0.38	0.47
Dimethylaniline	0.30	0.32
Undecanol	0.25	0.27

*30 m × 0.53 mm, 1.0-µm d_f.

I made 1000 injections at each temperature on each column. The bleed profiles and test mixes were run after 250, 500, and 1000 injections. The test mixes were Grob-type mixtures and specific to each phase type. (For test mix compounds, please contact the author.)

Results and Discussion

Tables II and III summarize the results of my studies. The data clearly show that no changes in any of the chromatographic parameters were detected for bonded and cross-linked stationary phases. Figure 1 shows identical chromatography after 2000 water injections on a polyethylene glycol column.

Furthermore, I observed no signs of increased activity (tailing) in any of the columns tested. Table III shows the asymmetry or skew numbers for the polyethylene glycol phase. I obtained virtually identical results for the other phase types.

The skew numbers are based upon a modified Gaussian peak model (7). This model relies more on the zeroth and first moment calculations, which are less influenced by baseline noise. Basically, the numbers represent the deviation from an ideal Gaussian peak, that is, from a perfectly symmetrical peak. Therefore, the smaller the number, the better. Noticeable tailing starts at ~0.8, and

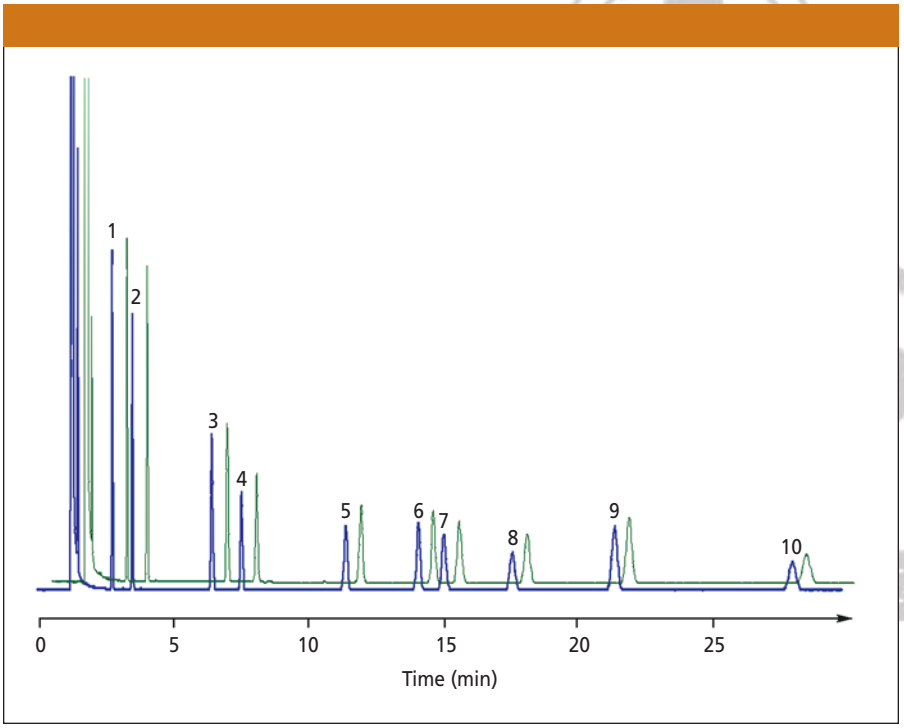

Figure 1: Separation of a test mixture before (lower trace) and after (upper trace) 2000 water injections. Column: 30 m × 0.53 mm, 1.0-µm d_f DB-Wax; carrier gas: helium at 45 cm/s; oven temperature: 130 °C (isothermal); injector temperature: 250 °C, 20:1 split; detection: flame ionization, 300 °C. Peaks: 1 = 2-octanone, 2 = tetradecane, 3 = 1-octanol, 4 = methyl decanoate, 5 = methyl undecanoate, 6 = naphthalene, 7 = 1-decanol, 8 = methyl dodecanoate, 9 = 2,6-dimethylaniline, 10 = 2,6-dimethylphenol.

obvious tailing appears at ~1.2. Column-to-column and run-to-run variations of skew numbers of ± 0.1 are normal.

The water injections had a negative effect on the 30% heptakis (2,3-di-*O*-methyl-6-*O*-*tert*-butyl dimethylsilyl)-β-cyclodextrin phase, which was the only nonbonded phase I tested. (The chiral selector is embedded only in a bonded stationary phase [Agilent DB-1701] and not bonded to the column.) The loss of resolution between the pair of enantiomers suggested that the chiral selector was washed from the column. This washout was gradual, however, and dependent upon temperature. At 130 °C, it was minimal. At 60 °C, it was easily noticeable, probably because the solubility of cyclodextrin is greater in liquid water. In general, I expected the amount of washout to be dependent upon the solubility of the phase material in water.

I observed no negative effects for the bonded porous-layer open tubular (PLOT) columns. Some PLOT columns (for example, molesieve and alumina) are unsuitable for water injections. Those columns tend to absorb water, which can lead to changes in retention times for other compounds. Chromatographers should follow manufacturers' recommendations for those columns.

I made an interesting general observation during these experiments. The time required to recondition the columns after injecting water was dependent upon the oven temperature. Longer conditioning times were necessary for injections made at lower temperatures than for injections made at higher temperatures, in particular with the polar columns. This relationship could be the result of the liquid water swelling the polar phases and allowing small polymer fragments (and possibly other materials) that had been entangled in the phase to come to the surface and to be eluted from the column. Based on this observation, I recommend that users periodically bake out their columns at temperatures greater than 200 °C if they make water injections at temperatures less than 80 °C.

Rinsing columns with water is recommended only for nonpolar columns. Rinsing the polar columns used in this test with water would cause a noticeable loss of stationary phase (Table IV), as indicated by lower retention factors. I observed no changes in any of the other parameters such as retention indices and efficiency, however. I observed no change at all for the nonpolar column. This outcome could be due to water's ability to penetrate into the polar stationary phases, in which bond cleavage by hydrolysis at the column wall could occur. Of course, this reaction also could occur with water injections. It isn't noticeable in this example because the amount of water from a 1-µL injection is several orders of magnitude smaller than the amount of water present during rinsing (3 mL). In addition, the residence time of the water in the column is much longer during the rinse. By contrast, nonpolar columns would repel the water, thus eliminating this mechanism for stationary-phase loss.

Table IV: Retention factor values (*k*) before and after rinsing

Phase Type	Before Rinsing	After Rinsing
100% Dimethylpolysiloxane	14.5	14.5
50% Cyanopropylphenyl polysiloxane	11.4	11.2
Polyethylene glycol	12.6	11.7

Conclusion

The data presented in this article clearly demonstrate that injecting water will not harm most columns' stationary phases. Although I tested only a few select stationary phases in this study, my extrapolation to other stationary phases with similar functional groups — such as 5% phenyl or 14% cyanopropylphenyl — should be valid, as long as the columns are bonded and cross-linked. Chromatographers must take some precautions when using nonbonded phases and rinsing polar columns. Many of the problems often associated with water injections are caused by other phenomena such as backflash.

References

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