

# Evaluation of Expelling of Mobile Phase from Pore of C18 Packing Material under Aqueous Condition

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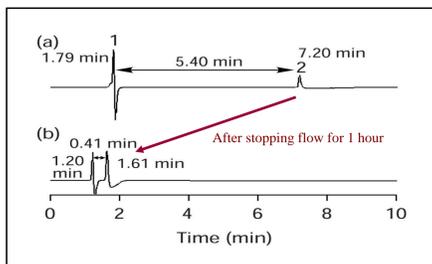
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## Abstract

It has been well known since publishing the paper by Nagae [1-5] that a mobile phase was expelled from the pore of packing materials for reversed phase liquid chromatography under an aqueous condition by capillarity, consequently, retention decreased. Capillarity depends on the contact angle of a liquid on the surface of a substance. In the case of more than 90 degree of the contact angle, in other words, non-wetting, the force brought by capillarity makes a liquid expel from the pore. Reversely in the case of less than 90 degree of the contact angle, in other words, wetting, the force brought by capillarity makes a liquid permeate onto the pore. In this study, it was revealed that even if the contact angle was more than 90 degree, a liquid was not always expelled from the pore. Only when the contact angle was more than 90 degree, and furthermore the force brought by capillarity was more than the atmospheric pressure, a liquid was expelled from the pore. Permeating or depermeating (expelling) of a mobile phase from the pore doesn't always depend on wetting or non-wetting of a liquid on the surface of packing materials. The expression of dewetting has been often used when a mobile phase was expelled from the pore of packing materials and retention reduced. This expression, however, was considered not to be factual. It was suggested that the expression of depermeating should be used in such a case.



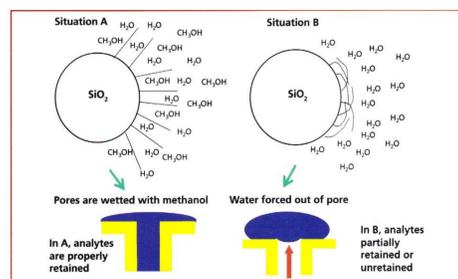
## Decrease in retention under aqueous condition



Condition  
Column: C18, 5 μm, 150 x 4.6 mm Mobile phase: Water  
Column back pressure: 6.0MPa Back pressure post column: 1.7MPa  
Flow rate: 1.0 ml/min Temperature: 40 °C  
Detection: RI  
Sample: 1. Sodium nitrite, 2. 2-Propanol

N. Nagae, T. Enami and S. Doshi, LC/GC North America October 2002.

## Explanation of dewetting

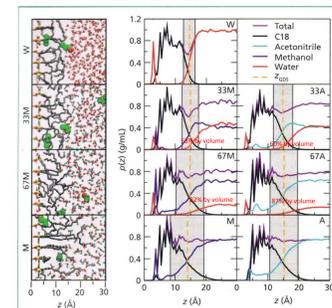


Is this true?  
An author feels...

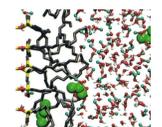
For the right hand side of the upper portion of Figure 3, situation B shows a C18 phase in a 100% water mobile phase. The C18 functionality prefers to be in a self-associated state (like prefers like) and folds upon itself in a collapsed state. The bottom portion of Figure 3 shows the situation as it actually happens. Because most of the interactions with an LC stationary phase occur inside the pores (rather than on the outer surface), when an organic solvent is present at higher concentrations (greater than 10%), the pores are filled with the water-organic mixture that allows the C18 bonded groups to be solvated, and everything behaves normally. However, when the solvent within a pore becomes unfriendly (for example, very low %B or 100% A), there is a tendency to force the water out of the pore, which results in a dewetting phenomenon.

Ronald E. Majors, LCGC North America, Jul 1, 2013.

## Structure of the Bonded-Phase Chains and Solvent Location



Mark R. Schure et al. reported the left figure. A bonded phase collapses under methanol:water=53/47(v/v) as well as under water.



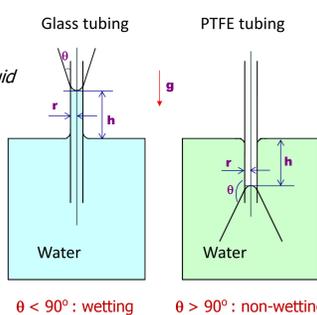
Methanol:water =33:67(M/M)=53:47(v/v)

Left: Snapshots from simulations in different solvents, C<sub>18</sub> chains (gray), and alkane and alcohol solutes (large spheres with green, red, and white indicating CH<sub>2</sub>, hydroxyl oxygen, and hydrogen, respectively). Middle and right: Density profiles of C<sub>18</sub> chains, methanol, water, and acetonitrile for pure water (W), pure methanol (M), pure acetonitrile (A), and solvent mixtures given as mole percent. T = 323 K, grafting density of 2.9 μmol/m<sup>2</sup>. Adapted, in part, from references 4 and 14.

Mark R. Schure, et al. LCGC North America 630 Volume 31 Number 8 August 2013.

## Capillarity, Capillary phenomenon

Capillarity equation:  
 $h = \frac{2\gamma \cos \theta}{r\rho g}$   
 $\gamma$ : surface tension of liquid  
 $\rho$ : density of liquid



## State of C18 packing materials in mixture of methanol and water



C18 packing materials were put into each solvent and stirred with ultra sonic vibration.

Methanol: water=70:30

C18 packing materials sedimented into the solvent.

(Methanol: water=70:30 solvent wet the surface of C18 packing material and permeated into the pore.)

Methanol: water=50:50

C18 packing materials almost floated on the solvent.

(Methanol: water=50:50 solvent didn't wet the surface of C18 packing material and couldn't permeate into the pore.)

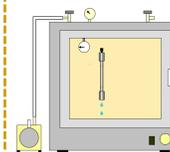
Methanol: water=30:70

C18 packing materials completely floated on the solvent

(Methanol: water=30:70 solvent didn't wet the surface of C18 packing material and couldn't permeate into the pore.)

T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.

## Mobile phase is expelled out of the column under 0.01 MPa pressure

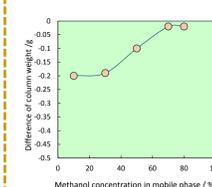


Column: C18, 5 μm, 250x4.6mm  
Simulated atmospheric pressure: 0.01MPa

Column temperature: 40 °C

C18 column was kept in the vacuum vessel at 0.01MPa for 20 min.

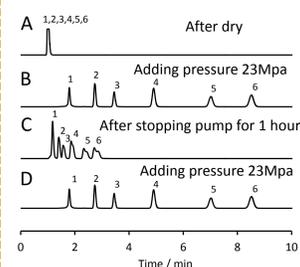
The weight of the column changes if the mobile phase is expelled out of the pore.



When methanol concentration in the mobile phase was 70% and 80%, in other word methanol:water (70:30) and (80:20), the mobile phase wasn't expelled out of the pore. However, when methanol concentration in the mobile phase was less than 70%, the mobile phase was expelled out of the pore and went out of the column. As a result, the column weight decreased. If the force brought by capillarity was more than 0.01 MPa, the mobile phase was expelled out of the pore under 0.01 MPa pressure. When atmospheric pressure is 0.1MPa, the mobile phase with more than 10% of methanol concentration wasn't expelled out of the pore as anybody knows.

N. Nagae, BUNSEKI KAGAKU Vol. 59, No. 3 (2010) 193.

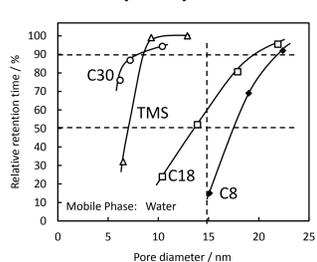
## Retention behavior of dried C18 column



The chromatogram A shows the first injection of the sample after the baseline became stabilized and the 6 kinds of components eluted without being separated. From this condition, a tubing of ID 0.1 mm was connected to the postcolumn outlet without stopping the pump and applied the back pressure of 23 MPa to the column outlet. It is now assumed that aqueous mobile phase mixed with air came out of the column and residual air that existed in pores of the dried C18 packing materials was expelled. The chromatogram B shows the result of injection after the baseline stabilized. The retention time of thymine was approximately 8.5 minutes and all the six different of components were completely separated. Later, we stopped the pump for one hour and pumped again without applying pressure at the postcolumn outlet. The obtained chromatogram is shown as C, and the retention decreased by around 70%. Then 23 MPa of back pressure was applied again at the postcolumn outlet with continuously pumping. The first time, air came out of the column, however we could not confirm if there was air in the mobile phase that came out of the column the second time. Chromatogram D, which was obtained once the baseline became stabilized, was almost the same as chromatogram B.

T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.

## C1 (TMS) shows decrease in retention



Relationship between pore size and relative retention time under 100% aqueous mobile phase  
Column, C30 (○), C18 (□), C8 (●), and TMS (△); column temperature, 40 °C; sample, thymine. Relative retention time was calculated as the ratio of the decrease in retention versus initial retention time.

As shown in Figure, different lengths of alkyl chain change the relative retention time against pore diameters and the decreasing ratio on retention time also differs. When comparing stationary phases using the same pore size of 15 nm, C8 decreased in retention time by 80% and C18 by 35%. However, C30 and TMS did not decrease the retention time. The longer the length of ligand for more than C8, the smaller the decrease in retention is with smaller pore diameters. That is, the longer alkyl chain is more stable on its retention even with small pore diameters. Generally the pore diameter of approximately 10 nm is used as packing material. However, the ligand length of C30 enables the analyses with stable retention time even using aqueous mobile phase. Although TMS is in the methyl group and has a short ligand, it gains high retention with a pore diameter of 9 nm. However, with 7 nm of pore diameter, the retention decreases by greater than 50%. No ligand collapse occurs on TMS phase. This means that ligand collapse has nothing to do with decrease in retention.

T. Enami and N. Nagae, Chromatography, 22 (2001) 33.

## Conclusion

Mobile phase	Wettability on C18	Structure of the C18 chains <sup>a</sup>	State of mobile phase under 0.01MPa pressure	State of mobile phase under 0.1MPa pressure	State of mobile phase under 23MPa pressure	Force brought by capillarity (expelling force)
Methanol:water (70:30)	Wetting	Almost collapsed state	Keep in the pore	Keep in the pore	Keep in the pore	---
Methanol:water (50:50)	Non-wetting	Collapsed state	Expelled out of the pore	Keep in the pore	Keep in the pore	More than 0.01MPa, Less than 0.1MPa
Methanol:water (30:70)	Non-wetting	Collapsed state	Expelled out of the pore	Keep in the pore	Keep in the pore	More than 0.01MPa, Less than 0.1MPa
Water	Non-wetting	Collapsed state	Expelled out of the pore	Expelled out of the pore	Keep in the pore	More than 0.1MPa, Less than 23MPa

a: Reported in Mark R. Schure, et al. LCGC North America 630 Volume 31 Number 8 August 2013

✓ What does "Dewetting" mean?

✓ A surface state changes from wetting to non-wetting due to ligand collapse? The surface of C18 is always non-wetting under an aqueous mobile phase with from 0% to 50% of methanol concentration even if such a mobile phase exists in the pore, so that expression of "dewetting" is not scientific. Depermeating is a scientific expression?

## Reference

- [1] N. Nagae, T. Enami, BUNSEKI KAGAKU, 49 (2000) 887.
- [2] T. Enami and N. Nagae, Chromatography, 22 (2001) 33
- [3] N. Nagae, T. Emani, S. Doshi, LC/GC, 20 (2002) 964.
- [4] T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.
- [5] T. Enami, N. Nagae, American Laboratory, 36 (2004) 10.
- [6] N. Nagae, BUNSEKI KAGAKU, 59 (2010) 193.