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# ***Study of State of Alkyl Chains for Reversed-Phase: Collapse or Brush up***

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# *Abstract*

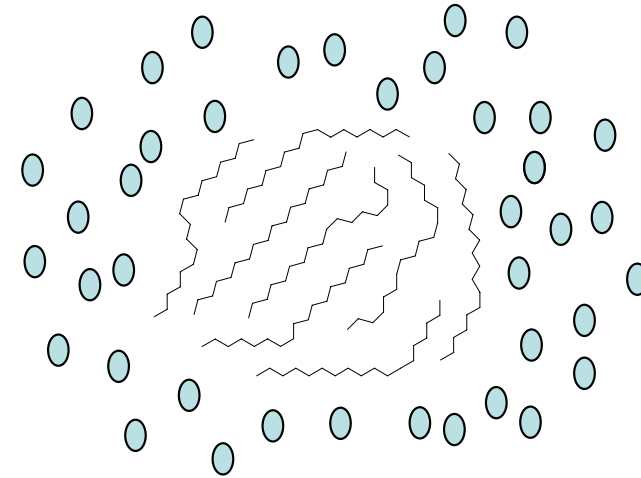
It had commonly been said that ligand collapse made retention decrease under 100% aqueous conditions for reversed-phase liquid chromatography. However Nagae et al. reported at 2000 that reducing retention under 100% aqueous conditions had nothing to do with ligand collapse and its cause was that a mobile phase like water expelled from the pore of the packing materials by capillarity. Solvation volume of an organic solvent in a mixture of organic solvent and water as a mobile phase to alkyl chain as a stationary phase was measured directly by retention of a organic solvent under lower concentration of organic solvent in a mobile phase, furthermore, indirectly by change of stationary volume obtained from measurement of elution

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volume of uracil as a non-retained substance under higher concentration of organic solvent in a mobile phase. Solvation volume of tetrahydrofuran (THF), acetonitrile and methanol were measured to be 0.35mL, 0.15mL and 0.06mL respectively on 0.35 mL of C18 stationary phase in the column in dimension of 150 x 4.6 mm i.d. THF solvated 6 times more than methanol on the C18 stationary phase, while acetonitrile solvated 2.5 times. Solubility of octadecane (C<sub>18</sub>H<sub>38</sub>) is not only in water but also in methanol is almost zero, so that C18 alkyl chain is considered to agglomerate on the surface even if 100% methanol is used as a mobile phase. These results suggested that THF solvated in the whole alkyl chain and alkyl chain brushed up while methanol solvated on the surface of agglomerating C18 phase.

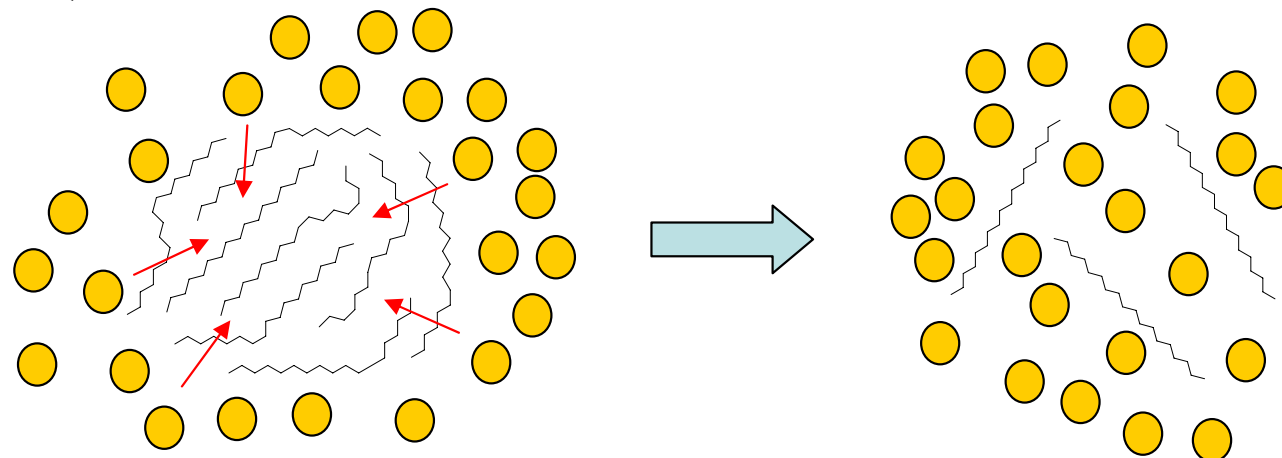
# State of octadecane in organic solvent

Octadecane ( $C_{18}H_{38}$ ) doesn't dissolve in methanol.

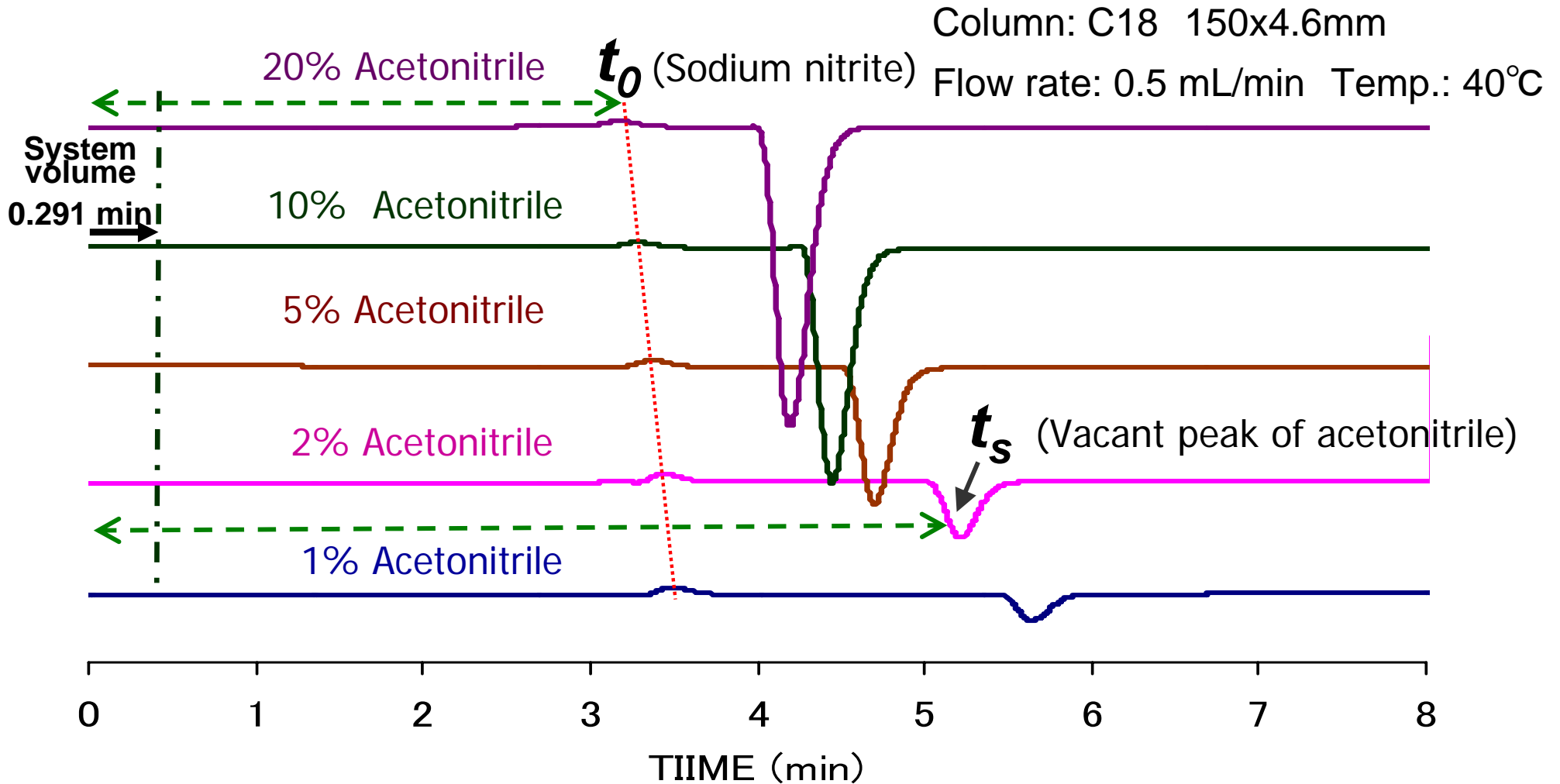


It is considered that octadecane aggregates in methanol and contact surface area is the least.

Octadecane dissolves in Tetrahydrofuran (THF).



# Chromatograms of Acetonitrile using Acetonitrile/Water mobile phases

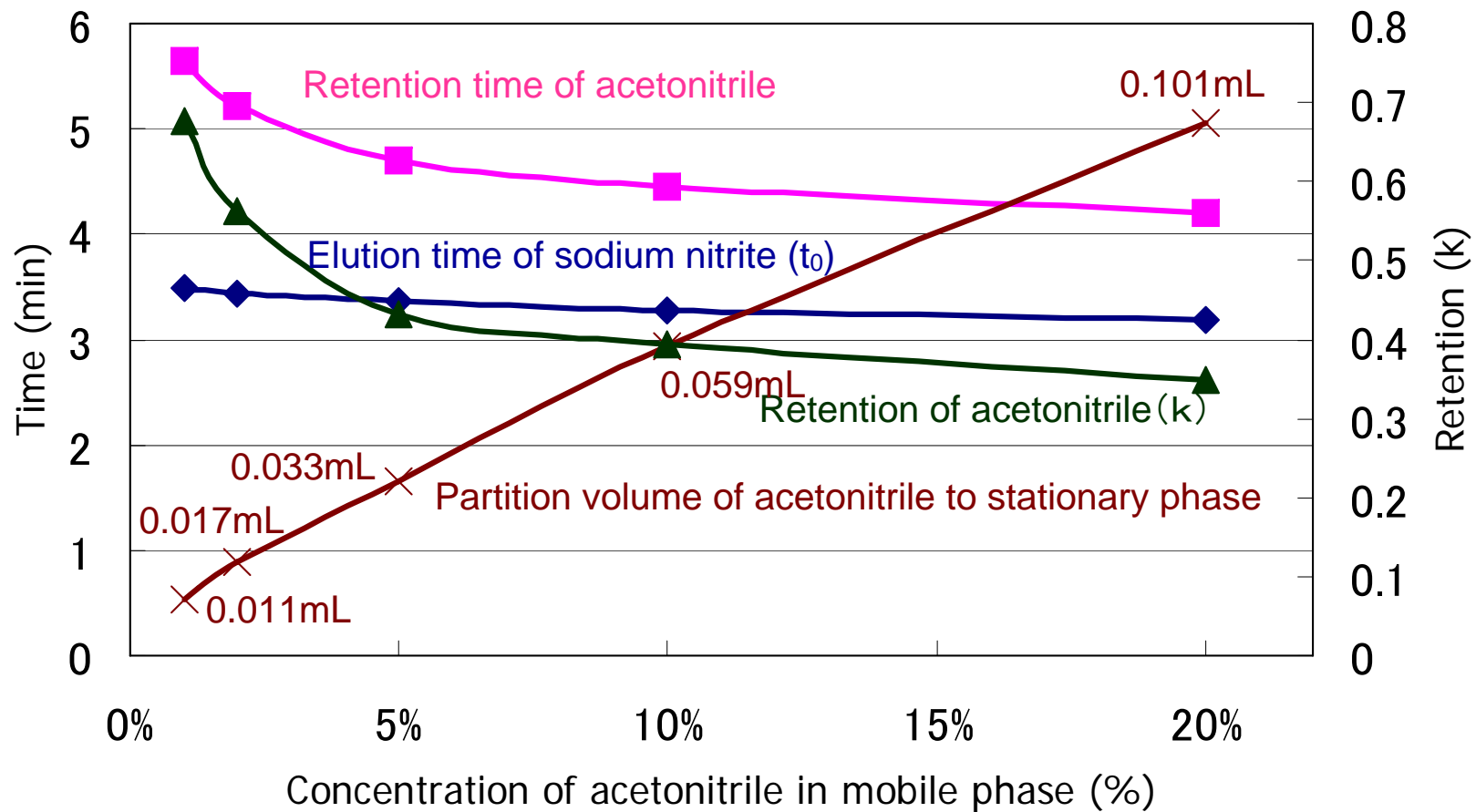


Acetonitrile retains using a mixture of acetonitrile and water as a mobile phase. In other word, acetonitrile partitons into a stationary phase. This patition volume is assumed to be solvation volume.

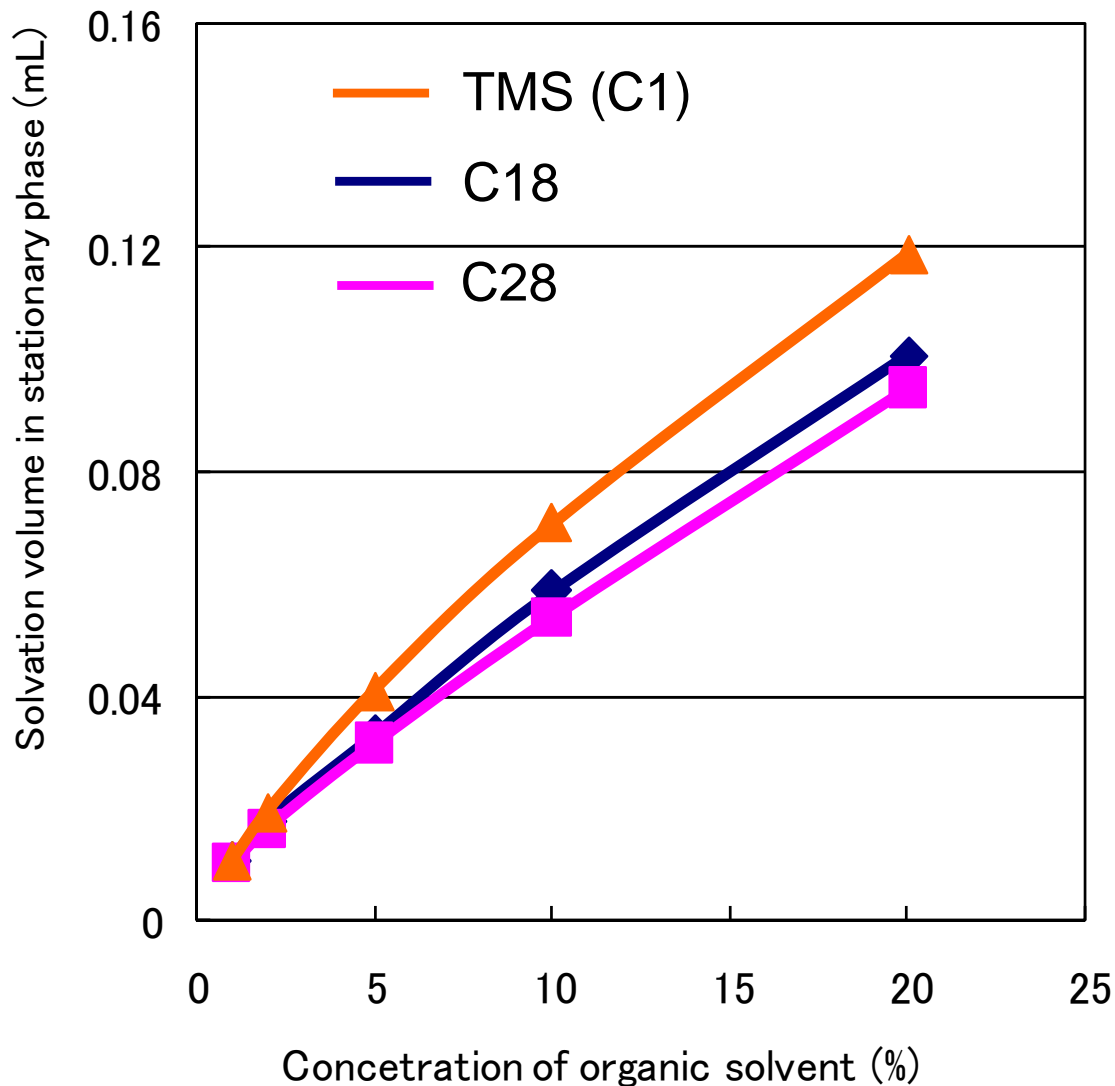
# Retention time, retention and partition volume to stationary phase using acetonitrile/water as mobile phase

$$k = (t_s - t_0) / (t_0 - \text{system volume}) \quad k = S / M \quad S = k \times M$$

Column: C18(%C: 16%) 150x4.6mm. About 1.5 g packings exists in a column. The C18 stationary phase in its packings is about 0.28 g or 0.35 mL.



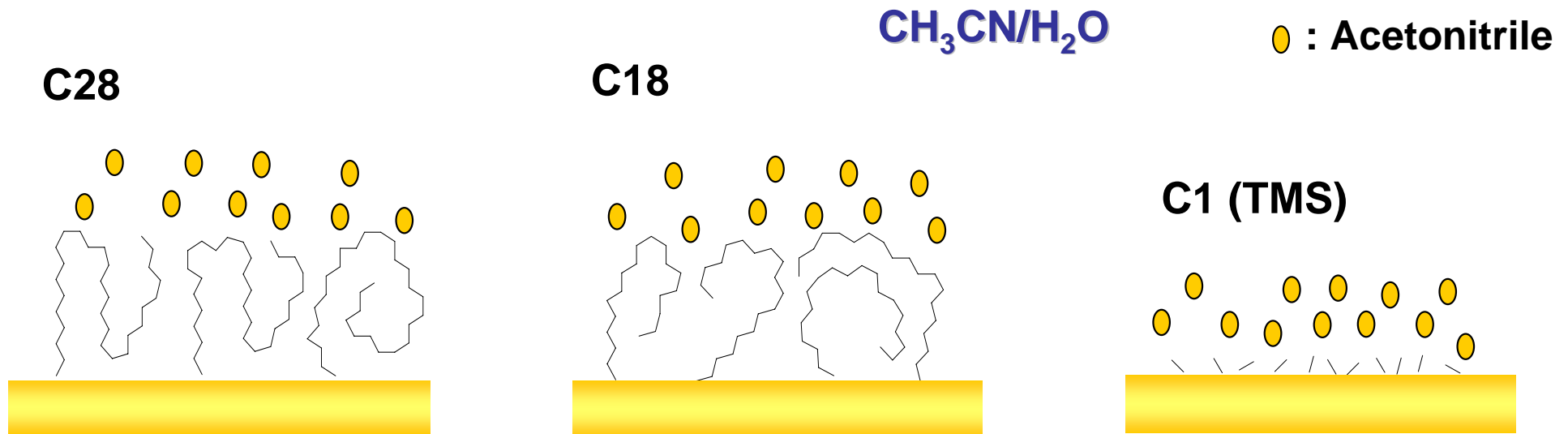
# Partition (solvation) volume of acetonitrile on C1, C18 and C28 stationary phases



Column: TMS (C1, %C: 5%)  
 C18 (%C: 16%)  
 C28 (%C: 16%)  
 150x4.6mm

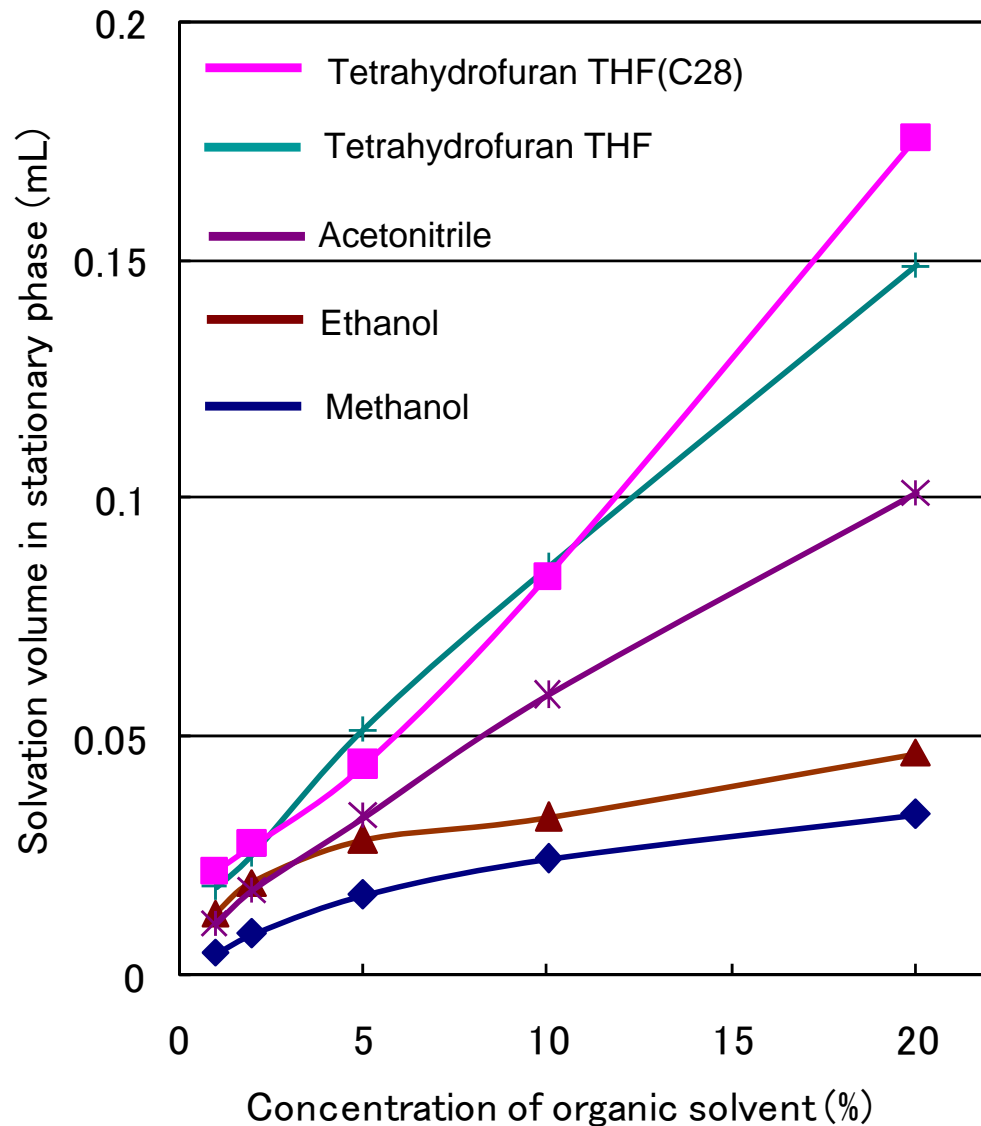
Partition volume of acetonitrile on each stationary phase is almost same.

# *Schematic diagram of solvation of acetonitrile under acetonitrile/water mobile phase*





# Partition (solvation) volume into C18



Column: C18, (C28) 150x4.6mm

1) 1.5 g of C18 packing material is packed into a column.

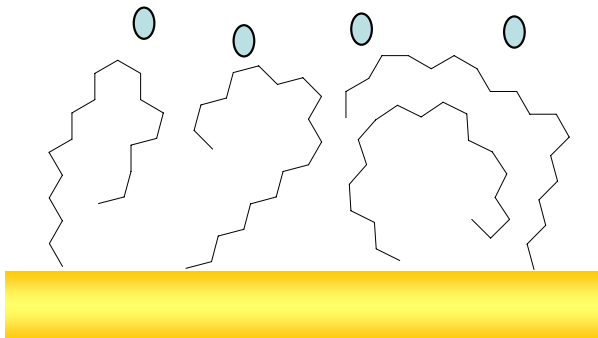
2) Carbon content of C18: 16%  
Carbon content of C28: 16%

Volume of octadecyl group in a column is calculated to be ca. 0.35mL.

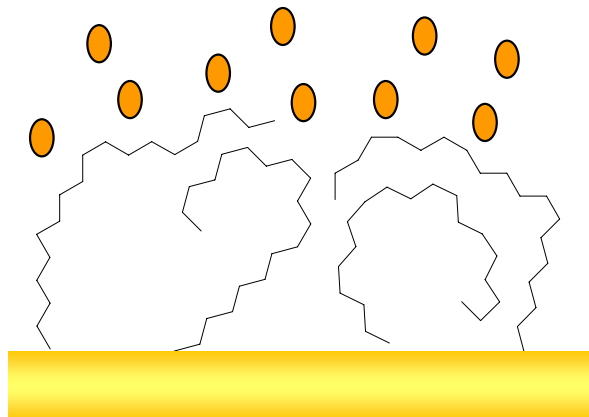
Methanol, acetonitrile and THF are solvated 0.04 mL, 0.10 mL and 0.15 mL respectively at 20% concentration of each organic solvent.

# Schematic diagram of solvation on C18 stationary phase under 20% organic solvent concentration

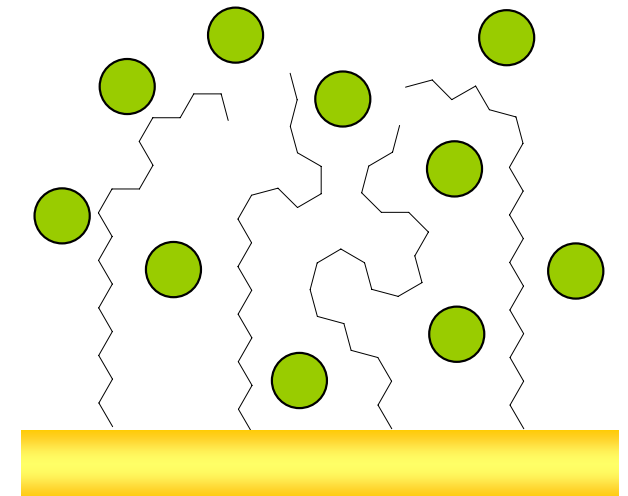
**Methanol**



**Acetonitrile**



**Tetrahydrofuran THF**



Solvation of methanol and acetonitrile occurred on the surface of agglomerated C18 stationary phase.

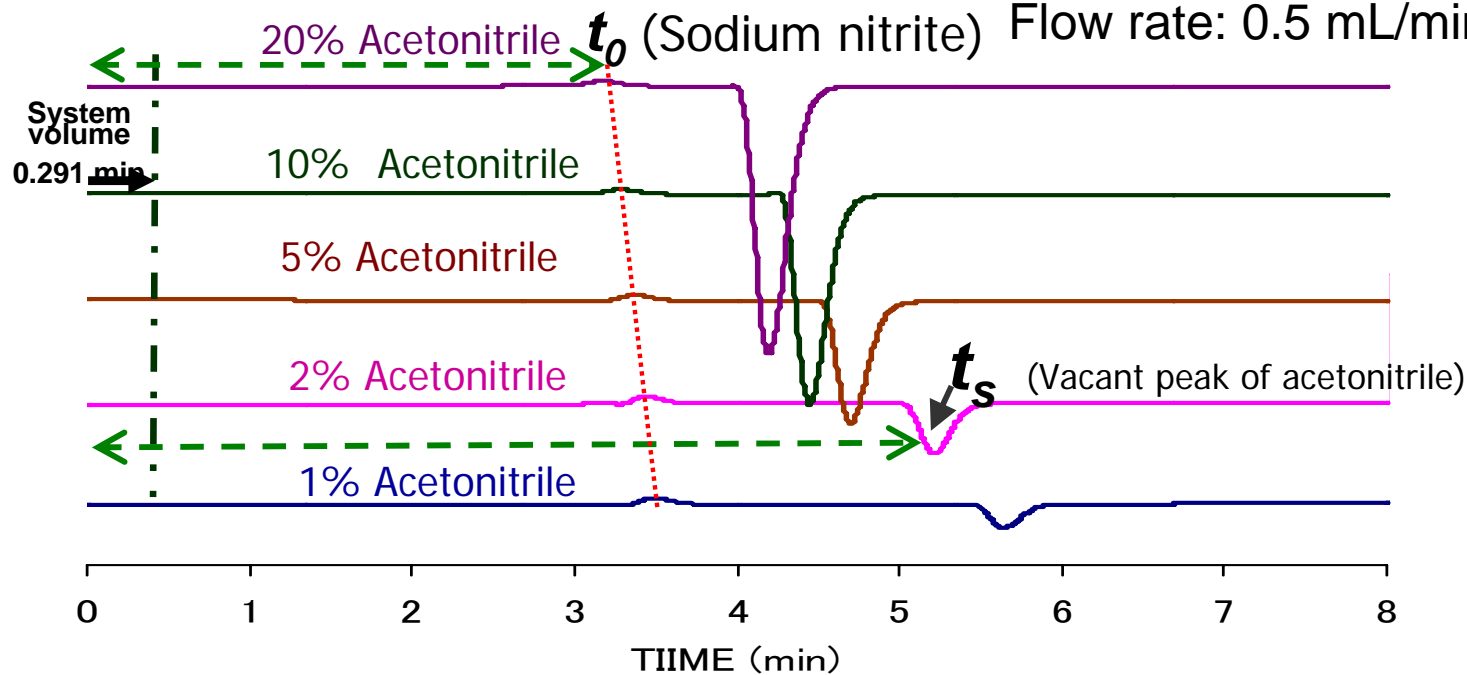
THF has swollen C18 Stationary phase.

# Solvated organic solvent works as a part of stationary phase

Chromatograms of Acetonitrile using **Acetonitrile/Water** mobile phases

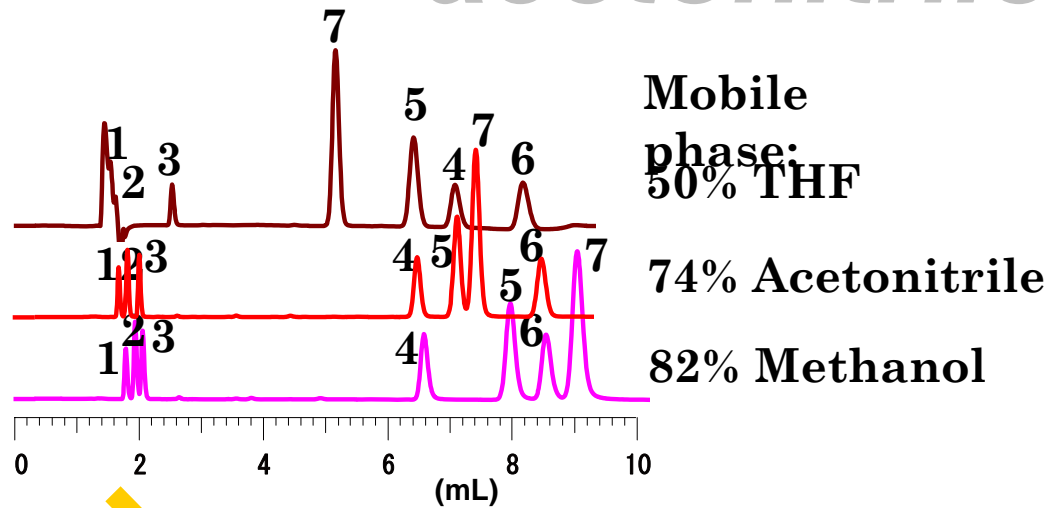
Column: C18 150x4.6mm

Flow rate: 0.5 mL/min Temp.: 40 °C



Elution time of sodium nitrite ( $t_0$ ) reduces as Organic solvent concentration in mobile phase increases. This result leads that solvated organic solvent works a part of stationary phase, so that volume of stationary phase grows larger and  $t_0$  decreases. In other words, the variation of  $t_0$  equals to that of stationary phase volume.

# Variation of $t_0$ among methanol, acetonitrile and THF



Column: C18, 5 $\mu$ m, 150 x 4.6 mm

Mobile phase :

THF/water (50:50), acetonitrile/water (74:26), methanol/water (82:18)

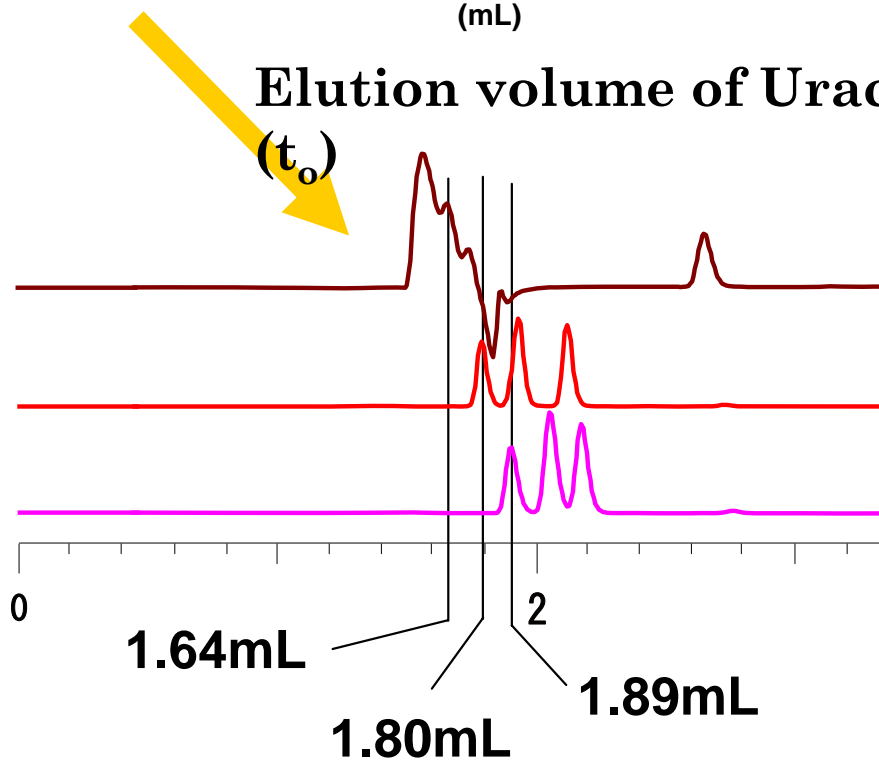
Detection: UV @ 250 nm.

Sample:

1=Uracil, 2=Caffeine, 3=Phenol, 4=Butylbenzene, 5=o-Terphenyl, 6=Amylbenzene, 7=Triphenylene

**Elution volume of Uracil**

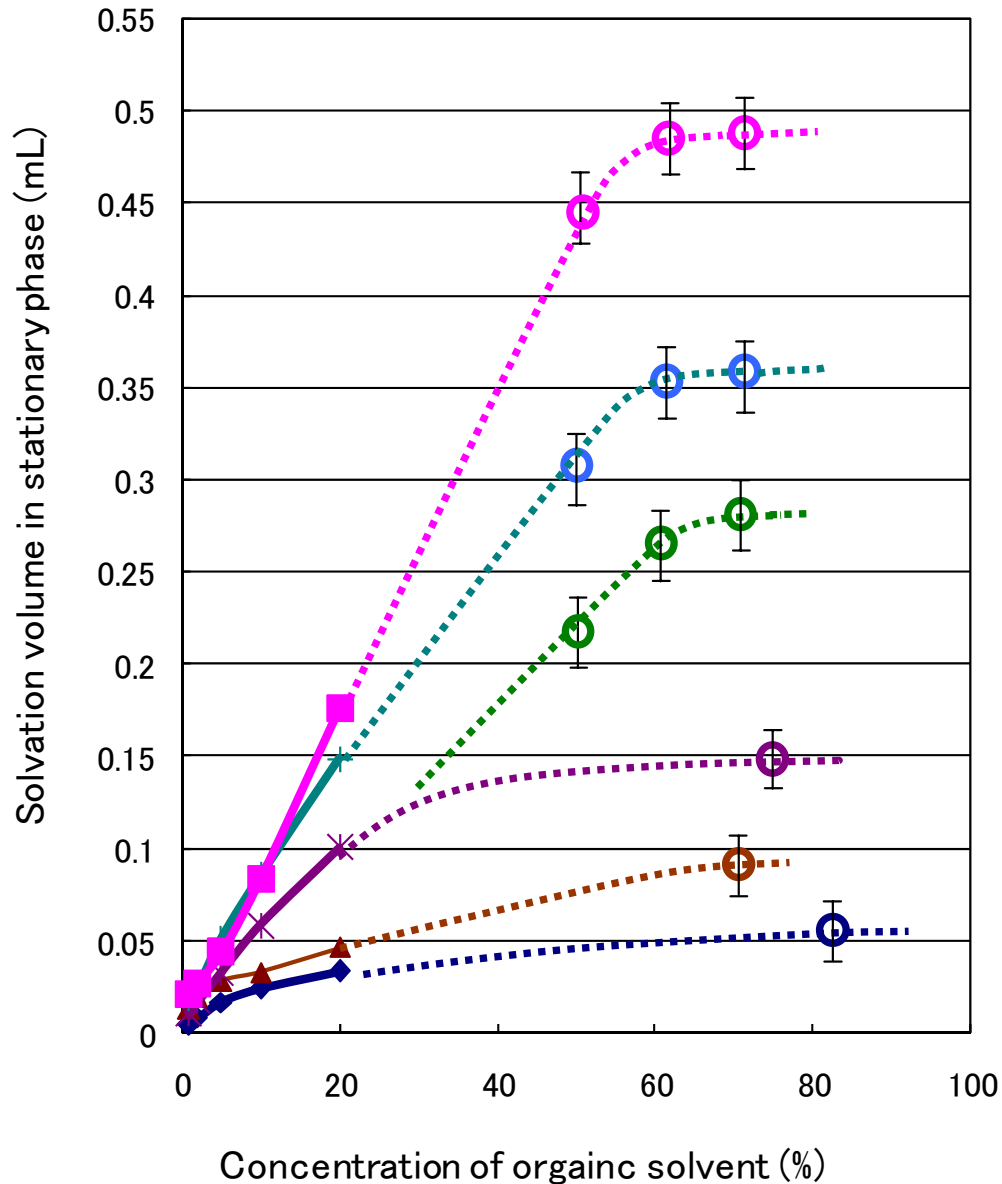
**( $t_0$ )**



Volume of stationary phase under 50%THF is larger than that under 82% methanol.

Variation of volume of stationary phase between 50% THF and 82% methanol is 0.25 mL.

# Solvation volume of organic solvent in stationary phase



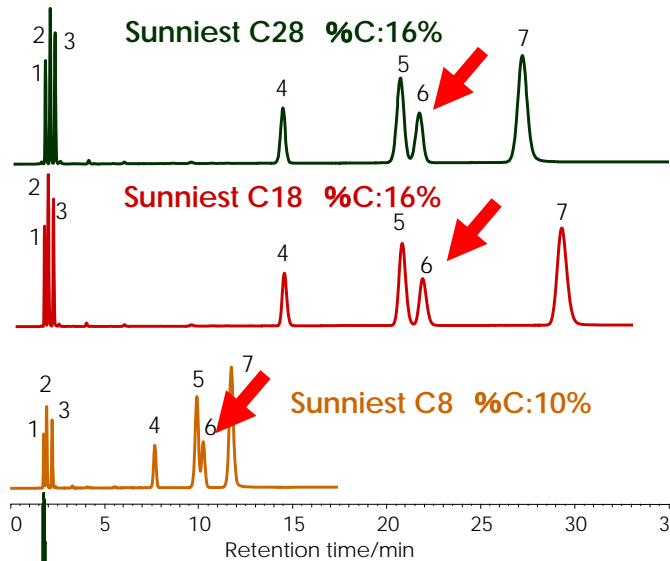
Column: C8, C18, C28  
150 x 4.6 mm

- THF (C28)
- THF (C18)
- THF (C8)
- Acetonitrile (C18)
- Ethanol (C18)
- Methanol (C18)

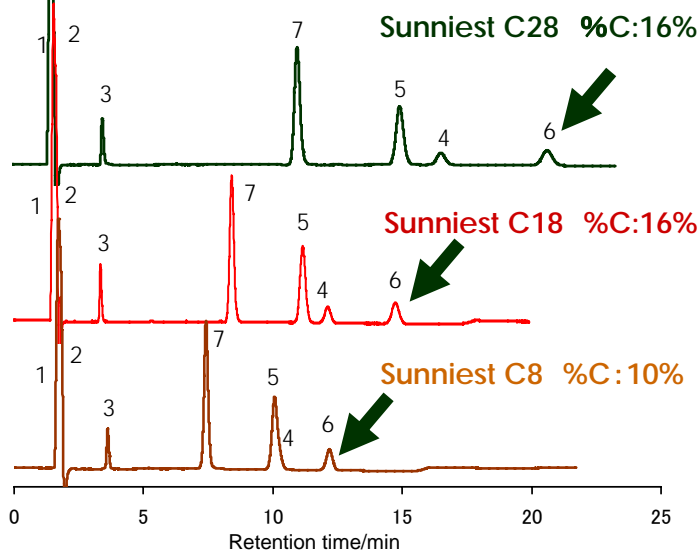
It was assumed that solvation volume of methanol under 82% methanol mobile phase was 0.06 mL. As this value was the criterion, the other values were determined by measurement of elution volume of Uracil.

# Separation of standard samples using methanol and THF

## Methanol



## THF



Column: Sunniest C18, C28, C8, 5 $\mu$ m  
4.6x150 mm

Mobile phase:

CH<sub>3</sub>OH/H<sub>2</sub>O=75/25

THF/H<sub>2</sub>O=50/50

Flow rate: 1.0 mL/min

Temperature: 40 °C

Sample: 1 = Uracil,

2 = Caffeine,

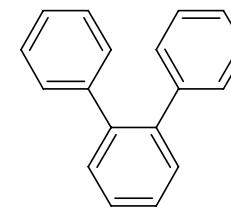
3 = Phenol,

4 = Butylbenzene,

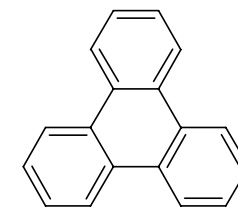
5 = o-Terphenyl,

6 = Amylbenzene,

7 = Triphenylene

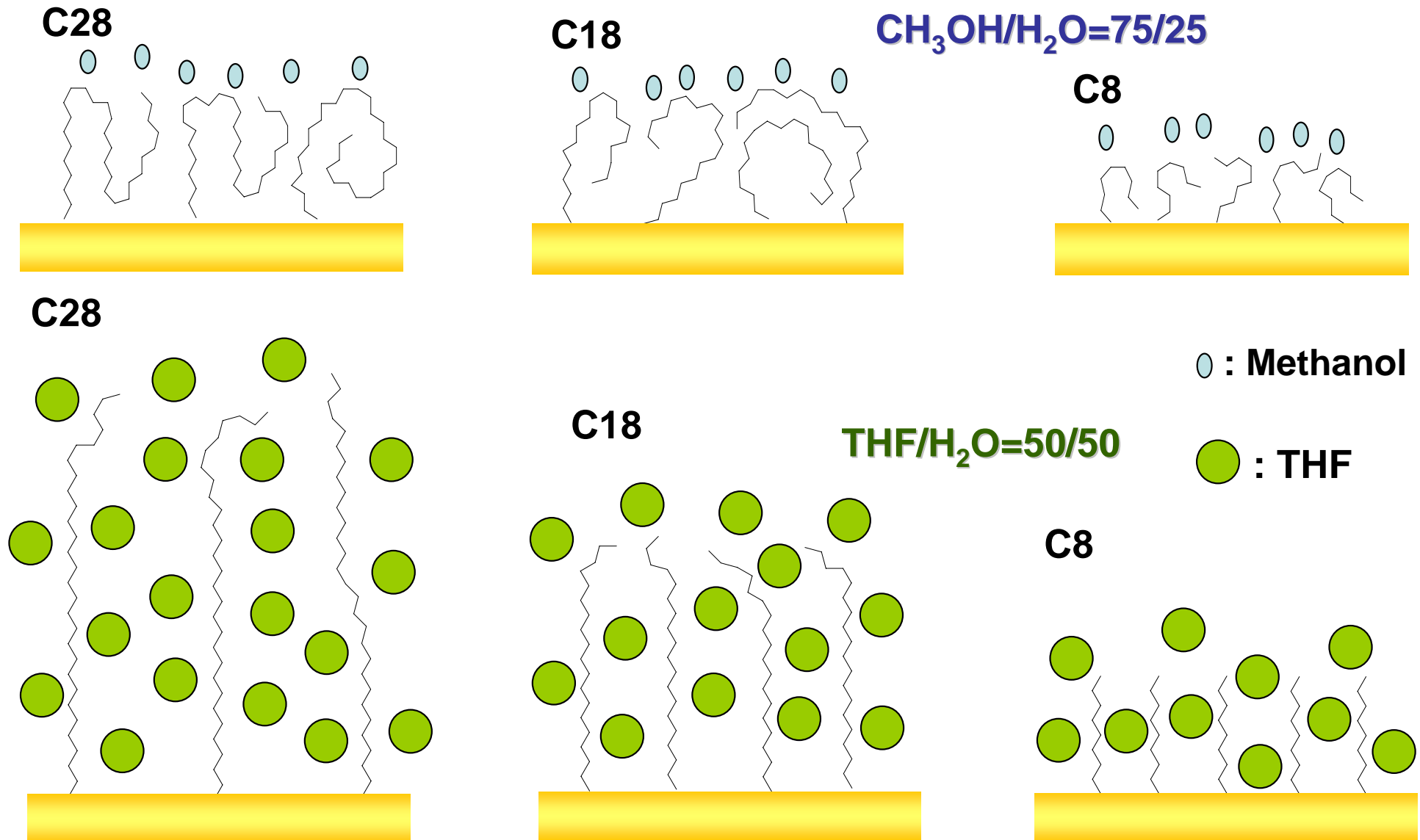


**Peak 5**



**Peak 7**

# Schematic diagram of solvation on C28, C18 and C8 stationary phases



# *Volume of C28, C18 and C8 stationary phases under methanol/water mobile phase*

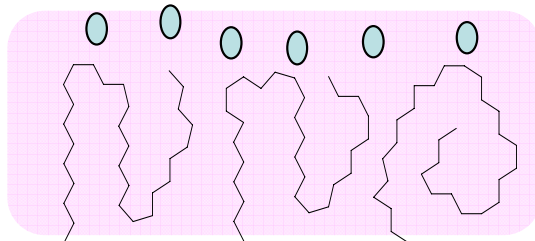
**CH<sub>3</sub>OH/H<sub>2</sub>O=75/25**

**t<sub>R</sub> (amylbenzene): 21.5 min**

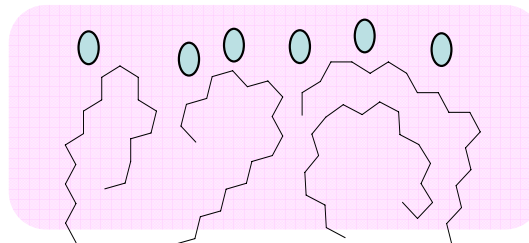
**t<sub>R</sub> (amylbenzene): 21.6 min**

**○ : Methanol**

**C28**

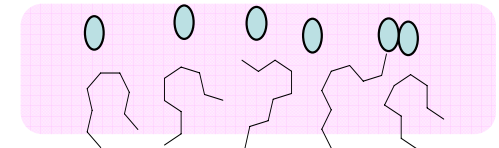


**C18**

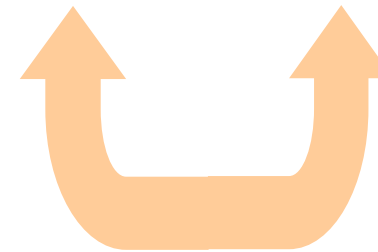


**t<sub>R</sub> (amylbenzene): 10.8 min**

**C8**



Volume of stationary phase is almost same.

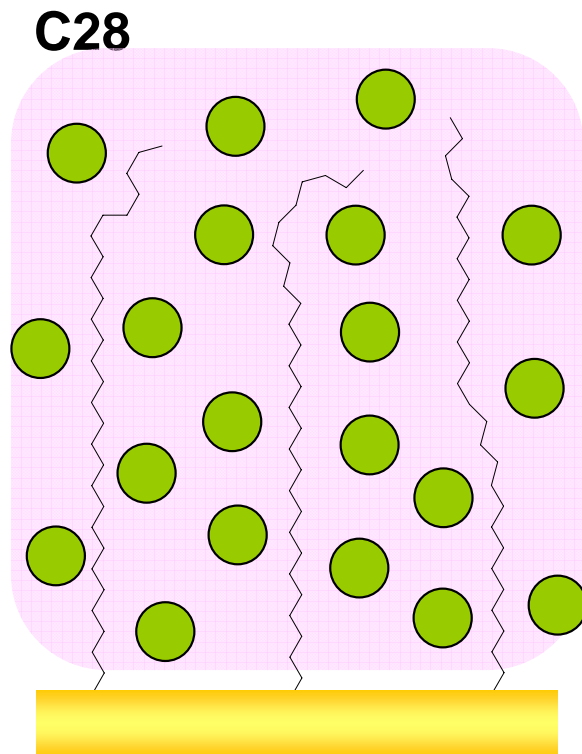


Volume of stationary phase is different.

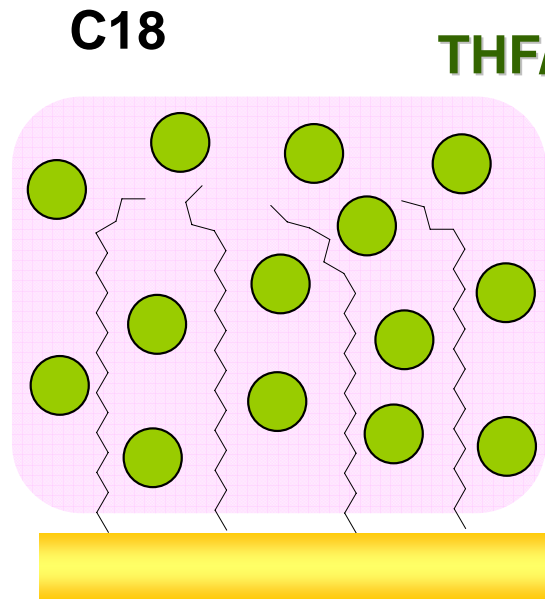


# Volume of C28, C18 and C8 stationary phases under THF/water mobile phase

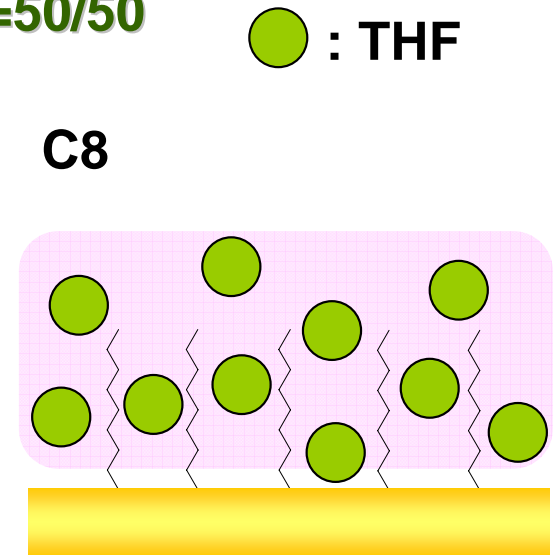
$t_R$  (amylbenzene): 20.8 min



$t_R$  (amylbenzene): 14.5 min



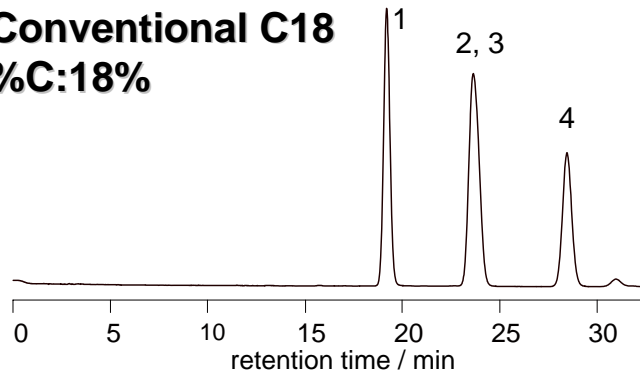
$t_R$  (amylbenzene): 12.2 min



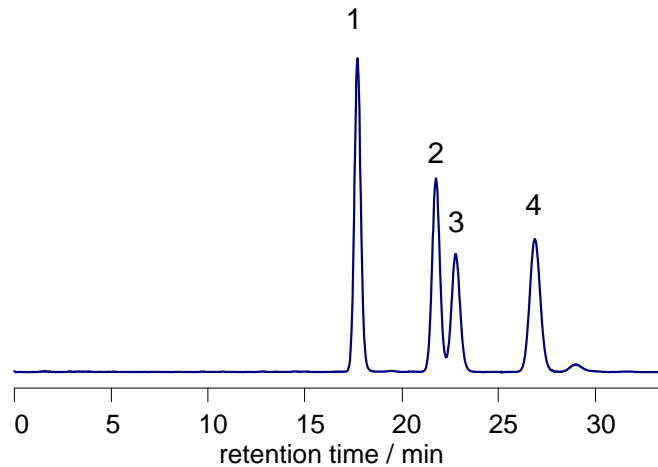
Volume of stationary phase is different.

# Almost same retention time

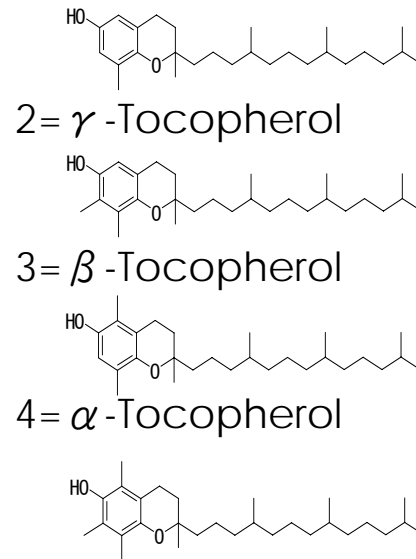
**Conventional C18**  
%C:18%



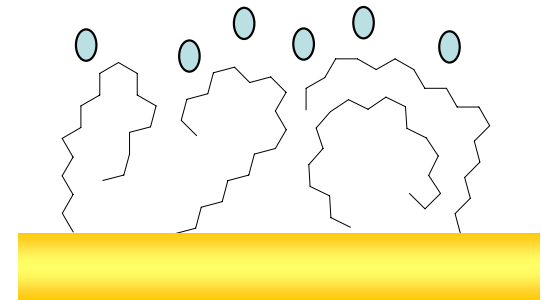
**Sunrise C28** %C:18%



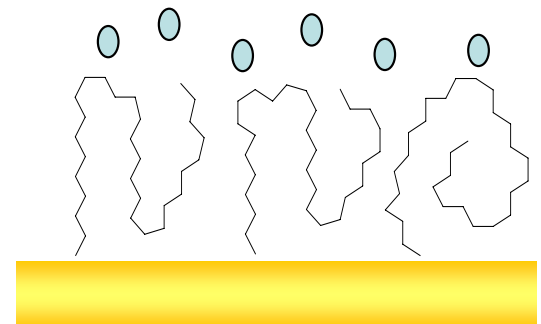
Column size: 4.6x250 mm  
Mobile phase: 97/3=CH<sub>3</sub>OH/H<sub>2</sub>O  
Flow rate: 1.0 mL/min  
Temperature: 25 °C  
Detection: UV at 295nm  
Sample: 1 =  $\delta$ -Tocopherol



**C18**

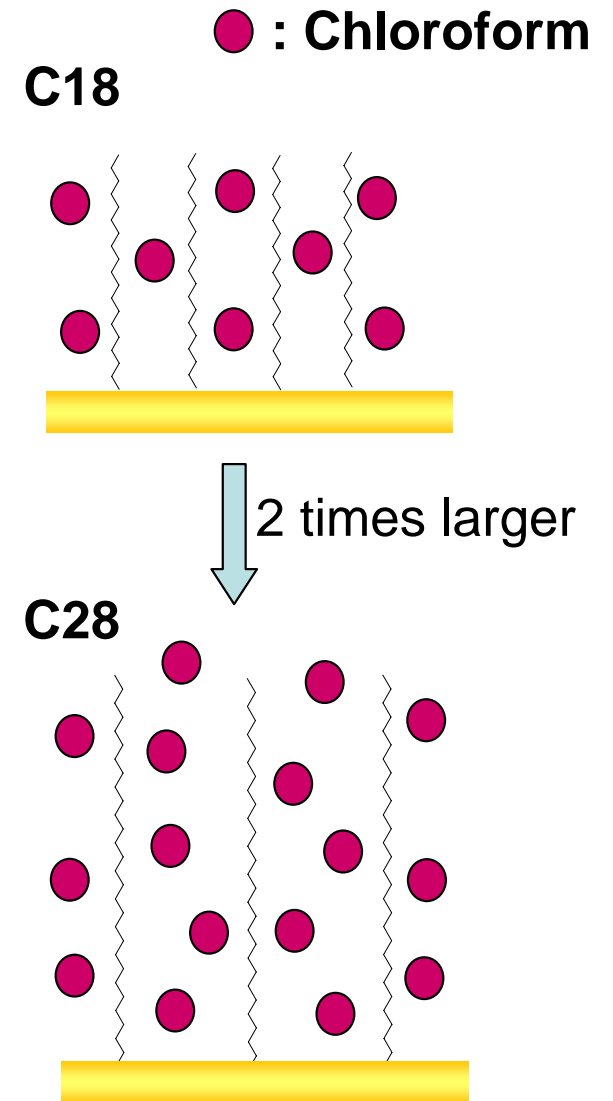
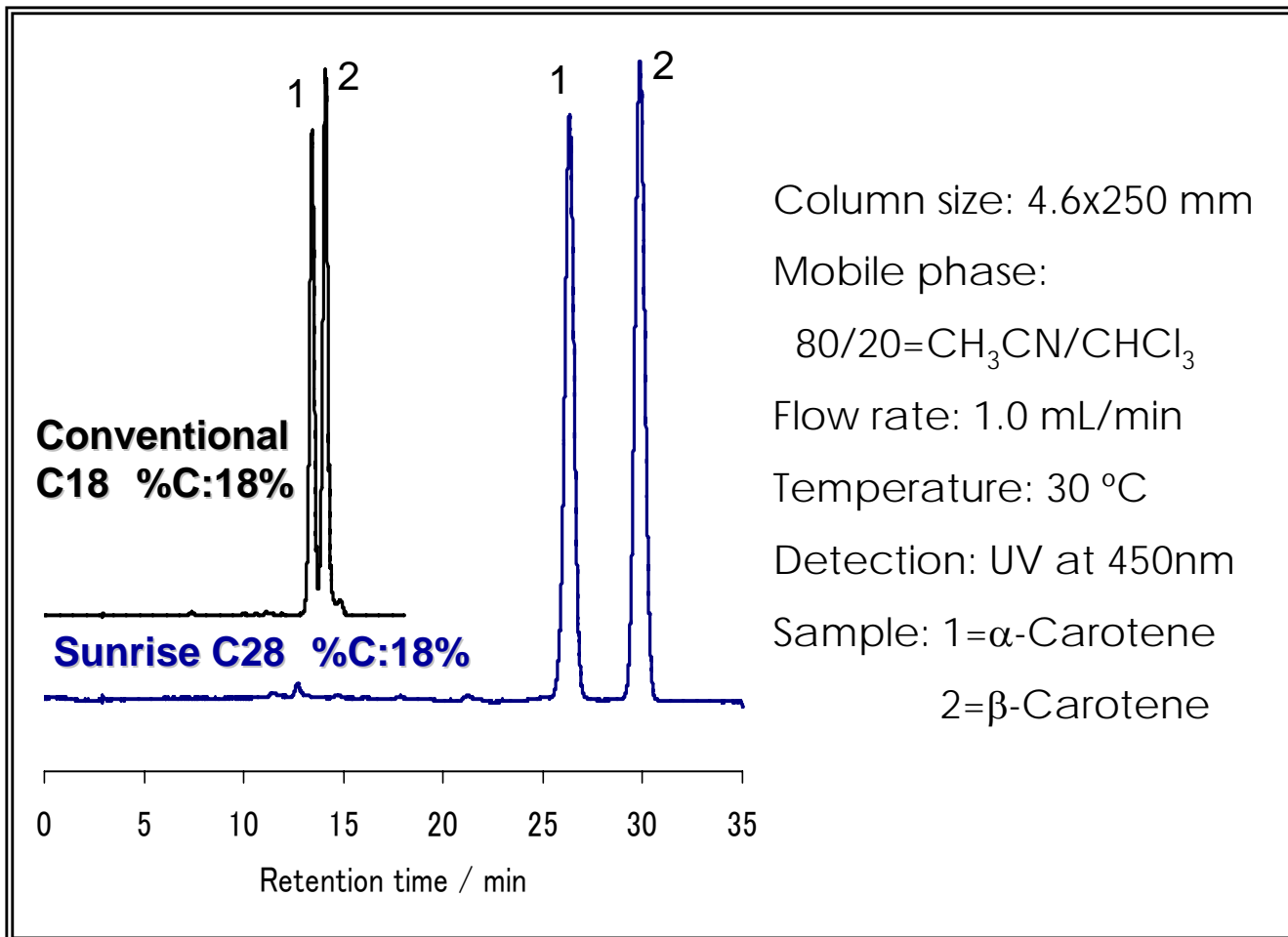


**C28**



Each stationary phase is almost same volume.

# Two times longer retention time



# Conclusion

- Solvation volume in stationary phase was obtained by measuring both retention of organic solvent and elution time of Uracil.
- Solvation volume of methanol and acetonitrile is almost same on the any stationary phases such as C8, C18 and C28.
- The longer an alkyl chain, the larger Solvation volume of THF.
- It is considered that alkyl chains are brush up under THF/water mobile phase, especially at high concentration of THF.