

A New Concept of End-capping for Reversed Phase Silica Material: Remaining of Water-Solvated Silanol Groups



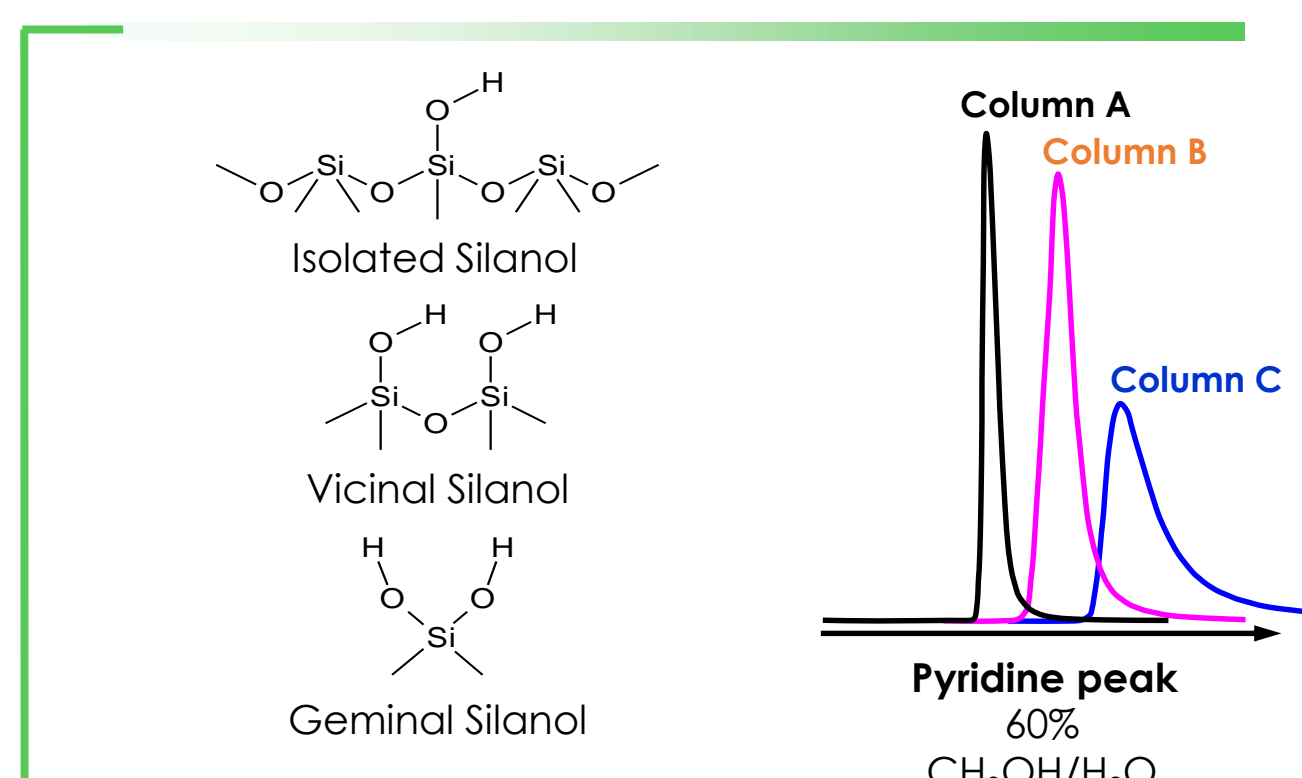
Etsuko Shearer¹, Norikazu Nagae², Tomoyasu Tuskamoto²
 1. BioNik Inc. 3397-19 Obuchi, Fuji, Shizuoka 417-0801 Japan
 2. ChromaNik technologies Inc. Namiyoke, Minato-ku, Osaka 552-0001 Japan
 For more information contact Scott Silver: scott@pyvot.tech



Phone: 212-204-0075
 Email: info@pyvot.tech
 Web: www.pyvot.tech

A reversed phase column like C18 has been widely used in various fields such as pharmaceutical, medical, environmental, industrial and food area. Especially silica based C18 column has showed high performance although an end-capping must be done enough in order to decrease a peak tailing of a basic compound caused by a residual silanol group on the silica surface. An End-capping has been recognized to be an important factor for a silica based reversed phase column. In the beginning residual silanol groups after C18 bonding were reduced by bonding with trimethylsilyl reagents (TMS). This end-capping method has been improved by various end-capping reagents with not only a monofunctional group but also a difunctional group, a high reaction temperature or a long reaction time. In the past ten years some column manufacturers have developed the unique method that positive charge added on the silica surface suppressed influence of residual silanol groups against a basic compound. In this study, an end-capping was tried by only heating as an unprecedented method. Silica gel bonded with only octadecyl group was applied heating at over 200 degree Celsius under a nitrogen atmosphere. After heating C18 silica gel was kept under the solution of methanol/water=50/50 at 50 degree Celsius for 2 hours. Then an activity of residual silanol groups were evaluated to separate basic compounds. The C18 silica gel heated under the optimum conditions showed a symmetrical peak of a basic compound such as amitriptyline, although it included silanol groups. It was considered that heat treatment controlled silanol activity against a basic compound. It was also observed that retention of basic compounds was more than ten times longer to compare with a conventional end-capped C18 stationary phase. Furthermore effect of both pH and salt concentration of a mobile phase for retention of samples was studied. As a result, an ion exchange interaction between a silanol group and a basic compound definitely occurred on the proposed C18 stationary phase. Heating could control silanol activity against a basic compound. Consequently residual silanol groups controlled its activity could change selectivity of basic compounds without peak tailing.

Form of silanol groups and peak shape of pyridine



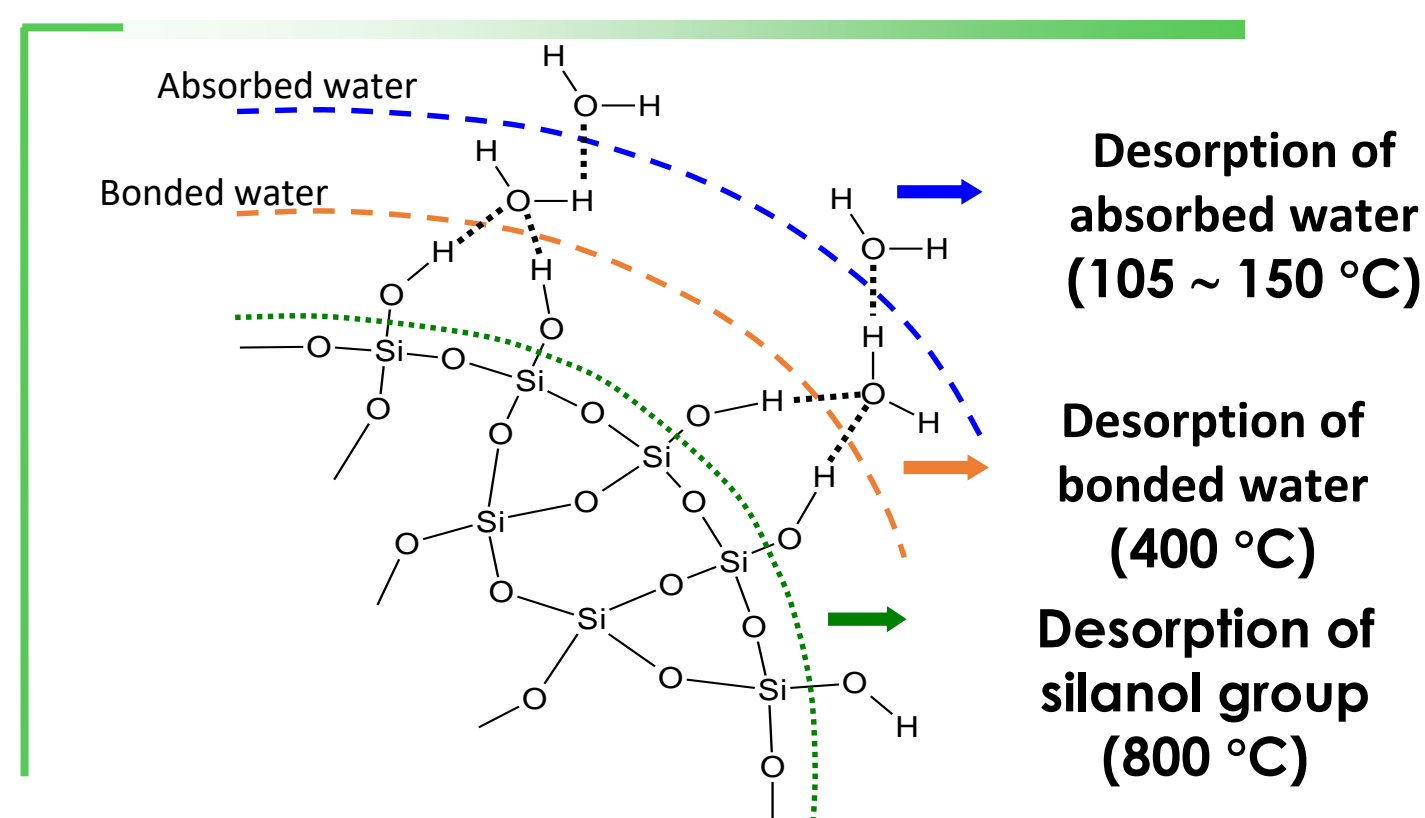
Our hypothesis

- Peak tailing of a basic compound is caused by the state of silanol groups, not by existence of silanol groups on silica surface.
- Silanol groups near a strong hydrophobic site don't occur an ion-exchange interaction etc. rapidly.

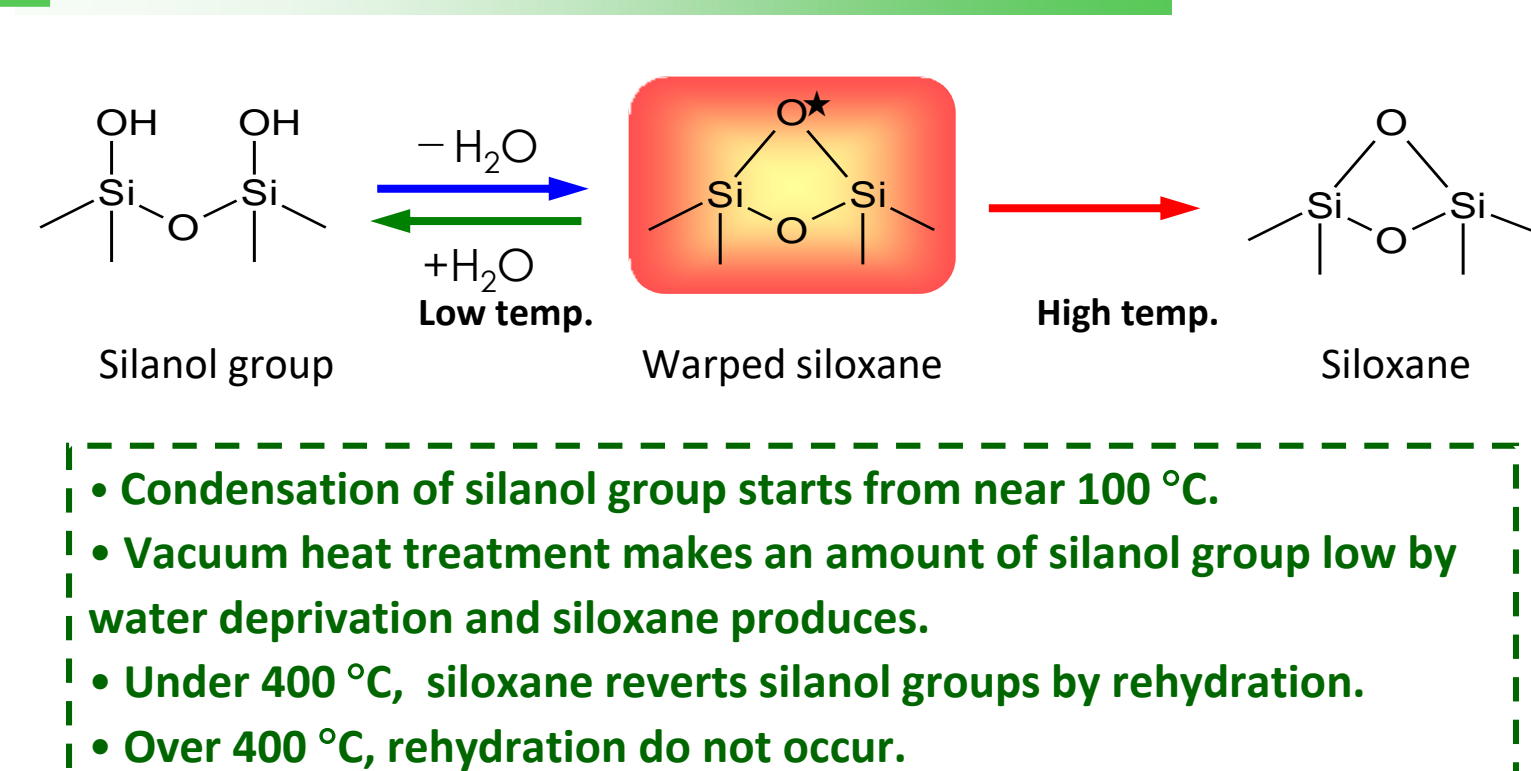
Consequence

- Decrease density of alkyl groups and make hydrophobicity low.
- Control activity of silanol groups near only hydrophobic site

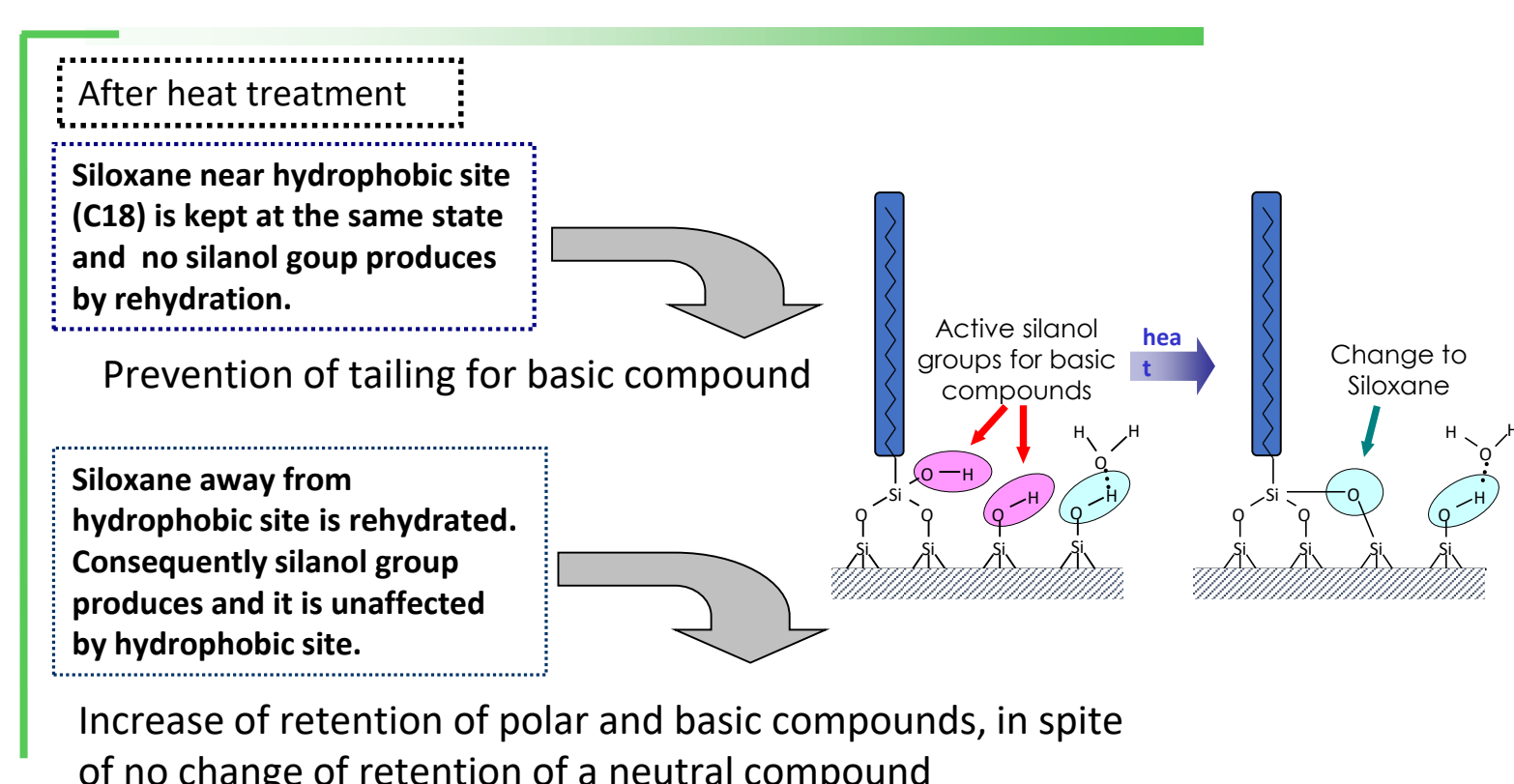
Variation of water on silica surface



Change of silanol groups by heat treatment



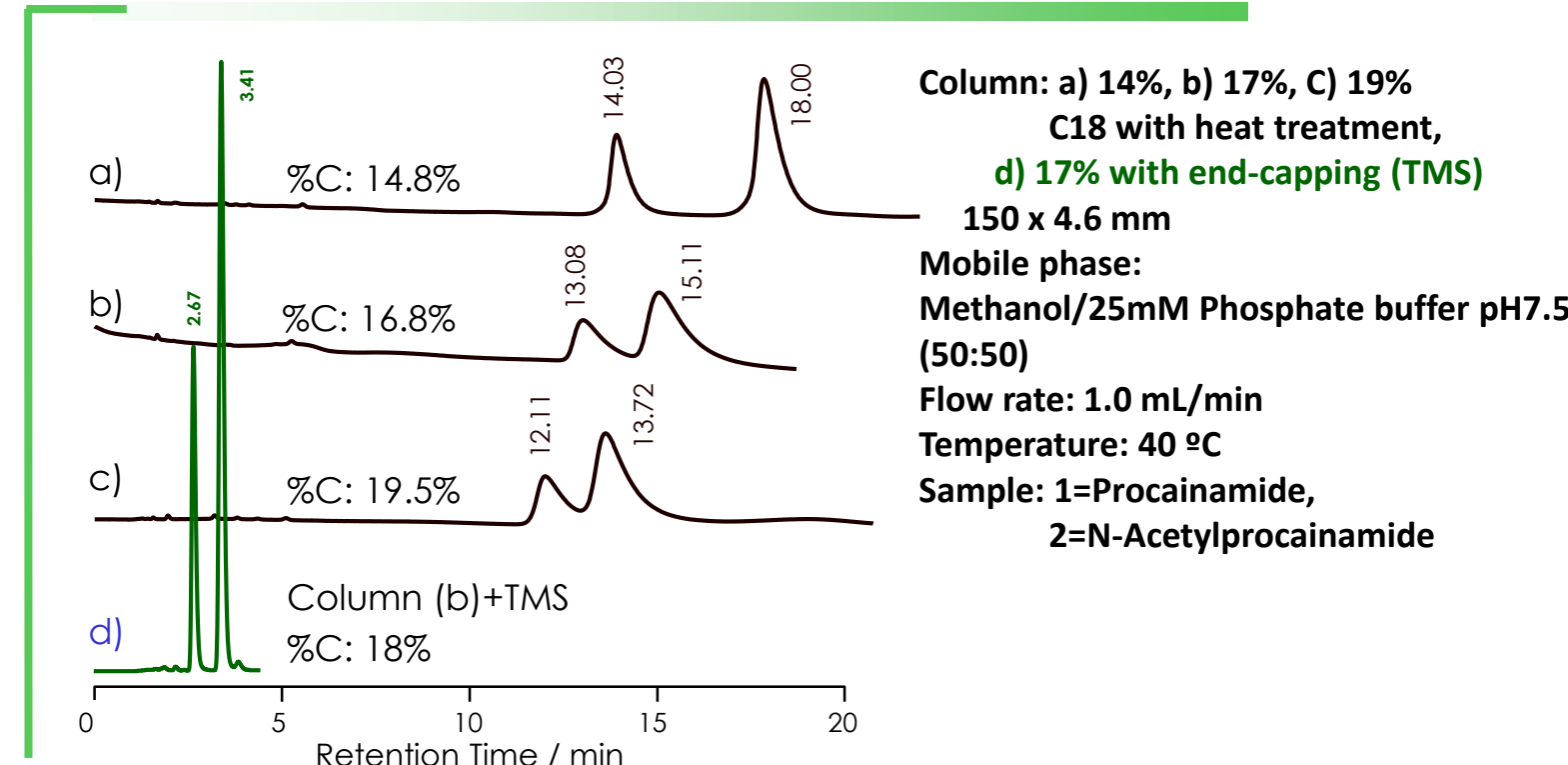
Control of silanol group



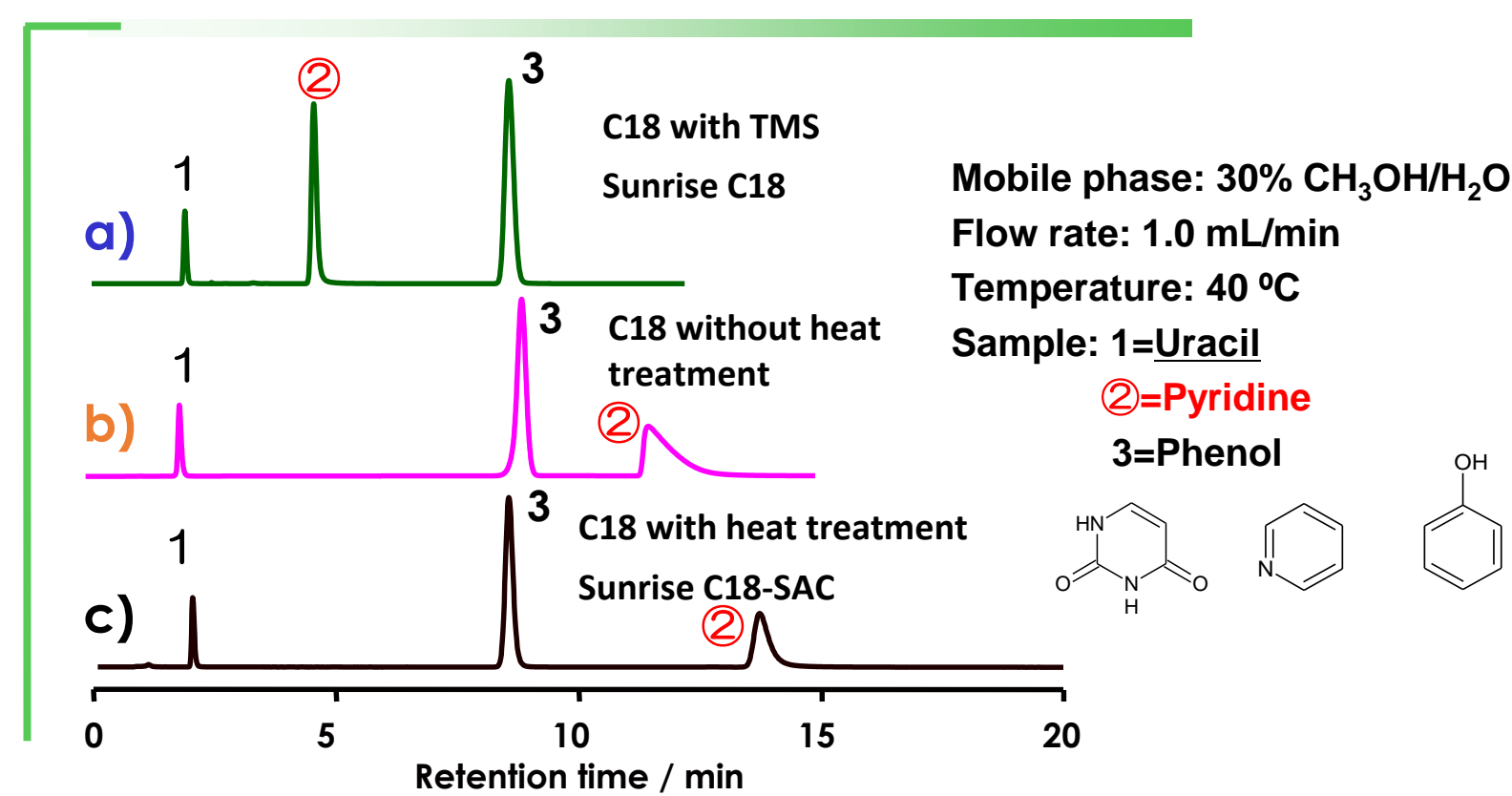
Experiment

- Silica gel**
 - Pore diameter: 12 nm, Specific surface area: 340 m²/g, Particle size: 5 μm
- Bonded Octadecylsilane**
 - Carbon content: 14%, 17%, 19%
 - As reference, prepare C18 phase with end-capping (TMS)
- Heat treatment in a vacuum**
 - Temperature: 200 °C, Treatment time: 48 hours
 - As reference, prepare C18 phase without heat treatment
- Evaluation**
 - Column: 14%: Sunrise C18-SAC (with heat treatment) Sunrise C18 (with end-capping)
 - Column dimension: 150 mm x 4.6 mm i.d.
 - Mobile phase: Acetonitrile or methanol/buffer solution

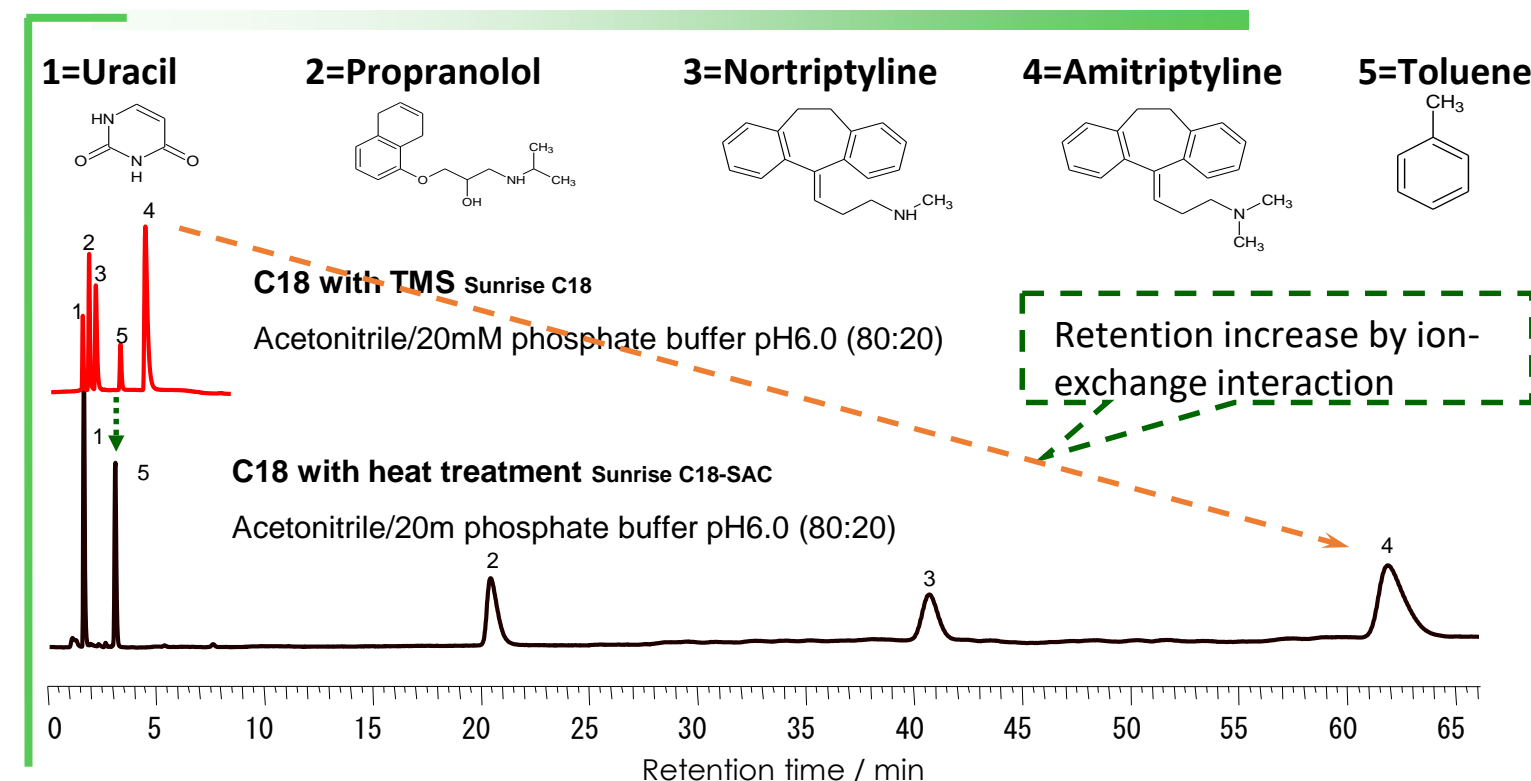
Comparison of carbon content



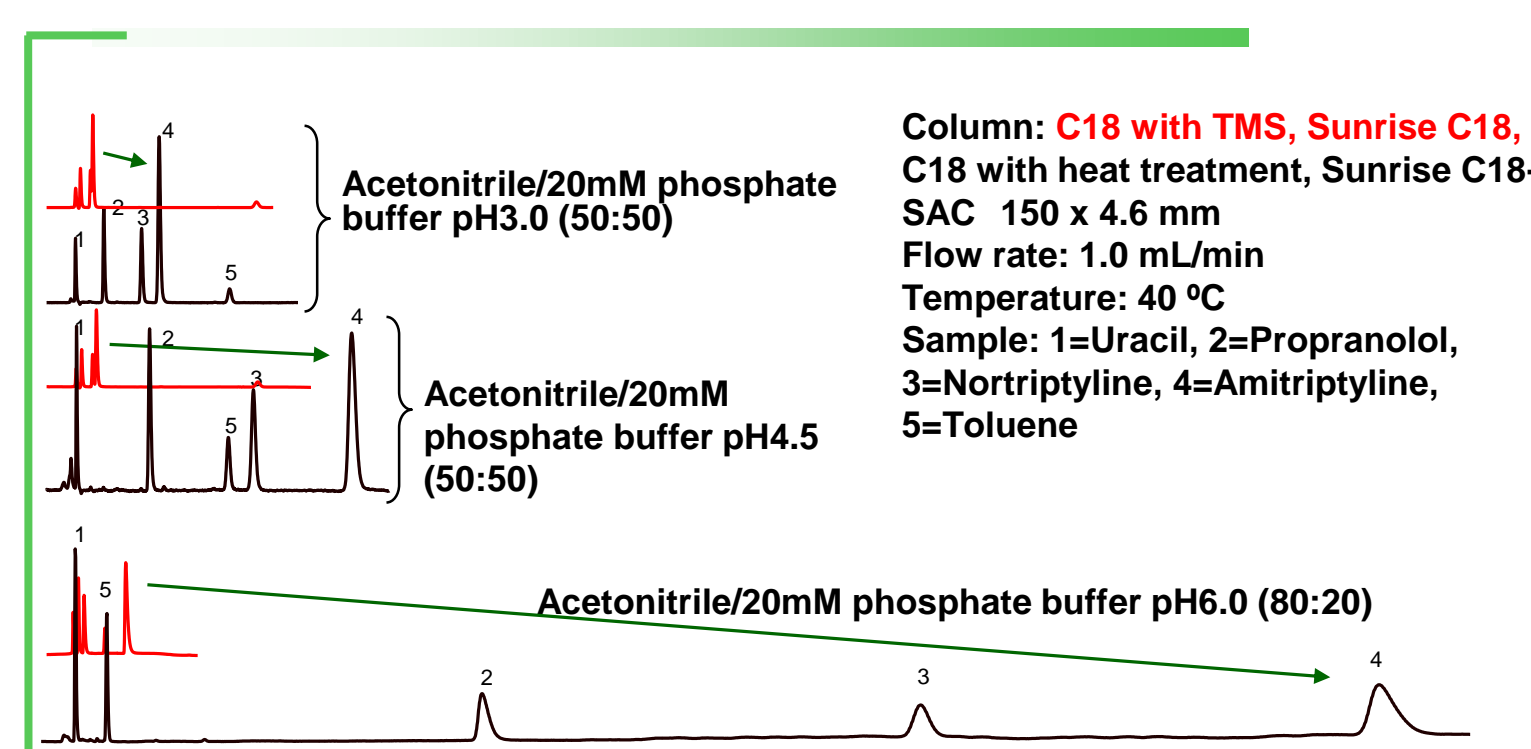
Comparison of pyridine peak shape



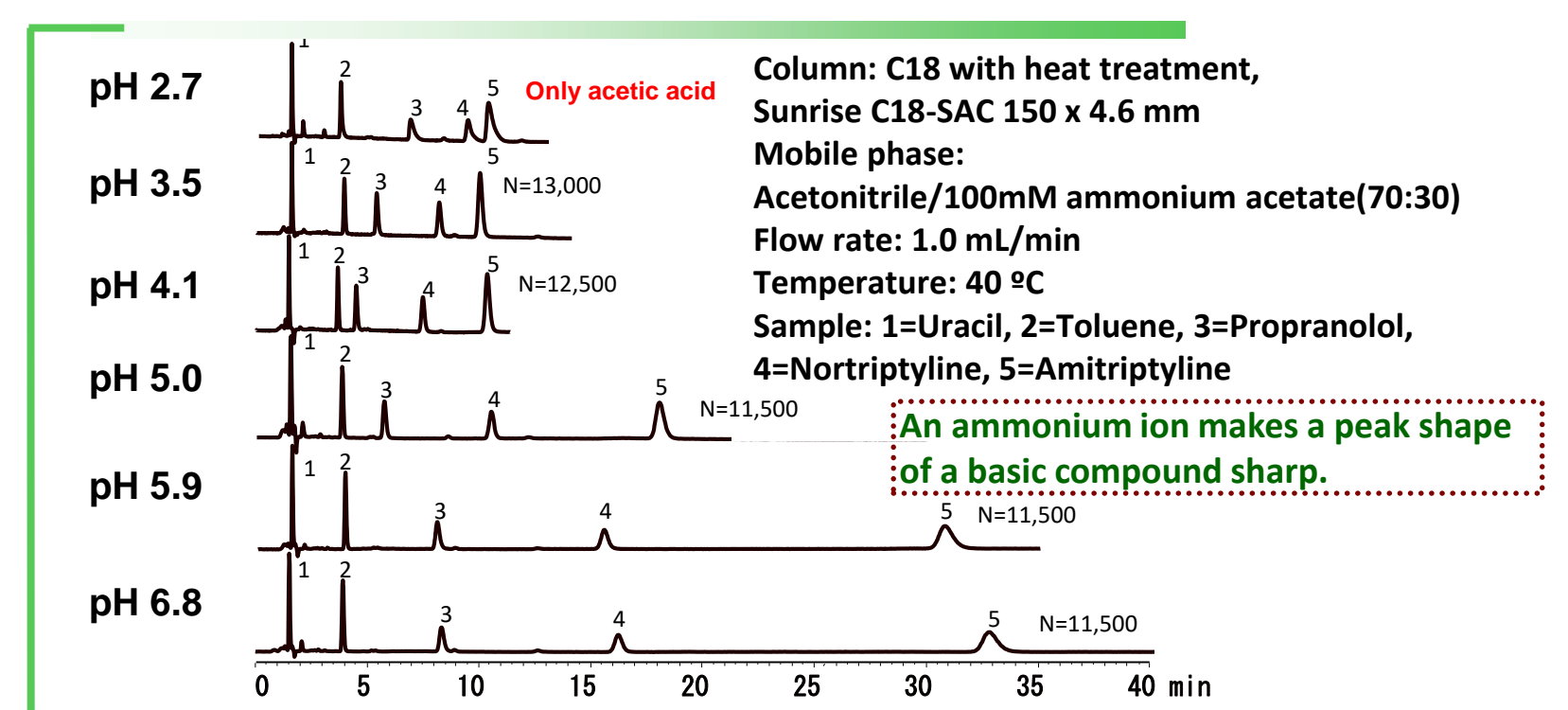
Separation of antidepressants



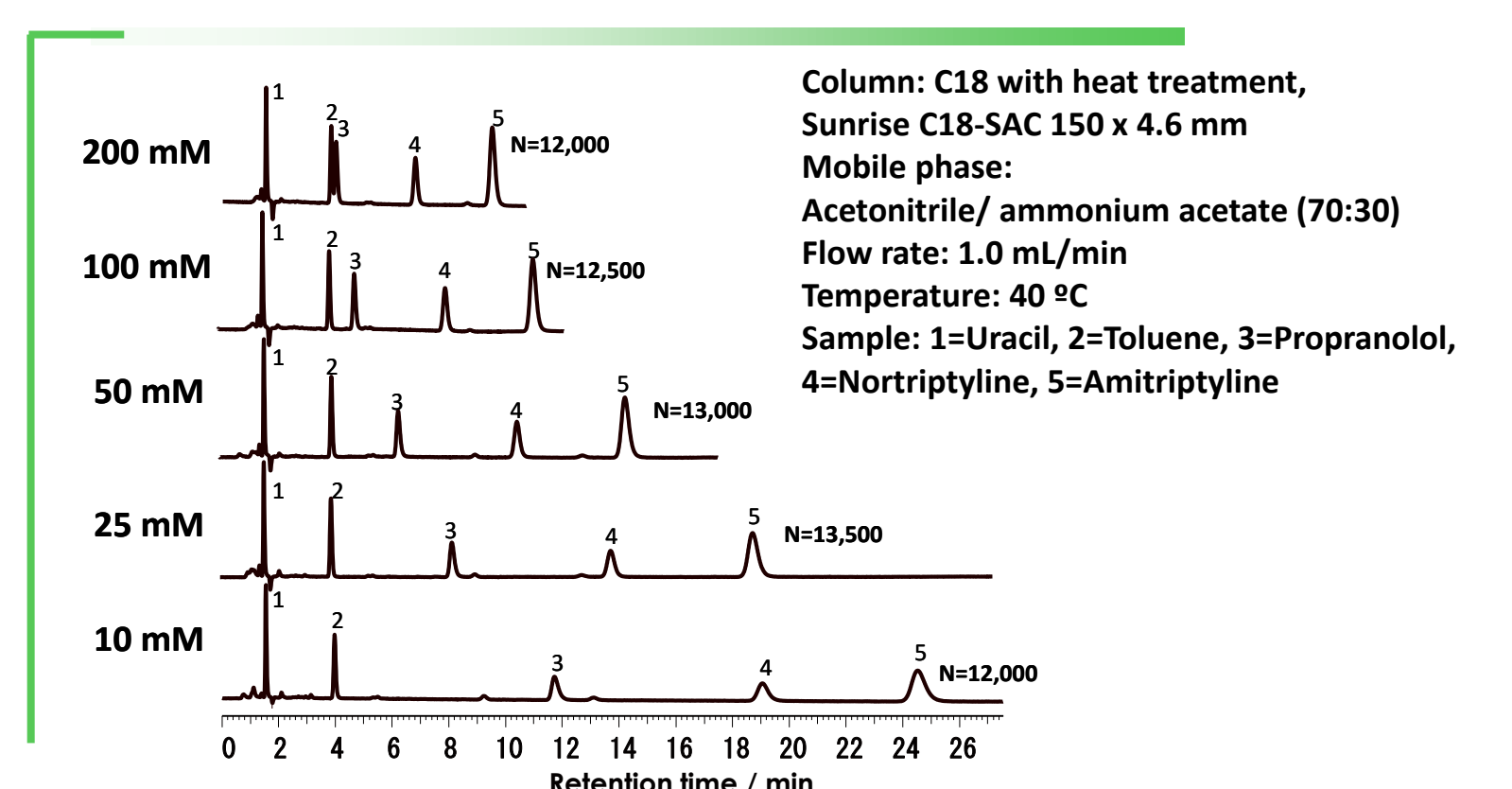
Control of retention by mobile phase pH



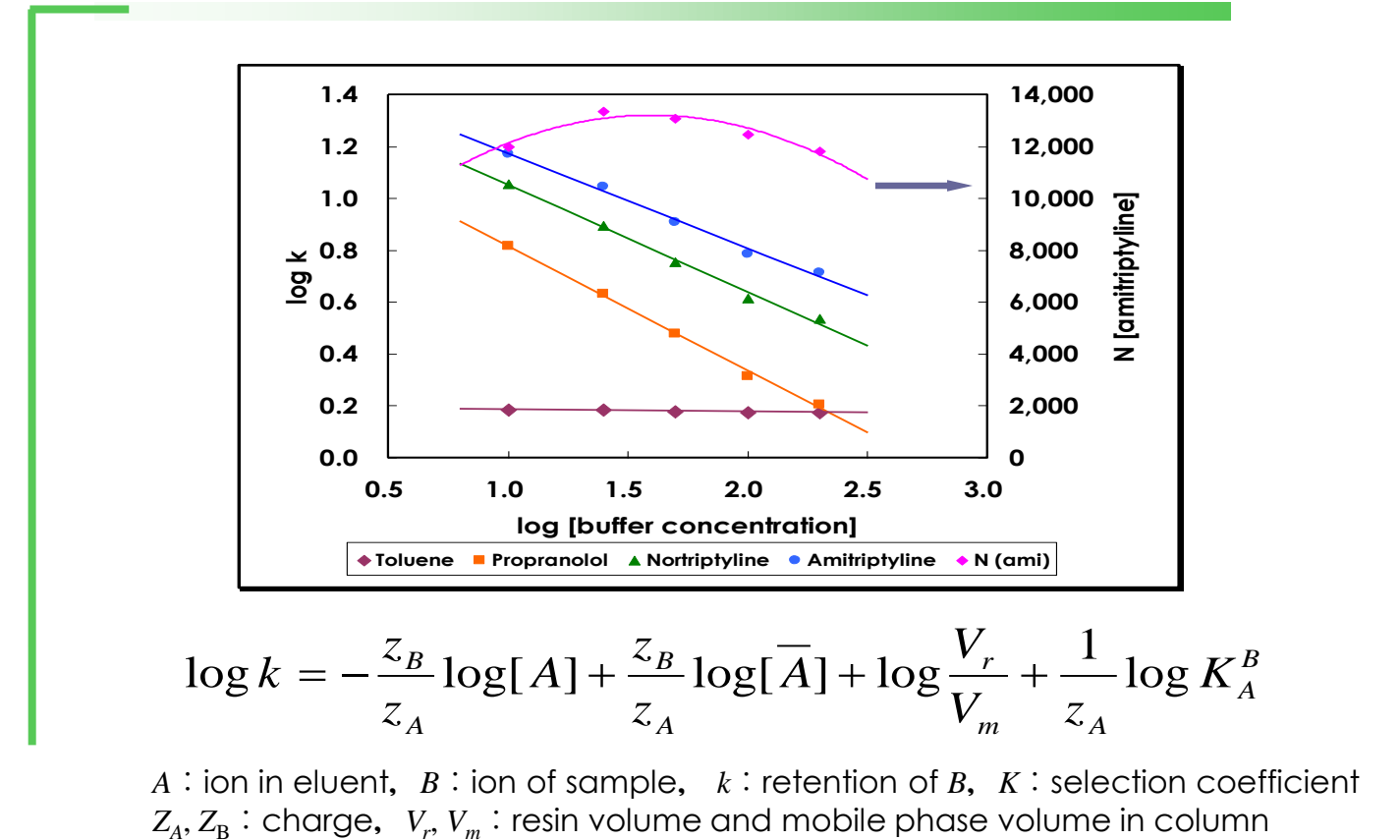
Separation of basic compounds with ammonium acetate: Effect of pH



Separation of basic compounds with ammonium acetate: Effect of salt concentration



Relationship between buffer concentration and retention



Conclusion

- Residual silanol group in proposed C18 phase made retention of polar compounds including basic compounds large.
- Silanol group controlled its activity by heat treatment did not make peak of basic compounds be tailing.
- Furthermore, ion-exchange interaction was recognized and retention of basic compounds could be changed by pH or salt concentration in a mobile phase.
- Proposed C18 phase was effective to separation of a metal chelating compound. It is surmised that hydration or change of state of silanol groups contributed to suppress influence of a metal impurity on a silica support.