Acylation of Aminopropyl-Bonded Silica Gel for Liquid Chromatography

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Acylation of aminopropyl-bonded silica gel for liquid chromatography with acid chlorides was investigated in terms of the pore structure of silica gel and the molecular size of acylating agents. Three types of aminopropyl bonded silica gel with different pore sizes, 40, 60 and 100 Å, were acylated with various acid chlorides. Silica gel with larger pore size generally reacted with a larger amount of acid chloride than that with smaller pore size. This trend was more remarkable when acylating agents with larger molecular size were used. Thus, the density of ligand on the silica surface can be controlled by the combination of pore structure of silica gel and molecular size of acylating agents. Unreacted amino groups on silica surface after the acylation with stearoyl chloride could be made to react when benzoyl chloride, with a smaller molecular size, was used as a second acylating agent.

Keywords  Surface modification, acylation, silica gel, liquid chromatography

In recent years, many workers have developed surface modified silica gel supports which perform well in separation and in selectivity for liquid chromatography. Reversed-phase chromatography using ODS-silica gel is now widely applied to various fields. In addition, diol, COOH, NH₂, SO₃H or N⁺R₄ type packings are now commercially available, mainly for ion-exchange chromatography. Polyacrylamide gels have been used to introduce a wide variety of functional groups by chemical modification for ion-exchange or affinity chromatography. Moreover, both commercial and domestically-modified aminopropyl-bonded silica gel (APS) have been not only directly applied to the chromatographic separation of sugars, for example, but also used as an intermediate to prepare various supports, such as ion-exchangers and optically active stationary phases. Functional groups can be introduced onto the APS surface by the acylation of the amino groups with ligands such as acid chlorides. The surface density of ligands often has to be highly controlled in ion-exchange or affinity chromatography for the separation of biopolymers. Thus, techniques for controlling the surface properties of chemically modified stationary phases will help to develop highly selective packings especially in the field of biochemistry.

The chemical modification of a silica surface is influenced by the pore structure of the silica gel. The effect of pore structure of silica gels and the molecular size of ligands on the coupling reaction has not been investigated in detail.

This paper describes the acylation procedure of APS with various acid chlorides which was used to develop useful silica-based stationary phases for liquid chromatography by controlling surface density of ligands; different pore sizes of silica gels and different molecular sizes of ligands were tried.

Experimental

Materials

Three types of silica gel for column chromatography (particle size: 0.063 - 0.200 mm) purchased from Merck, i.e. Si-40 (nominal pore diameter, 40 Å; surface area, 800 m²/g; specific pore volume, 0.6 ml/g), Si-60 (60 Å; 500 m²/g; 0.7 ml/g) and Si-100 (100 Å; 200 m²/g; 1.0 ml/g) were used for the surface modification. 3-Aminopropyl triethoxy silane (APTES) was from Chisso, and propionyl chloride, benzoyl chloride and stearoyl chloride from Wako were used. Other reagents were of reagent grade.

Preparation of APS

Fifty grams of silica gel and 500 ml of toluene were placed in a 1 l flat bottom flask provided with a reflux condenser and a magnetic stirrer. Fifteen grams of APTES was added to the flask, and the mixture was heated under reflux for 1 h with stirring. After being cooled to room temperature, the reacted silica gel was separated from the solvent and the unreacted APTES by vacuum filtration using a sintered glass funnel, washed with 100 ml of benzene for three times, followed by 100 ml of dichloromethene for three times,
and dried under vacuum at 110°C over night. The reacted APTES was determined by elemental analysis 
(C,H,N) using CHN Corder type MT-3 (Yanagimoto Co., Ltd.).

**Acylation of APS**

Five grams of APS was placed in a 200 ml flat bottom flask provided with a reflux condenser and a 
magnetic stirrer; then 50 ml of a toluene solution 
containing a given amount of an acid chloride was 
added to the APS, and the mixture was heated under 
reflux for 1 h with stirring. After being cooled to room 
temperature, the reacted silica gel was separated from 
the solvent by vacuum filtration, washed with 30 ml of 
benzene for three times, followed by 30 ml of 
dichloromethane for three times, and dried under 
vacuum at 110°C over night. The reacted acylating 
agent was determined by elemental analysis as 
mentioned above.

**Results and Discussion**

Reaction of silica gel with APTES and acylation of 
the resulted APS with acid chloride are summarized 
below:

\[
\text{OH} + (\text{C}_2\text{H}_5\text{O})_3\text{SiC}_3\text{H}_6\text{NH}_2 \xrightarrow{\text{C}_2\text{H}_5\text{OH}} \xrightarrow{\text{in toluene, under reflux}} \text{O-Si-C}_3\text{H}_6\text{NH}_2
\]

\[
\text{O-Si-C}_3\text{H}_6\text{NH}_2 + \text{RCOC}_1 \xrightarrow{\text{HCl}} \xrightarrow{\text{in toluene, under reflux}} \text{O-Si-C}_3\text{H}_6\text{NHCOR}
\]

The carbon content of aminopropyl group bonded to 
the silica surface is shown in Fig. 1. The amount of 
added APTES to silica gel is represented as carbon % 
of aminopropyl group per g of silica gel. Reacted 
aminopropyl groups were evaluated from the carbon 
and nitrogen contents by means of C,H,N elemental 
analysis. Hydrogen contents were considered only as a 
reference, because it is difficult to distinguish the 
reacted hydrogen from the hydrogen derived from 
adsorbed water on the silica surface or from the silanol 
groups of the silica gel. The amount of aminopropyl 
group increased as the APTES concentration increased, 
as shown in Fig. 1. Since commercial APS for 
HPLC typically contains about 4 - 6% of carbon, silica 
gel packings used in this work were treated so that they 
would contain comparable amounts of carbon. The 
data of elemental analysis for the obtained APS are 
summarized in Table 1. Since aminopropyl groups are 
introduced to a silica gel surface by a coupling reaction 
via silanol groups, and since the density of silanol 
groups is approximately proportional to the surface 
area of silica gel, more aminopropyl groups should be 
introduced to the silica gels having larger surface area. 
Moreover, if one aminopropyl group is introduced 
onto a silica surface by an elimination reaction of three 
ethanol molecules from ethoxy groups of APTES, the 
ratio of C/N in APS will be 3. According to the C/N 
ratio in Si-40, 1.65 ethoxy group carbon atoms remain

<table>
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<th>Table 1 Elemental analysis of APS</th>
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<td>Elemental analysis, %</td>
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<td>C</td>
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a. Calculated from nitrogen content.

Fig. 1 Effect of APTES amount on coupling reaction of 
silica gel. ●, Si-40; ○, Si-60; O, Si-100.

Fig. 2 Acylation of APS by benzoyl chloride. ●, Si-40; ○, 
Si-60; O, Si-100.
in the bonded aminopropyl group. Therefore, APS from Si-40 has 0.83 ethoxy groups per aminopropyl group, and 0.60 and 0.62 ethoxy groups are contained in APSs of Si-60 and Si-100, respectively.

The acylation of the APS with various amounts of benzoyl chloride is shown in Fig. 2. The amount of reacted benzoyl chloride is represented by the ratio of the reacted benzoyl radical to the nitrogen atom of the aminopropyl group. So when all the amino groups are acylated by the acylating agent, the ratio should be 1.0. Reacted benzoyl chloride increased as the amount of benzoyl chloride added increased. Benzoyl chloride reacted better on the wider pore size silica gels, presumably because of less geometric hindrance between the acylating agent and the aminopropyl groups bonded on the wider pore surface. The effect of the reaction time is shown in Fig. 3 when 2.5 mmol/g of benzoyl chloride is used as the acylating agent. Since a reaction time of 1 h was enough for the acylation as shown in this figure, the following acylation experiments were carried out by 1 h reaction under reflux.

The acylation of amino groups bonded to silica gels with various pore sizes was investigated using propionyl chloride whose molecular weight is smaller than that of benzoyl chloride in order to examine the effect of molecular size on the acylation. The smaller acid chloride had been expected to permeate easily into larger pores of silica gel and to react with the amino groups in much larger amounts compared with the larger acid chloride. As shown in Fig. 4, however, the acylation did not increase so much as expected, presumably because the reactivity of propionyl chloride with amino group was lower than that of benzoyl chloride. The reactivity of propionyl chloride with APS of Si-60 was greater than with that of Si-100, which suggests that in the case of relatively smaller molecules, interaction between the amino group and acid chloride molecule affects their reactivity more remarkably than the pore structure of the silica gel.

The acylation by stearoyl chloride is shown in Fig. 5. The differences in the degree of acylation among those three silica gels were remarkable in the case of using large stearoyl chloride. Acylation of Si-40 is assumed to be prevented to some extent by geometric hindrance caused by both the narrow pore structure of Si-40 and the large molecular size of stearoyl chloride. The acylating agent has easier access to the amino group bonded to the surface of larger pore structural silica gel such as Si-100 than in the case of Si-40. Unreacted amino groups remained on the Si-40 APS surface in an

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**Fig. 3** Effect of reaction time on acylation using benzoyl chloride. ●, Si-40; ○, Si-60; ◦, Si-100.

**Fig. 4** Acylation of APS by propionyl chloride. ●, Si-40; ○, Si-60; ◦, Si-100.

**Fig. 5** Acylation of APS by stearoyl chloride. ●, Si-40; ○, Si-60; ◦, Si-100.
amount of about 60% of original amino groups at the 2.5 mmol/g addition of stearoyl chloride, whereas 40% and 30% of original amino groups remained unreacted on Si-60 and Si-100 APS surfaces, respectively. Thus, the degree of acylation of amino groups on a silica surface can be controlled by changing the molecular size of acid chloride, that is, acyl groups can be partially introduced onto the silica surface. This result also suggests that at least two different kinds of acyl groups can be introduced on the silica surface by varying the molecular size of acylating agents.

Moreover, the amino groups remaining after the acylation with stearoyl chloride could be further reacted with a smaller acid chloride, such as benzoyl chloride as shown in Fig. 6. The amount of the reacted second acylating agent with Si-40 APS was larger, presumably because a larger amount of amino groups remained unreacted on the silica surface of Si-40 APS than on the other APSs. This result suggests that at least two different kinds of acyl groups can be introduced on the silica surface by varying the molecular size of acylating agents.

References