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FT-IR SPECTROSCOPY OF CHEMICALLY BONDED SILICA GEL FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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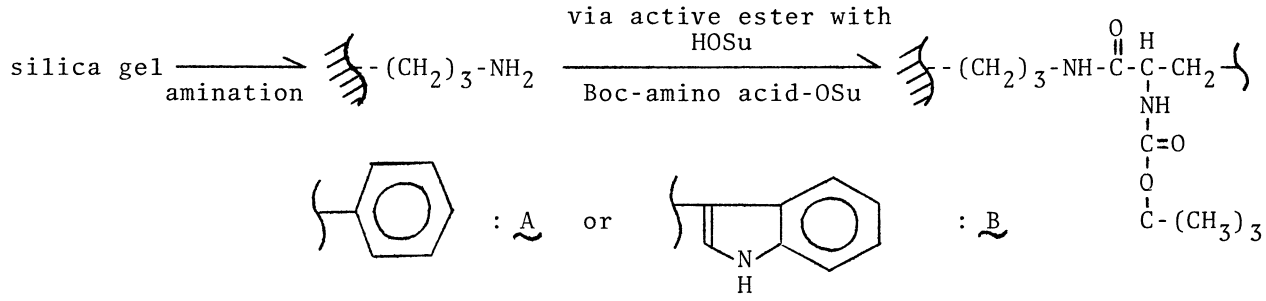
The surfaces of the silica gels modified with Boc-amino acid were characterized by using FT-IR spectroscopy. It was proved that the formation of covalent bonding between Boc-amino acid and aminated silica gel occured.

Chemically bonded silica gels have prevailed in high performance liquid chromatography(HPLC). Most of them are so called ODS-silica or amino-silica gel[1]. In recent few years, amino acid bonded silica gel has been studied to resolve enantiomeric amino acids[2]. However, the surface characterization of chemically modified silica gel is not necessarily sufficient. To characterize the modified gel is indispensable in order to prepare the gel having good quality and to proceed theoretical study. A qualitative study of the silica gel modified with chromophores was undertaken by photoacoustic spectroscopy in visible region[3]. High resolution NMR spectroscopy for solid was shown to be fairly promising in this field[4].

In this letter, it is shown that FT-IR spectroscopy is a considerably powerful means to investigate the chemically modified silica gel for HPLC. Surface moieties of the silica gel modified with N-protected amino acid were directly observed by FT-IR spectroscopy. Bonded amino acids were Boc-L-phenylalanine or Boc-L-tryptophan. The modified gels were synthesized as following sequence;



These amino acids were coupled via the active ester of them with N-hydroxysuccinimide(HOSu) to the aminated silica gel, which was prepared from silica gel(Toyo Soda, LS310, 5μ) and 3-aminopropyltriethoxysilane. After the coupling reaction of Boc-amino acid, the gel was thoroughly washed and dried under vacuum. So far as the coupling reaction proceeds, the moiety coming from HOSu should be removed away by washing. The details of the synthesis will be given elsewhere. The surface bonding should be mono-layer. The BET surface areas of these gels are shown in Table. The chemical modification caused only slight reduction in BET surface area, suggesting

Table BET Surface Area of Modified Silica Gels

	silica gel	amino-silica gel	A	B
BET surface area, m^2/g	456	424	415	438

that a basic structure of the original silica gel may be retained after the modification. The amounts of bonding were determined by Kjeldohl nitrogen analysis as 0.30 and 0.42 mmole/g-dry gel for Boc-L-phenylalanine(A) and Boc-L-tryptophan-amino-silica gel(B), respectively. IR diffuse reflectance spectra were measured by using Digilab FTS-15 FT-IR spectrometer. The samples were prepared as powder mixtures in KCl(silica gel or modified silica gel : KCl = 1 : 10) and were contained in shallow disk cell with a cavity volume of about 0.1 ml. KCl powder was used as reference. IR diffuse reflectance spectra of the original silica gel, its aminated one and further phenylalanine-bonded gel are shown in Fig.1.(The ordinates are shifted to each other for convenience of view.) By attaching the aminopropyl functionality, obvious changes were observed over three regions of wavenumbers as indicated by arrows.(a) to b) in Fig.1.) Absorptions at 3376 and 3305 cm^{-1} could be assigned to the asymmetric and symmetric NH stretching vibrations of primary amine, respectively. The broad band at 1595 cm^{-1} also was assigned to NH_2 deformation vibration. This band vanished resulting from the formation of amide bond between amino-silica gel and Boc-amino acid as shown later in Fig.3. The two bands at 2870 and 2940 cm^{-1} came from CH stretching vibration of propyl group. Remarkable changes were observed by the further bonding of Boc-phenylalanine or tryptophan as

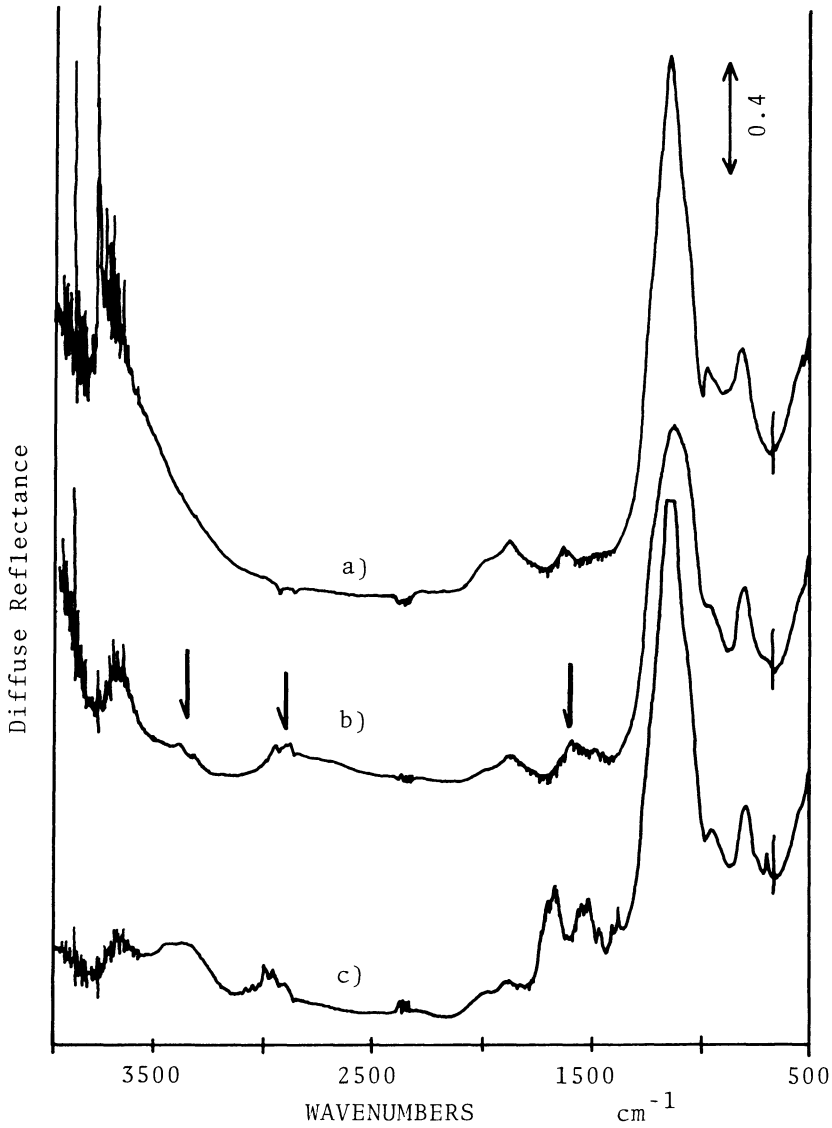


Fig.1. The diffuse reflectance spectra of FT-IR. scans: 124, resolution: 1 cm^{-1}, a) silica gel, b) amino-silica gel, c) Boc-phenylalanine-silica, A

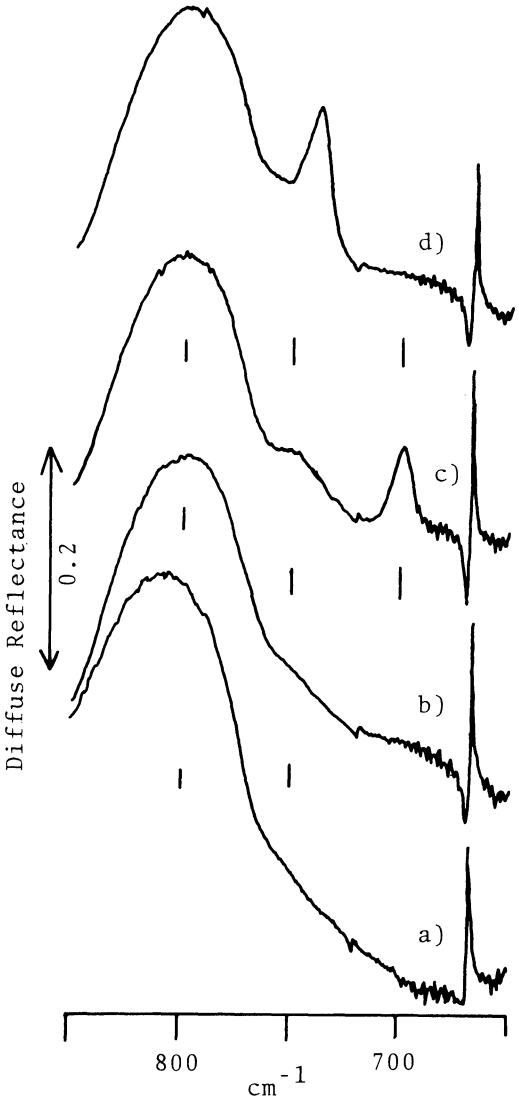


Fig.2. The expanded diffuse reflectance spectra of FT-IR. a) silica gel, b) amino-silica gel, c) A , d) B

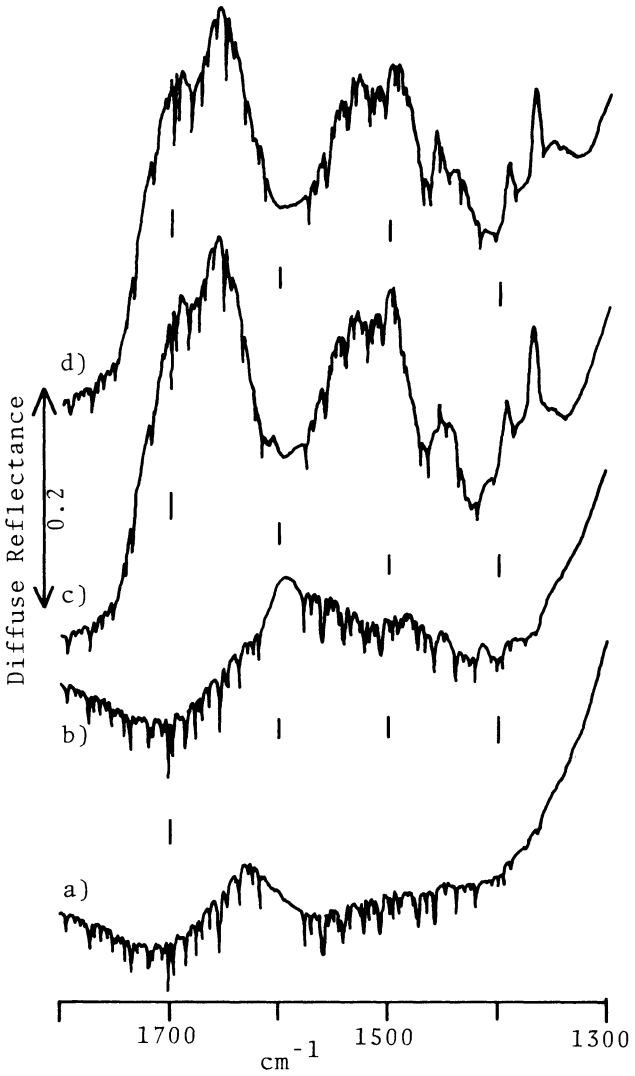


Fig.3. The expanded diffuse reflectance spectra of FT-IR. a) silica gel, b) amino-silica gel, c) A , d) B

shown in Fig.1 c). A sharp doublet appeared commonly to both at 1395 and 1368 cm^{-1}. The intensity of the second peak is about twice that of the first. These two peaks were attributed to CH deformation vibrations of t-butyl groups. Bands at 700 and 740 cm^{-1} of A are characteristics of mono-substituted aromatic ring, while the band characteristic to 1,2-di-substituted aromatics was observed at 740 cm^{-1} for B . The expanded spectra of this region are shown in Fig.2. The expanded spectra of the region 1800 to 1300 cm^{-1} are shown in Fig.3. Above-mentioned sharp doublet due to t-butyl group is particularly noticeable in the expanded spectra. Bands around carbonyl region for A and B were intricate. The Boc-amino acids themselves have no peak in the region 1510 to 1630 cm^{-1}. So, the peak at 1530 cm^{-1} may be ascribed to the amide II band newly formed. Bands at 1700 and 1660 cm^{-1} were assigned to carbonyl in Boc group and amide I band, respectively. These assignment may be tenta-

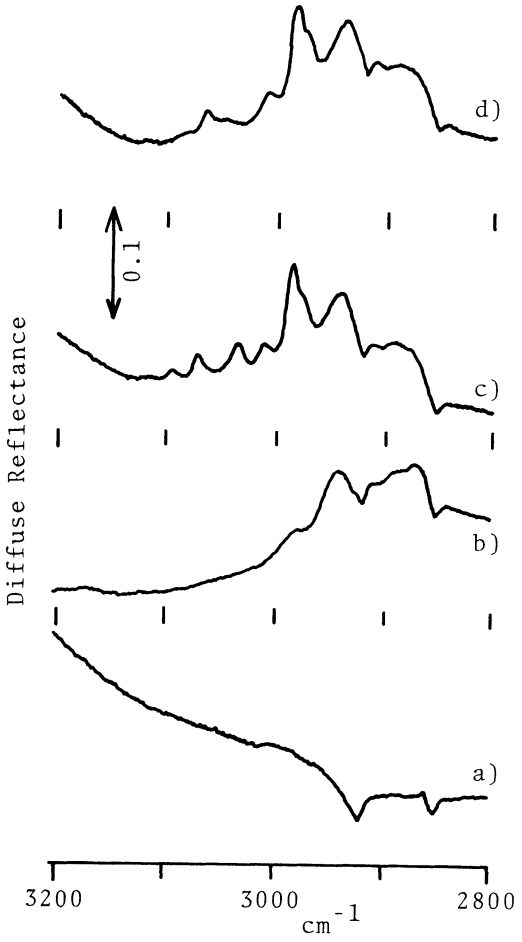


Fig.4. The expanded diffuse reflectance spectra of FT-IR. a) silica gel, b) amino-silica gel, c) A, d) B

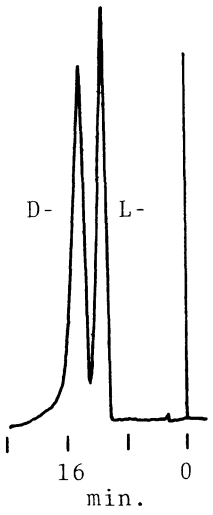


Fig.5. Resolution of D,L-histidine. column: A, eluent: 1/15M phosphate buffer containing 10^{-5}M Cu^{++} (pH 5.28)

tive, being open to be clarified. The small peak at 1350 cm^{-1} for B came from CN stretching vibration in the indole ring of tryptophan. Also the fact that the absorption of NH stretching region for B is more intense than that for A indicates a contribution of additional NH group from the indole ring. CH stretching region over 3100 to 3000 cm^{-1} for A and B were rather complicated, but a distinction between mono- and di-substituted aromatics seems feasible. The expanded spectra of this region are shown in Fig.4.

The appearance of amide band and the disappearance of primary amine proved definitely the formation of covalent bonding between the amino-silica gel and Boc-amino acid. The active ester of HOSu shows strong bands around 1800 cm^{-1} ascribable to the ester carbonyl and sterically strained carbonyl in succinimide ring. While, any trace of them could not be observed in the spectra of Boc-amino acid bonded gel. These results provide further evidence of the covalent bonding of Boc-amino acid to the silica gel.

After deprotecting Boc group, the gel A could resolve completely the enantiomer of histidine with good efficiency by high performance liquid chromatography as shown in Fig.5. Experiments with respect to the gel B are under investigations.

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