Articles

Molecular Recognition: α-Cyclodextrin and Aspirin Inclusion Complexation

Hee-Sook Choi

Animal Resources Research Center, College of Animal Husbandry,
Kon-Kuk University, Seoul 133-701.
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Molecular interaction between α-cyclodextrin and aspirin was studied by UV, ^{1}H-NMR and ^{13}C-NMR spectroscopy analyses for solution complex and by FT-IR analyses for solid complex. The inclusion structure provides a basic understanding of the aspirin and α-cyclodextrin interaction.

Introduction

Aspirin, as well as the salts of salicylic acid (for example, sodium salicylate), is employed as an analgesic-antipretic and as an effective nonsteroidal anti-inflammatory agent[1-3].

The most common side effects from aspirin are gastrointestinal disturbances (dyspepsia, nausea, vomiting). Prolonged adminstration of large doses induces gastric bleeding and frequently leads to blood loss in the stool and occasionally to an iron-deficiency anemia. Since the acetyl group of aspirin is quickly removed, the plasma half-life of aspirin is about 15 minutes. Twenty minutes after ingesting aspirin, only 30% of the drug in the blood is present as the acetylsalicylates; the rest is mostly salicylate[4].

The study of the mechanism of aspirin hydrolysis therefore, is important in determining the factors controlling the blood aspirin concentration. The structure of aspirin and β-cyclodextrin inclusion complex was studied by H. Choi[5].

This paper investigates an accurate determination of the structure of aspirin and α-cyclodextrin inclusion complex.

Materials and Methods

Materials. α-Cyclodextrin was obtained from Anspec. Aspirin was obtained from Sigma Chemical Co., St Louis, Mo.

NMR Spectroscopy. All structural studies of complexes by ^{1}H-NMR were recorded on Varian VXR-500 spectrometer with 32 K computer memory operating at 499.8 MHz. DMSO-d_6 (Aldrich Chemical Co.) was used as external reference with a signal at 3.03 ppm relative to TMS (at 0.0 ppm) for ^{1}H-NMR spectra. A 5 mm sample tube was used.

Proton decoupled ^{13}C-NMR spectra were run on a Nicolet NT-200 spectrometer operating at 50.31 MHz. DMSO-d_6 (Aldrich Chemical Co.) was used as an external reference, and was referenced at 41.010 ppm relative to DSS (0.0 ppm) for ^{13}C-NMR spectra. More than 5000 interferograms were collected, using 32 K data points. A 12 mm sample tube was used.

UV/VIS Spectroscopy. UV/VIS measurements were made on a Beckman DU-7HS spectrophotometer controlled by a built-in high speed microprocessor. The UV/VIS spectra obtained were recorded on a Beckman video copier interfaced with the spectrometer.

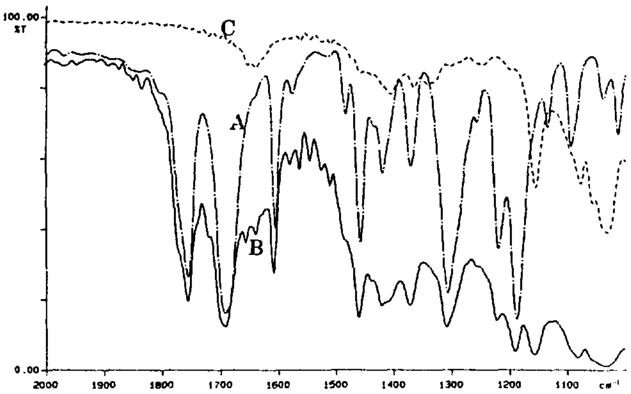


Figure 1. IR absorption spectra of (A) aspirin, (B) α-cyclodextrin-aspirin freeze dry complex, (C) α-cyclodextrin.

IR Spectroscopy. IR spectra were obtained with the Perkin-Elmer Model 1600 FT-IR spectrophotomer which included 16 K of battery-backed memory, software with extensive graphics and data processing capability, based on Perkin-Elmer's CDS-3 Infrared data system. The model 1600 can operate in single beam ratio, single beam, or interferogram mode. The IR spectral total range was from 7800-100 cm^{-1} with 4 cm^{-1} resolution. Solid samples for IR spectral analysis were prepared by the KBr (Aldrich Chemical Co.) disc method at room temperature and spectra were obtained from 4000 to 600 cm^{-1}.

Attempted Preparation of the α-Cyclodextrin Aspirin Inclusion Complex. One mmole (1.080 g) of α-cyclodextrin was dissolved in 20 m*l* of double distilled water at 35℃. An equimolar amount of aspirin (0.18015 g) was added to this solution. This mixture was stirred, and kept at 5℃ overnight. The precipitated crystals were filtered, washed with a small amount of water and ether, then dried in vacuum at 70℃ for 15 hours. This compound was not an aspirin α-cyclodextrin complex. Free aspirin could be seen from IR and NMR spectra. Freeze drying was tried in attempt to form a α-cyclodextrin aspirin inclusion complex. The IR spectrum of the freeze dried product is shown in Figure 1. The

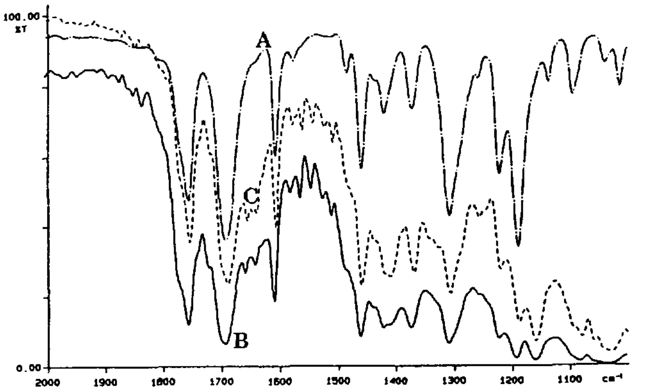


Figure 2. IR absorption spectra of (A) aspirin, (B) α-cyclodextrin-aspirin complex by freeze dry, (C) α-cyclodextrin-aspirin adsorption complex.

spectrum did not indicate any significant changes from that of the adsorption complex of aspirin and α-cyclodextrin.

Preparation of the α-Cyclodextrin Aspirin Adsorption Complex. Aspirin (0.5 mmole) was dissolved in 5 m*l* of chloroform at room temperature. An equimolar amount of α-cyclodextrin was added to form a suspension. The chloroform was removed completely under vacuum, yielding an adsorption complex of cyclodextrin and aspirin. The IR spectrum of this complex is shown in Figure 2. Detailed analysis of these spectral data is in the Results and Discussion section.

Structural Studies of the Aspirin Complex with α-Cyclodextrin in Solution State. The structure of aspirin complexed with α-cyclodextrin in solution state under an alkaline pH condition was studied by ^{1}H-NMR and ^{13}C-NMR in order to correlate physical structure with future kinetic studies. Aspirin complexed with equimolar cyclodextrin was dissolved in 0.2 M pD 11.4, deuterated phosphate buffer for ^{1}H-NMR studies. All samples were prepared at the same concentration (10 mM) to avoid any interference due to concentration differences. For ^{13}C-NMR studies a DMSO and D_2O (1 : 5) mixture was used as the solvent system.

Effect of π-π Interaction on the Aspirin Aromatic Proton Chemical Shifts. Chemical shifts of the aspirin aromatic protons under various concentrations were measured by ^{1}H-NMR to study changes in π-π interactions. Different aspirin concentrations, 15 mM, 10 mM, 5 mM, were prepared in a 0.5 m*l* phosphate buffer. The chemical shifts changes of aromatic protons against the concentration of aspirin are shown in Table 2.

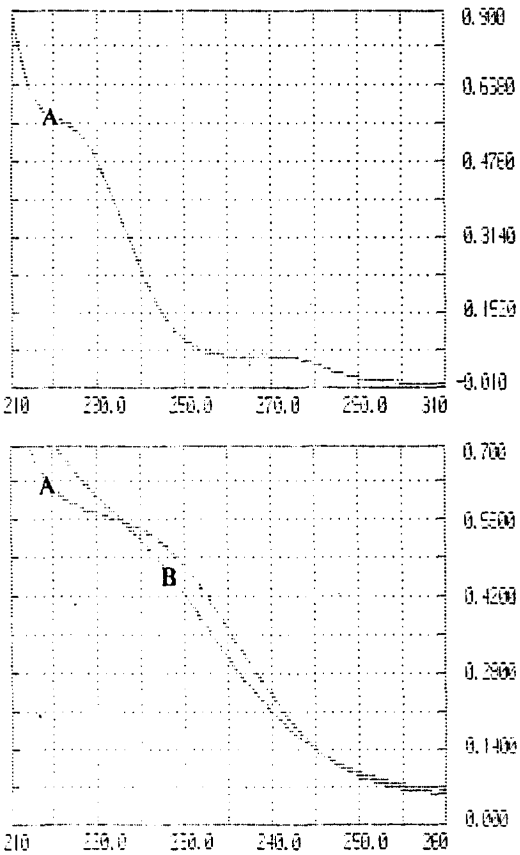


Figure 3. UV absorption spectra of (A) aspirin and (B) α-cyclodextrin-aspirin complex.

Results and Discussion

Infrared and Ultraviolet Spectroscopy. The structure of α-cyclodextrin-aspirin complex was studied by IR and UV spectroscopy. A solid aspirin and α-cyclodextrin complex could not be prepared by the precipitation co-crystallization method due to a large difference in their solubilities. Therefore the preparation of aspirin-α-cyclodextrin inclusion complex was attempted by the freeze drying method, but this complex did not show any significant difference from the adsorption complex and aspirin alone (Figures 1, 2, Table 1).

In the X-ray study of aspirin crystals, the carboxyl groups are dimerized[6] by hydrogen bonding. The shift of the carboxyl carbonyl stretching band to lower frequency implies the dissociation of the dimer resulting in the complexation state with α-CD.

Table 1. Infrared Spectral Analysis of Aspirin before and after Complexation with α-Cyclodextrin, with KBr

	Acetoxyl carbonyl C=O stretching	Carboxyl carbonyl C=O stretching	Aromatic C=C stretching
Aspirin	1753.2 (s)	1692.0 (s)	1605.5 (s)
Aspirin-α-cyclodextrin adsorption complex	1753.2 (s)	1687.4 (s)	1605.8 (s)
Aspirin-α-cyclodextrin freeze drying complex	1753.1 (s)	1688.4 (s)	1606.3 (s)

s: strong

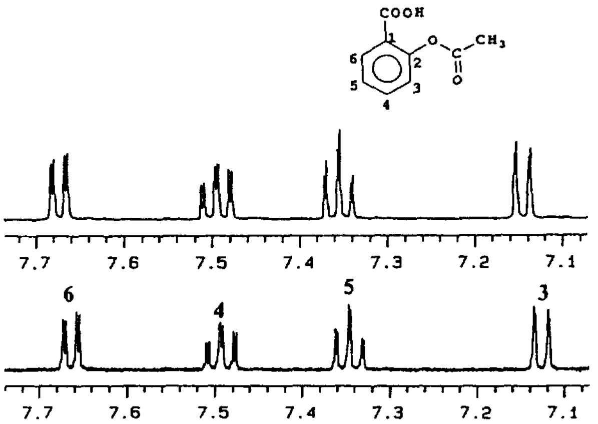


Figure 4. 500 MHz ^{1}H-NMR spectra of aspirin (bottom) and aspirin-α-cyclodextrin.

Table 2. Aromatic Protons Chemical Shifts Changes of Aspirin under Various Concentrations (in Hz)

Protons	Concentrations		
	0.015 M	0.01 M	0.005 M
H_6	1610.82	1611.27	1613.14
H_4	1547.72	1548.98	1551.32
H_5	1497.63	1498.70	1504.00
H_3	1454.39	1455.58	1457.92

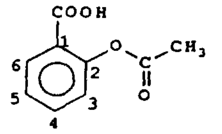
In the UV spectrum in 0.2 M phosphate buffer at pH 11, the absorption showed only a shoulder between 210 and 260 nm and disappeared when an equimolar quantity of α-cyclodextrin was present (Figure 3).

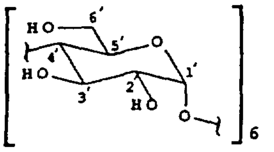
^{1}H-NMR Spectroscopy. The 500 MHz ^{1}H-NMR spectrum of the α-cyclodextrin-aspirin complex in alkaline solution showed that 1 : 1 complex was present. Significant downfield shifts of the aspirin protons after complexation was observed (Figure 4). To demonstrate that the downfield shift of the phenyl protons were a result of the absence of π-π interactions in the complex, the aromatic proton chemical shifts were measured under various aspirin concentration (Table 2).

As the concentration of aspirine increased, the aromatic proton resonance shifted upfield. This phenomenon may result from the anisotropic ring current effect due to aromatic ring stacking at a high aspirin concentration. However, the ring current effect can only partially account for the downfield shift. The shift must mainly come from the changes of the dielectric environment and/or the induced dipole-dipole interactions due to complexation.

Compared to the CH_3 protons, larger chemical shift changes of C_3-H, C_5-H and C_6-H protons of the aromatic ring of aspirin were observed after complexation with α-cyclodextrin. The 500 MHz ^{1}H-NMR spectra were analyzed with the Raccoon spin simulation program and computer-simulated spectra were generated as the final test of the chemical shift assignments and coupling constant. The 500 MHz ^{1}H-NMR chemical shift and coupling constant data for α-cyclodextrin and α-cyclodextrin-aspirin 1 : 1 complex are presented in Table 3 and Figure 5. The real and simulated spectra are shown in Figure 6, 7 and Table 4.

Table 3. 500 MHz ^{1}H-NMR Chemical Shift of Aspirin before and after Complexation with α-Cyclodextrin in pD 11.4 Phosphate Buffer (DMSO-d_6 was used as an external references)





Protons	α-CDX Aspirin complex(ppm)	α-CDX (ppm)	Aspirin (ppm)	Difference (Hz)
CH_3	2.3170		2.3150	1.0
3	7.1460		7.1260	10.0
4	7.4960		7.4930	1.5
5	7.3560		7.3460	5.0
6	7.6740		7.6635	5.3
1'	5.0385	5.0425		−2.0
2'	3.6108	3.6185		−3.9
3'	3.9470	3.9720		−12.5
4'	3.5670	3.5730		−3.0
5'	3.8260	3.8280		−1.0
6_a'	3.8985	3.8970		0.8
6_b'	3.8510	3.8530		−1.0

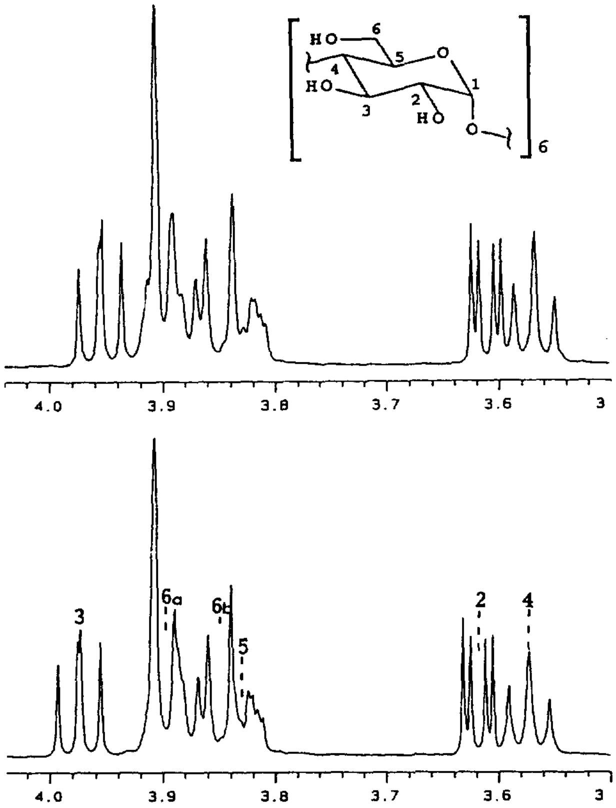


Figure 5. 500 MHz ^{1}H-NMR spectra of α-cyclodextrin (bottom) and α-cyclodextrin-aspirin complex (top).

The upfield shifts observed for H-3' may be due to the aromatic ring current effect (Figure 5). The magnitudes of H-3' (−12.5 Hz) and H-5' (−1.0 Hz) chemical shift changes

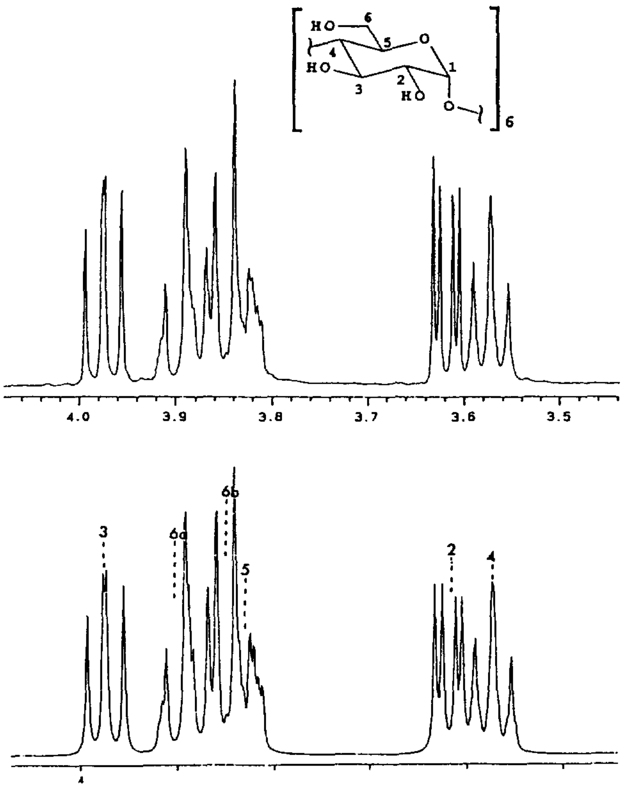


Figure 6. 500 MHz ^{1}H-NMR spectrum of α-cyclodextrin (top) and its spin simulated spectrum (bottom).

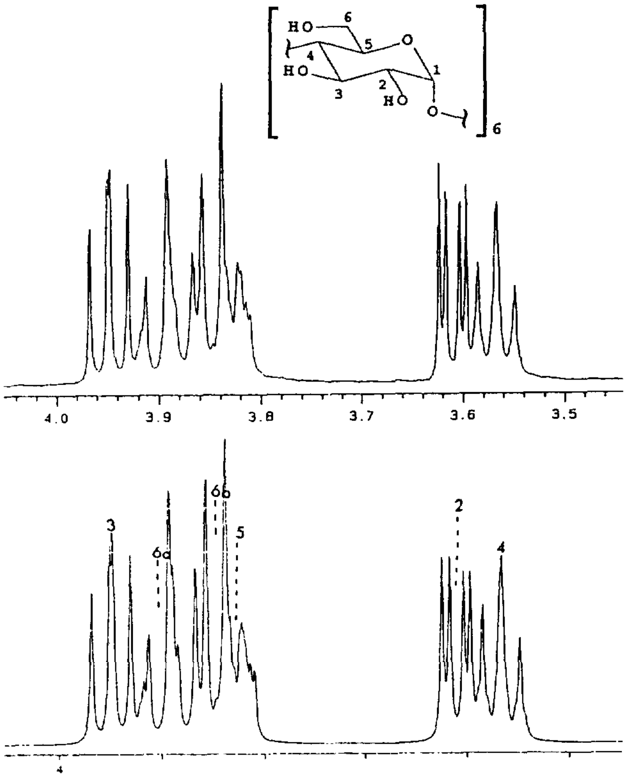
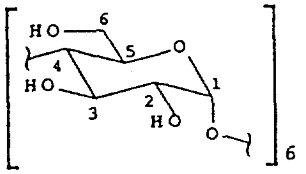


Figure 7. 500 MHz ^{1}H-NMR spectrum of α-cyclodextrin-aspirin complex (top) and its spin simulated spectrum (bottom).

Table 4. 500 MHz ^{1}H-NMR Computer-Simulation Spectra Data for α-Cyclodextrin and α-Cyclodextrin-Aspirin Complex



Protons	α-CDX	α-CDX-Aspirin
		Coupling constant (Hz)
J_{12}	3.5	3.5
J_{15}	−0.7	−0.7
J_{23}	10.2	10.2
J_{34}	8.6	8.6
J_{45}	10.3	10.3
J_{46a}	−0.3	−0.3
J_{46b}	−0.7	−0.7
J_{56a}	2.1	2.2
J_{56b}	4.7	5.0
J_{6a6b}	−12.1	−12.4

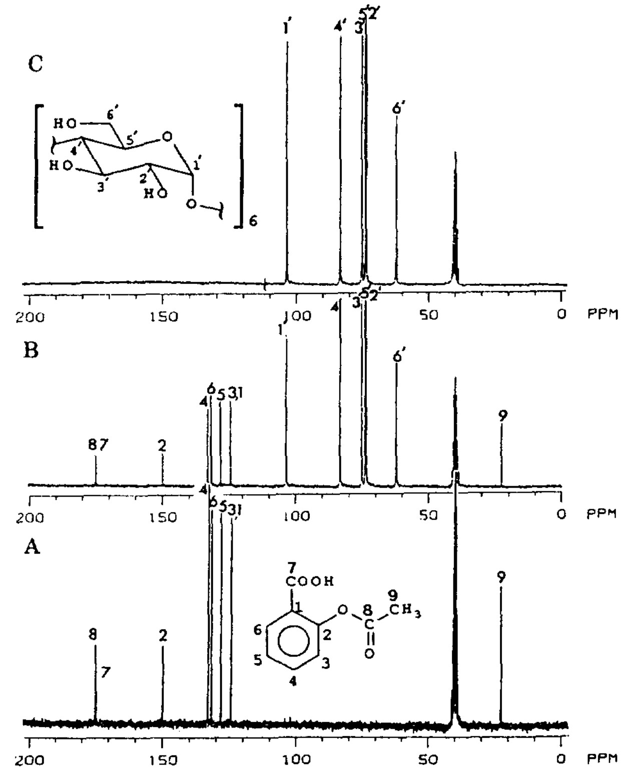
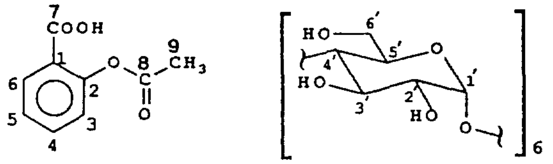


Figure 8. 50.3 MHz ^{13}C-NMR spectra of (A) aspirin, (B) α-cyclodextrin-aspirin complex, and (C) α-cyclodextrin.

indicated that the phenyl ring could penetrate into the broader end of α-cyclodextrin cavity. The phenyl ring could not penetrate deep enough to shield H-5′ protons due to samll diameter of the α-cyclodextrin cavity.

^{13}C-NMR Spectroscopy. The structure of the α-cyclodextrin aspirin complex in the solution state was studied by solution ^{13}C-NMR (Figure 8, Table 5). Overall, the ^{13}C-

Table 5. 50.43 MHz ^{13}C-NMR Carbon Chemical Shifts of Aspirin before and after Complexation with α-Cyclodextrin in 83% D_2O, 0.1 M Phosphate buffer pD 7.4 and 17% DMSO-d_6



Carbons	α-CDX 1 : 1 complex (ppm)	α-CDX (ppm)	Aspirin (ppm)	Difference (Hz)
8	174.495		174.601	−5.33
7	174.350		174.326	1.18
2	149.411		149.427	−0.79
1	132.705		132.691	0.71
4	132.627		132.691	−3.25
6	131.408		131.446	−1.88
5	127.913		127.933	−0.96
3	124.115		124.119	−0.18
9	22.242		22.241	0.05
1'	103.119	103.104		0.77
4'	82.975	82.970		0.29
3'	74.850[a]	74.846[b]		0.17[c]
5'	73.589[a]	73.563[b]		1.31[c]
2'	73.157[a]	73.128[b]		1.48[c]
6'	61.858	61.837		1.04

[a,b] These assignment might be interchangeable. [c] The differences are highly tentative because of the uncertainties in the chemical shift assignments.

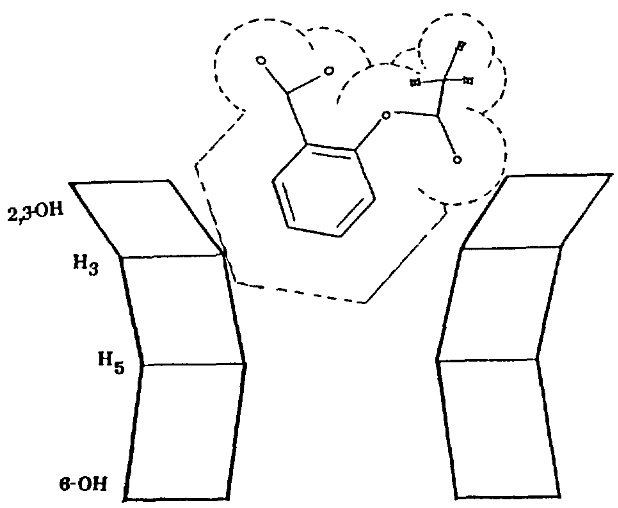


Figure 9. Proposed structure of aspirin and α-cyclodextrin complex in the solution state.

NMR spectral changes were much smaller than the ^{1}H-NMR spectral changes. As was discussed in the previous section, the included lead (head) carbons are largely shielded compared to the tail carbons[7,8]. These upfield shifts have been attributed to the changes of the electric environment of aqueous solvent (high dielectric constant) to that of the nonpolar cavity (low dielectric constant)[7]. Slightly larger upfield shift of the C_4-carbon in the inclusion complex as compared with C_1-carbon was observed (Table 5). Also the acetoxy carbonyl carbon showed a large upfield chemical shift change due to intermolecular interactions with α-cyclodextrin.

These spectral data suggested that the phenyl ring of aspirin might be partially included into the α-cyclodextrin cavity with the para carbon (C_4) of aspirin which was included deepest in the cavity.

Proposed Structure of α-Cyclodextrin-Aspirin in Solution State. The ^{1}H-NMR and ^{13}C-NMR spectral data indicated that the phenyl ring aspirin is the leading group inserted into the center of α-cyclodextrin cavity from broader end. The magnitude of the upfield shifts of these H-3, H-5 on α-cyclodextrin allowed to estimate the position of the aromatic ring center in the cavity based on the anisotropic ring-current effect using the plots calculated by Komiyama and Hirai[9]. In this inclusion complex, the center of the aromatic ring is approximately 2 Å above the H-3 plane of α-cyclodextrin and the proposed structure is shown in Figure 9.

In summary, this research shows that aspirin forms an inclusion complex with the hydrophobic cavity of α-cyclodextrin in the solution state but not in the solid state, while aspirin forms a state inclusion complex with the hydrophobic cavity of β-cyclodextrin in both the solution and the solid state[5].

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Synthesis of Amphiphilic Poly(alkyl methacrylate-*b*-methacrylic acid) by Group Transfer Polymerization and Selective Hydrolysis

Soon-Ki Kwon*, Weon-Jung Choi, Yun-Hi Kim, and Sam-Kwon Choi

**Department of Polymer Science and Engineering, Kyeongsang National University, Jinju 660-701*
Department of Chemistry, Korea Advanced Institute of Science and Technology,
P.O. Box 150, Cheongryangri, Seoul 130-650.

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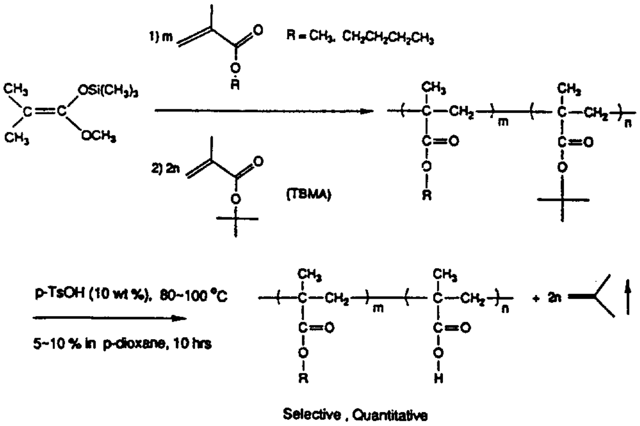
Several poly(alkyl methacrylate-*b*-*t*-butyl methacrylate) diblock copolymers were synthesized by group transfer polymerization. The molecular weight of poly(*t*-butyl methacrylate) segments and the composition of the resulting block copolymers were controlled by the monomer feed ratios and mole ratios of monomer to initiator. The poly(*t*-butyl methacrylate) block was quantitatively hydrolyzed to poly(methacrylic acid) block by refluxing with a catalytic amount of *p*-toluenesulfonic acid in dioxane at 100℃ for 12 hrs. The thermogravimetric analysis of poly(alkyl methacrylate-*b*-*t*-butyl methacrylate) exhibited the lose of isobutylene and subsequent anhydride formation in the range of 205-310℃.

Introduction

Since Webster *et al.*, have announced a new method of a living polymerization termed "group transfer polymerization (GTP)", it has been a considerable interest to many research groups due to superiority to other polymerization methods.[1] We had reported a difunctional initiator for GTP and the novel synthesis of acrylic based triblock copolymers.[2]

Block copolymers containing hydrophobic and hydrophilic segments are of interest as amphiphilic materials which are used as detergent, compatibilizer, and surface modifier etc.[4-6] Direct synthesis of polymers with well-defined structure containing hydroxyl or carboxyl group functionality is quite difficult because the termination in anionic polymerization was caused by the active hydrogen of related monomers. One of the possible means of circumventing this difficulty is to introduce a protecting group to the monomer, masking reactive site. One of the synthetic methods for amphiphilic block copolymer is to copolymerize a protected monomer and a hydrophobic monomer and hydrolyze the resulting block copolymer selectively. Although various block copolymers had been reported,[4-7] there is little report on acrylic based amphiphilic block copolymer. Recently, we reported the synthesis and properties of the novel amphiphilic triblock copolymers.[3]

This article deals with the preparation of poly(alkyl methacrylate-*b*-*t*-butyl methacrylate) (PAMA-*b*-PTBMA) by GTP and amphiphilic poly(alkyl methacrylate-*b*-methacrylic acid) (PAMA-*b*-PMAA) by selective hydrolysis of the block copolymer. The characterization of the resulting amphiphilic block copolymer will be discussed.



Scheme 1.

Experimental

Materials and Instruments. Solvents were purified by normal procedure and handled under moisture free atmosphere. The polymerization solvent, tetrahydrofuran (THF) was distilled over sodium and benzophenone immediately prior to use.

Methyl methacrylate (MMA) and *n*-butyl methacrylate (BMA) (Aldrich) were purified by distillation over calcium hydride. TBMA was prepared from the reaction of methacry-

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*To whom correspondence should be addressed.