약제학회지(1991), 제21권 제4호
J. Kor. Pharm. Sci., Vol. 21, No. 4, 223-230(1991)

Structure Study of Inclusion Complex of β-Cyclodextrin and Aspirin

Hee-Sook Choi
Department of Medicinal Chemistry, Purdue University, IN. 47907, USA
(Received October 10, 1991)

β-시클로덱스트린과 아스피린의 포접화합물의 구조에 관한 연구

최 희 숙*
퍼듀대학교 약학대학
(1991년 10월 10일 접수)

The structural specificity and the chemical dynamics between β-cyclodextrin and aspirin were studied by FT-IR, UV, ^{1}H NMR, ^{13}C NMR, and FAB-MS spectroscopy in solution and solid state. A stable solid inclusion complex was prepared by the recrystallization method. From the spectral changes of the host and guest molecules, orientational preference for binding in the cyclodextrin cavity was determined.

Keywords—β-cyclodextrin, aspirin, complex.

Aspirin (acetylsalicylic acid), the most commonly used salicylate, is employed as an analgesic-antipyretic and as an effective nonsteroidal antiinflammatory agent.[1-4] The most common side effects from aspirin are gastrointestinal distrubances.

Cyclodextrin forms inclusion complexes with many kinds of molecules.[5,6] Inclusion compounds of selected cyclodextrin have been studied extensively in recent years with regard to their structure, conformation and use in enzyme modeling.

In order to design better aspirin dosage form, the structure of β-cyclodextrin and aspirin inclusion complex was determined in this study.

Materials and Methods

Materials

β-Cyclodextrin was obtained from Chemical Dynamic Corp., South Plainfield, NJ. Aspirin was obtained from Sigma Chemical Co., St. Louis, MO in U.S.A.

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. The spectra were measured with 11.2 μsec (90°) pulse width and 5 seconds repetition time. 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. A pulse width of 11 μsec (45°) and a pulse delay of four seconds were utilized. DMSO-d_6 (Aldrich Chemical Co.) was used as an

*Current address: Ewha Womans University, Seoul 120-750

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.

Solid-state ^{13}C NMR spectra were measured on a Brucker CXP-100 spectrometer with cross-polarization/magic angle spinning mode operating at 25.2 MHz. The spinning speeds ranged from 3.2 to 3.6 kHz, which was sufficient to suppress most unwanted spinning side bands. All chemical shifts are expressed externally referenced to the ^{13}C resonance of Me_4Si. The actual referencing material used was hexamethylbenzene, either periodically mixed with the sample or run separately in the rotor prior to running the sample alone. The high-field peak of solid hexamethylbenzene is assumed to be at 17.6 ppm downfield from Me_4Si. The cross-polarization contact times ranged from 1.25 to 3.0 ms and the recycle times from 1 to 2 s. These were determined experimentally for the best sensitivity results for each sample. The dipolar-dephased data were obtained by using a 50 μs delay.

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.

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}.

Mass Spectrometry

Fast atom bombardment(FAB) mass spectra were obtained with a Kratos MS-50 sector mass spctrometer utilizing 3:1 dithiothreitol/dithioerythritol as the matrix. An accelerating voltage of 8 kV and a 100 sec/dec scanning rate were used for the experiments.

Preparation of the β-Cyclodextrin Aspirin Inclusion Complex

One mmole(1.351 g) of β-cyclodextrin was dissolved in 68 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 complex was filtered and washed with a small amount of water and ether. The crystalline β-cyclodextrin-aspirin complex was dired in vacuum at 70℃ for 15 hours to yield 1.14 g 75%; mp 275℃.

The 470 MHz ^1H NMR spectral integration showed that this was a 1:1 complex of β-cyclodextrin and aspirin.

Prepartion of the β-Cyclodextrin-Aspirin Adsorption Complex

Aspirin (0.5 mmole) was dissolved in 5 m*l* of chloroform at room temperature. An equimolar amount of β-cyclodextrin was added to this solution. β-cyclodextrin did not dissolve in chloroform under these conditions and therefore, this mixture was a suspension. The chloroform was removed completely under vacuum and the remaining solid was the adsorption complex of β-cyclodextrin and aspirin.

Structural studies of the Aspirin Complex with β-cyclodextrin in solution state

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

Results and Discussion

Infrared and Ultraviolet Spectroscopy

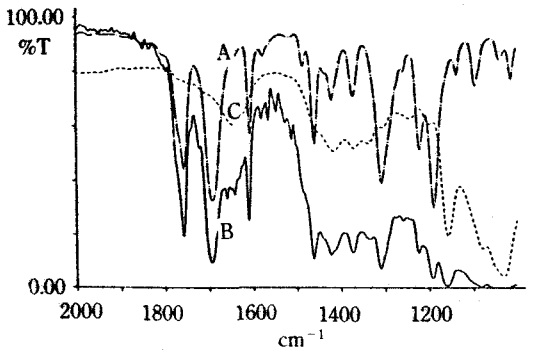


Figure 1—IR absorption spectra of (A) aspirin, (B) aspirin-β-cyclodextrin adsorption complex, (C) β-cyclodextrin.

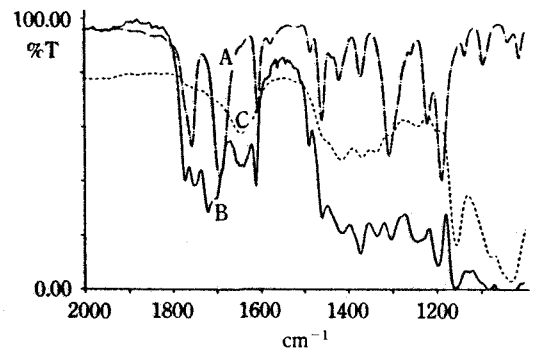


Figure 2—IR absorption spectra of (A) aspirin, (B) aspirin-β-cyclodextrin inclusion complex (by recrystalization), (C) β-cyclodextrin.

The FT-IR KBr disc spectra of the aspirin, aspirin-β-cyclodextrin inclusion complex and aspirin-β-cyclodextrin adsorption complex are summarized in Fig. 1, 2 and Table I. The wavenumber of the aspirin-acetoxyl group of β-CDX-aspirin complex also shifted to smaller wavenumber from the free acetoxyl carbonyl stretching band. In the x-ray study of aspirin crystals, the carboxyl groups are dimerized[7] by hydrogen bonding. Using conventional IR methods, Nakai *et al.*[8] observed marked shifts to larger wavenumber for the carboxyl carbonyl stretching band, indicating dissociation of the dimer. This difference can be attributed to the intermolecular hydrogen bond formation with the hydroxyl groups of β-cyclodextrin as proton donor.

Our FT-IR results showed the splittings of the

Table I—*Infrared spectral analysis of aspirin before and after complexation with β-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-β-cyclodextrin adsorption complex	1752.7(s)	1691.3(s)	1606.1(s)
Aspirin-β-cyclodextrin recrystalization complex	1767.3(s) 1750.3(s)	1717.0(s) 1701.0(s)	1607.4(s)

a: strong, m: medium

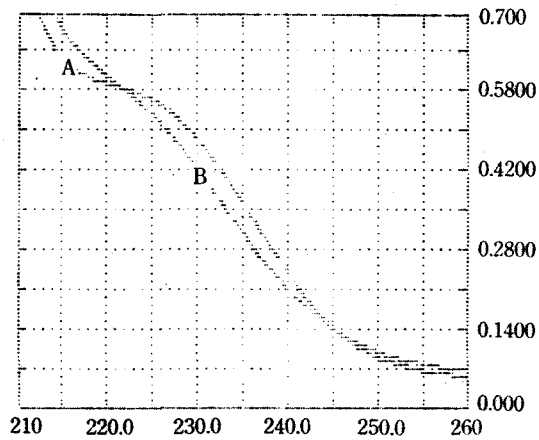


Figure 3—UV absorption spectra of (A) aspirin and (B) β-cyclodextrin-aspirin complex in 0.2 M phosphate buffer (pH 11).

carbonyl peaks into two peaks, suggestive of the possible existence of two conformers for the carboxyl and acetoxyl carbonyl groups. Nakai *et al.*[8] observed the IR spectra of aspirin and β-cyclodextrin complex in dilute CCl_4 solution and solid ground mixtures of those compounds. In their solid inclusion complex, acetonyl C=O band and carboxyl C=O band are observed at 1774 cm^{-1} an 1724 cm^{-1}, respectively. Also the carbonyl bands did not show any splittings and it is different from our inclusion complex results.

Fig. 3 shows the UV spectrum of aspirin with and without β-cyclodextrin in 0.2 M phosphate buffer at pH 11. The UV absorption showed only a shoulder between 210 and 260 nm and disap-

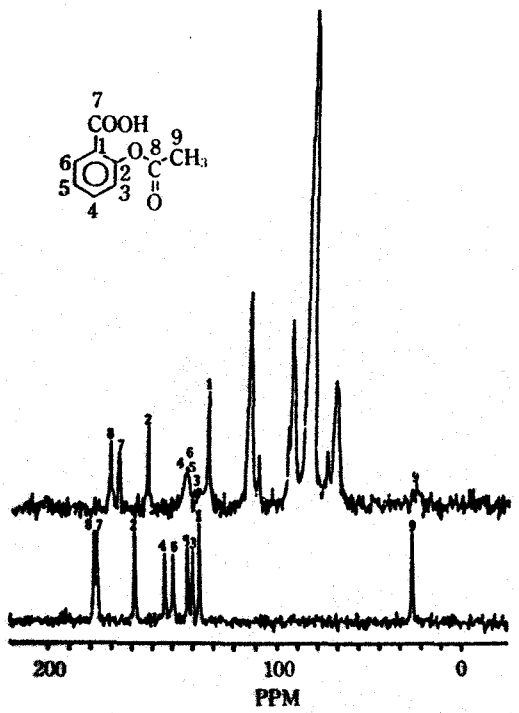
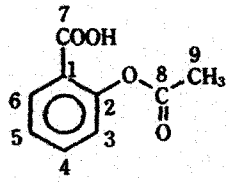


Figure 4—25 MHz CP/MAS solid state ^{13}C NMR spectra of aspirin (bottom) and aspirin-β-cyclodextrin complex (top).

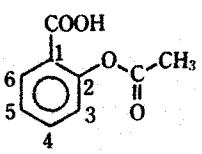
Table II—*Solid state ^{13}C NMR spectra of aspirin and β-cyclodextrin-aspirin complex*

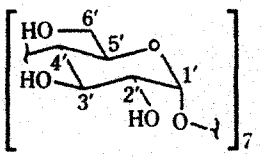


Carbon	Aspirin	β-cyclodextrin -aspirin	Difference in ppm
C_8	178.59 ppm	170.43 ppm	−8.16 ppm
C_7	177.13 ppm	166.43 ppm	−10.70 ppm
C_2	159.32 ppm	152.70 ppm	−6.62 ppm
C_4	144.99 ppm	134.12 ppm	−10.87 ppm
C_6	141.08 ppm	134.12 ppm	−6.95 ppm
C_5	134.28 ppm	134.12 ppm	−0.16 ppm
C_1	128.45 ppm	124.17 ppm	−4.28 ppm
C_3	131.75 ppm	128.45 ppm	−3.30 ppm

peared to short wavelength when an equimolar quantity of β-cyclodextrin was present. This shift

Table III—*500 MHz ^1H NMR chemical shift of aspirin before and after complexation with β-cyclodextrin in pD 11.4 phosphate buffer (DMSO-d_6* was used as an external reference)





Protons	β-CDX Aspirin (ppm)	β-CDX (ppm)	Aspirin (ppm)	Difference (Hz)
CH_3	2.3200		2.3150	2.5
3	7.1146		7.1260	−5.7
4	7.4915		7.4930	−0.8
5	7.3365		7.3460	−4.8
6	7.6930		7.6635	14.8
1′	5.0520	5.0575		−2.8
2′	3.6280	3.6357		−3.9
3′	3.9300	3.9525		−11.3
4′	3.5650	3.5720		−3.5
5′	3.8166	3.8500		−16.7
6a′	3.8747	3.8710		1.9
6b′	3.8443	3.8510		−3.4

in UV absorption upon complex formation may be explained by a partial shielding of the excitable electrons in the cyclodextrin cavity.

Solid State ^{13}C NMR Spectroscopy

The 25 MHz CP/MAS solid state ^{13}C NMR spectra of aspirin and aspirin-β-cyclodextrin complex are shown in Fig. 4 and the chemical shift differences are summarized in Table II. The peak assignments of solid state ^{13}C NMR spectra were based on the solution spectral data. The large upfield shift of the C_7 carbon indicated an absence of the dimeric hydrogen bonding. Also the large upfield shift of the C_8 carbon can be explained by intermolecular interactions with β-cyclodextrin. All protonated carbons C_3, C_4, C_5 and C_6 showed line broadening resulted from the ineffective proton dipolar decoupling due to the rapid molecular motion of the aromatic ring within the cavity.[9]

Solid state ^{13}C NMR, and FT-IR spectral changes provided strong evidence for an inclusion complex formation between aspirin and the hyd-

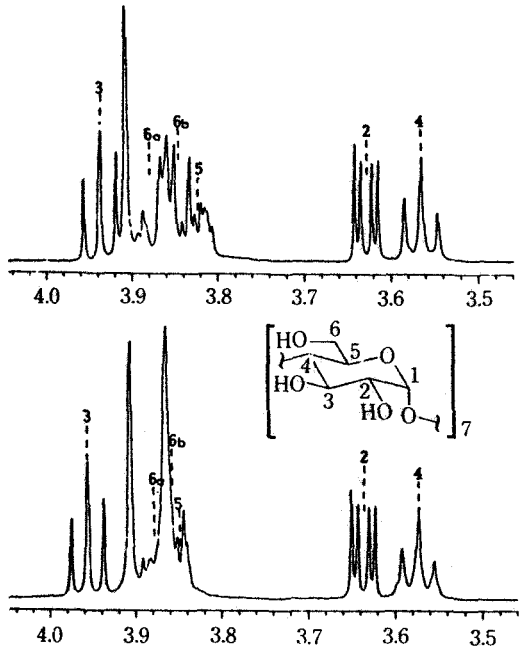


Figure 5—500 MHz ^{1}H NMR spectra of β-cyclodextrin-aspirin complex (top) and β-cyclodextrin (bottom).

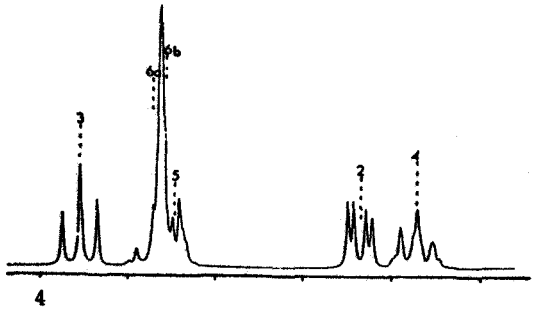


Figure 6—500 MHz ^{1}H NMR spectrum of β-cyclodextrin (top) and its spin simulated spectrum (bottom).

rophobic cavity of β-cyclodextrin in solid state.

^{1}H NMR Spectroscopy

The structure of β-cyclodextrin-aspirin complex in solution state was studied by 500 MHz ^{1}H NMR spectroscopy. Integration of ^{1}H NMR spectrum of the β-cyclodextrin-aspirin complex in alkaline solution showed a 1:1 complexation. The ^{1}H NMR chemical shift changes are presented in Table III and Fig. 5. Chemical shifts and coupling constants of β-cyclodextrin and its aspirin complex spectra were obtained by the Raccoon spin simulation

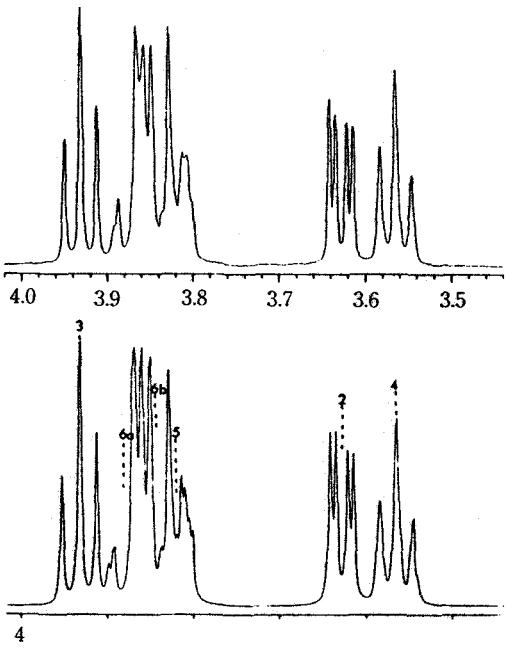


Figure 7—500 MHz ^{1}H NMR spectrum of β-cyclodextrin-aspirin (top) and its spin simulated spectrum (bottom).

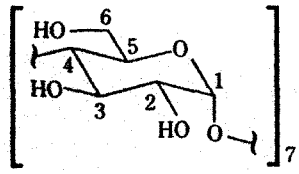
program. These seven-spin spectra are shown in Fig. 6, 7 and the data are summarized in Table IV. In comparing the spectra of aspirin and the β-CDX-aspirin inclusion complex, a downfield shift of C_6-H, and CH_3, and an upfield shift of C_3-H and C_5-H aspirin protons were observed in the inclusion complex spectrum Fig. 8. These shifts are probably due to the modulation of the coplanarity between the carboxyl and phenyl groups. The upfield shift of H-3′, and H-5′, can also be explained by the ring current effect.

The magnitudes of H-3′(−11.3 Hz) and H-5′ (−16.7 Hz) chemical shift changes indicating that the phenyl ring could penetrate into the wider end of the β-cyclodextrin cavity. Also due to larger diameter of the β-cyclodextrin cavity, the phenyl ring can penetrate deep enough to shield H-5′ protons.

^{13}C NMR Spectroscopy

Solution state ^{13}C NMR spectroscopy was used to further study the solution state structure of the β-cyclodextrin aspirin complex. These spectra are shown in Fig. 9 and spectral data are summa-

Table IV—*500 MHz ^1H NMR computer-simulated spectra data for β-cyclodextrin and β-cyclodextrin-aspirin complex*



protons	β-CDX	β-CDX-Aspirin
	chemical shift (ppm)	
1	5.0575	5.0520
2	3.6357	3.6280
3	3.9525	3.9300
4	3.5720	3.5650
5	3.8500	3.8166
6a	3.8710	3.8747
6b	3.8510	3.8443
	coupling constant (Hz)	
J_{12}	3.5	3.6
J_{15}	−0.7	−0.6
J_{23}	10.2	10.1
J_{34}	9.8	9.9
J_{45}	10.0	10.0
J_{46a}	−0.7	−1.0
J_{46b}	−0.7	−1.0
J_{56a}	2.2	0.9
J_{56b}	5.0	5.2
J_{6a6b}	−13.3	−13.6

rized in Table V. The larger upfield shift of C_5-carbon with relative to C_1 and C_4 carbons indicated that this carbon is located at inside end of the cavity. The C_7 and C_8 carbons also showed an upfield shift, which can be explained by intermolecular steric interactions with β-cyclodextrin.

Mass Spectrometry

The aspirin-β-cyclodextrin inclusion complex and aspirin-β-cyclodextrin adsorption complex were studied by fast atom bombardment mass spectrometry (Fig. 10). The objective of this study was to determine if FAB mass spectrometry could be used to monitor a solid inclusion complex formation. In the inclusion complex, the m/z of 1315 and 1353 could be assigned to the pseudomolecular ion of the β-cyclodextrin-aspirin adduct ($M^+ + H^+$) and its potassium adduct, respectively. None of these peaks appeared in the adsorption

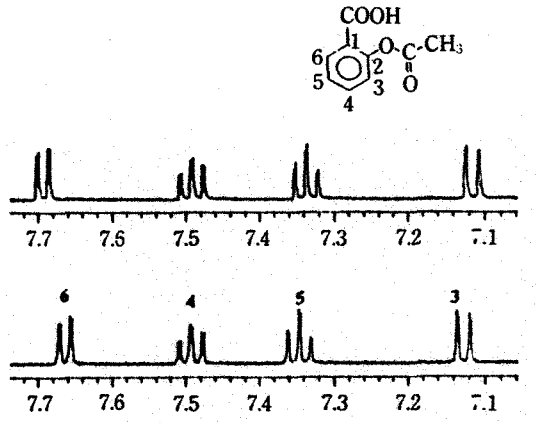


Figure 8—500 MHz ^1H NMR spectra of aspirin (bottom) and its β-cyclodextrin complex (top).

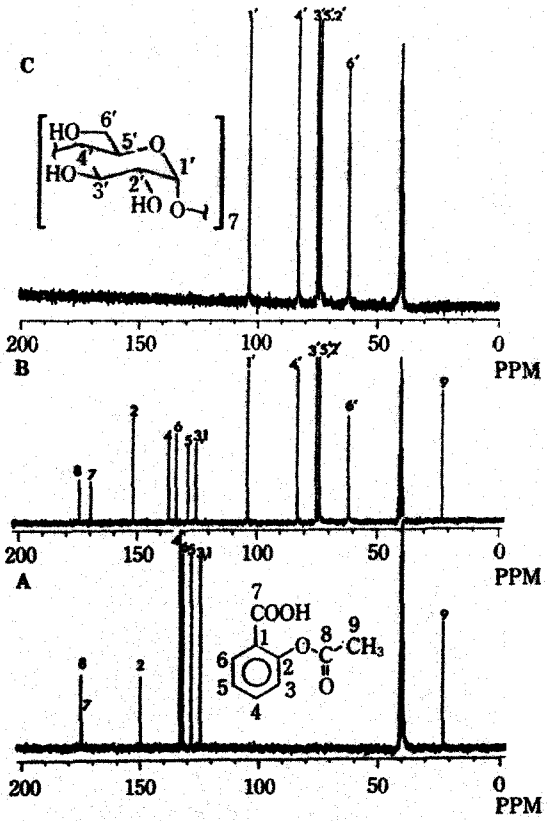
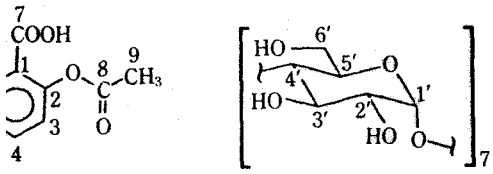


Figure 9—50.3 MHz ^{13}C NMR spectra of (A) aspirin, (B) β-cyclodextrin-aspirin complex, and (C) β-cyclodextrin.

: V—50.43 MHz ^{13}C NMR carbon chemical shifts
)irin before and after complexation with β-cyclode-
in 83% D_2O, 0.1 M phosphate buffer pD 7.4 and
DMSO-d_6.



ons	β-CDX Aspirin 1;1 Complex (ppm)	β-CDX (ppm)	Aspirin (ppm)	Difference (Hz)
	174.511		174.601	−4.56
	174.205		174.326	−6.09
	149.500		149.427	3.71
	132.657		132.691	−1.72
	131.505		131.446	2.79
	127.859		127.933	−3.60
	124.079		124.119	−1.98
	124.079		124.119	−1.98
	22.245		22.241	0.18
	103.506	103.506		0
	82.495	82.702		−10.40
	74.720	74.734		−0.68
	73.548 a	73.521 b		1.40 c
	73.364	73.521		−7.89
	61.491	61.681		−9.57

These assignments might to interchangeable
he differences are highly tentative because of the
rtainties in the chemical shift assignments.

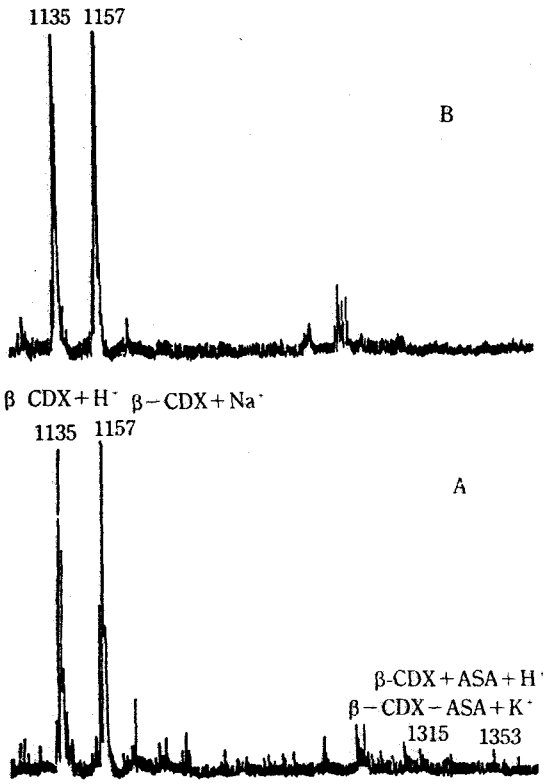


Figure 10—FAB mass spectra of (A) aspirin-β-cyclodextrin complex and (B) aspirin-β-cyclodextrin adsorption complex.

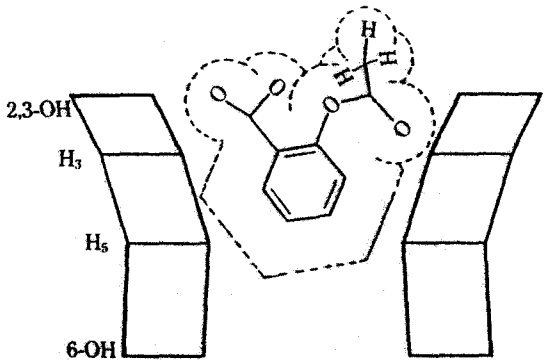


Figure 11—Proposed structure of aspirin and β-cyclodextrin complex in the solution state.

iplex. Therefore, FAB mass spectrometry is a ful method to determine solid inclusion comp- formation.

Proposed Structure of β-Cyclodextrin-Aspirin In ution State

The ^1H NMR and ^{13}C NMR spectral data indi- ing that the phenyl ring of aspirin is the lea- g(head) group inserted into the center of broa- end of β-cyclodextrin cavity.

The magniture of the upfield shifts of H-3', H- on β-cyclodextrin allowed to estimate the posi- n of the aromatic ring center in the cavity.[10] this β-cyclodextrin-aspirin complex, the center the aromatic ring is approximately 2Å above : H-5' plane of β-cyclodextrin (Fig. 11).

In summary, this research shows that aspirin ms a stable inclusion complex with the hydro- obic cavity of β-cyclodextrin in both the solution and the solid state.

Acknowledgement

This research was supported partially by the

Purdue Research Foundation, Purdue University, West Lafayette, Indiana, U.S.A. and partially by Grant GMO8521-29 from the Institute of Genernal Medical Sciences of National Institutes of Health, PHS, U.S.A.

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