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Interaction of Tri-*O*-methyl-*β*-cyclodextrin with Drugs. I. Effect of Tri-*O*-methyl-*β*-cyclodextrin on the Partition Coefficients of Drugs[1]

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The partition coefficients of *p*-nitrophenol between 0.05 N HCl and $CHCl_3$ were measured with or without tri-*O*-methyl-*β*-cyclodextrin (methyl-*β*-CD) at 15.5, 20.0, and 25.0 °C. The partition coefficients increased linearly with methyl-*β*-CD concentration. In the presence of 3.00×10^{-2} M methyl-*β*-CD, the value was about 2.5 times larger than that measured in the absence of methyl-*β*-CD. Methyl-*β*-CD was not detected in the aqueous phase by polarimetry, and most of the methyl-*β*-CD molecules were present in the $CHCl_3$ phase. The increase of the partition coefficients can be interpreted in terms of 1 : 1 complex formation between methyl-*β*-CD and *p*-nitrophenol.

The complexes of methyl-*β*-CD and *p*-nitrophenol in the aqueous solution and the $CHCl_3$ solution were studied quantitatively by ultraviolet absorption measurements. The formation of inclusion complex in D_2O was also investigated by proton magnetic resonance spectroscopy.

Keywords——tri-*O*-methyl-*β*-cyclodextrin; partition coefficient; *p*-nitrophenol; UV; NMR; inclusion; complex

Tri-*O*-methyl-*β*-cyclodextrin (methyl-*β*-CD) is a permethylated *β*-cyclodextrin derivative and has some unusual physical properties compared with *β*-cyclodextrin. Methyl-*β*-CD is soluble not only in water but also in organic solvents, especially in CCl_4 and $CHCl_3$. The authors reported previously that methyl-*β*-CD interacted with some medicinals in CCl_4.[2] Since methyl-*β*-CD has complexing ability,[3] it is suggested that the addition of methyl-*β*-CD affects the partition coefficients of drugs between water and organic solvents, like polysorbate 80,[4] and influences the bioavailability of drugs.

In this study, the effect of methyl-*β*-CD addition on the partition coefficients of *p*-nitrophenol was investigated. *p*-Nitrophenol is known to form inclusion complexes with cyclodextrins in solution,[3a,5] and in the crystalline state.[6] Furthermore, the formation of the inclusion complex between methyl-*β*-CD and *p*-nitrophenol was studied by UV and PMR spectroscopies.

Experimental

Materials——Methyl-*β*-CD was synthesized by a modification of the reported procedure[7] and recrystallized twice from hot water, mp 156—158°C, $[\alpha]_D^{19}$ +160.0° ($CHCl_3$). *p*-Nitrophenol was purchased from Wako Pure Chemical Industries, Ltd. and recrystallized from $CHCl_3$. *β*-Cyclodextrin was obtained from Seikagaku Kogyo and recrystallized from water, $[\alpha]_D^{19}$ +160.0° (H_2O). Deuterium oxide (99.8%) was purchased from Nakarai Chemicals Ltd. All other materials and solvents were of anyaltical reagent grade.

Procedure for the Determination of the Partition Coefficients——Aqueous phases were prepared by dissolving *p*-nitrophenol (1.00×10^{-4}—1.00×10^{-2} M) in 0.05 N HCl. Organic phases were prepared by dissolving methyl-*β*-CD (0—3.00×10^{-2} M) in $CHCl_3$. The concentrations before distribution were denoted as $[PNP]_{init}$ and $[MCD]_{init}$, respectively. Ten ml of each solution was taken in a 50 ml glass-stoppered flask and shaken at 130 rpm for 1 h in a thermostated water bath at 15.0, 20.0, or 25.0°C. The flask was left to stand, then the aqueous phase was withdrawn and the concentration of *p*-nitrophenol was determined at 317 mm with a Hitachi 124 double-beam spectrophotometer. The partition coefficient was defined as follows:

$$\text{Partition coefficient} = \frac{[PNP]_{init} - [PNP]_{aq}}{[PNP]_{aq}}$$

where $[\mathrm{PNP}]_{\mathrm{aq}}$ indicates the concentration of *p*-nitrophenol in the aqueous phase after the distribution.

The partition of methyl-β-CD between 0.05 N HCl and $CHCl_3$ was determined by polarimetry. A Nihon Bunko DIP-SL machine was used and the lower limit of detection was of the order of 10^{-5} M.

UV Absorption Studies——The UV absorption spectra of *p*-nitrophenol were recorded at various methyl-β-CD concentrations with a Hitachi model 340 spectrophotometer.

PMR Studies——PMR spectra in D_2O were measured with a JEOL JNM-MH-100 spectrometer. Tetramethylsilane was used as an external reference.

Results and Discussion

The Effect of Methyl-β-CD on the Partition Coefficients of *p*-Nitrophenol

Table I shows the partition coefficients obtained at 25.0°C. The values increase linearly with the addition of methyl-β-CD at each initial concentration of *p*-nitrophenol. All the partition coefficients at 3.00×10^{-2} M initial concentration of methyl-β-CD are about 2.5 times larger than those in the absence of methyl-β-CD. At constant initial concentration of methyl-β-CD, the partition coefficients show little variation among samples regardless of the initial concentration of *p*-nitrophenol. After the distribution, methyl-β-CD could not be detected in the aqueous phase and was recognized to be almost wholly in the $CHCl_3$ phase. It was also confirmed that *p*-nitrophenol did not influence the distribution behavior of methyl-β-CD.

Table I. Effect of Methyl-β-CD on the Partition Coefficients of *p*-Nitrophenol at 25.0°C

Concentration of *p*-nitrophenol (M)[a]	Concentration of methyl-β-CD (M)[a]						
	0	5.00×10^{-3}	1.00×10^{-2}	1.50×10^{-2}	2.00×10^{-2}	2.50×10^{-2}	3.00×10^{-2}
1.00×10^{-4}	1.83±0.02[b]	2.28±0.03	2.78±0.05	3.23±0.05	3.68±0.05	4.12±0.05	4.52±0.05
6.00×10^{-4}	1.86±0.05	—	2.81±0.06	—	3.83±0.02	—	4.79±0.06
1.00×10^{-3}	1.83±0.02	—	2.82±0.05	—	3.83±0.02	—	4.77±0.03
2.00×10^{-3}	1.84±0.04	—	2.79±0.04	—	3.81±0.02	—	4.75±0.05
4.00×10^{-3}	1.85±0.05	—	2.80±0.03	—	3.79±0.06	—	4.74±0.04
1.00×10^{-2}	1.83±0.02	—	2.75±0.06	—	3.73±0.03	—	4.76±0.06

a) Initial concentration.
b) Standard deviation (*n*=5).

Figure 1 shows plots of the partition coefficients *versus* the initial concentration of methyl-β-CD at 15.5, 20.0, and 25.0°C at constant initial concentration of *p*-nitrophenol (1.00×10^{-4} M). At each temperature, an approximately linear relationship exists between the partition coefficients and the initial concentration of methyl-β-CD, and the effect of methyl-β-CD addition is more significant at lower temperature than at higher temperature. This suggests that, in the $CHCl_3$ phase, decreasing temperature enhances the interaction between methyl-β-CD and *p*-nitrophenol. The stability constants of the complex between methyl-β-CD and *p*-nitrophenol were determined to be 68, 60, and 51 M^{-1} at 15.5, 20.0, and 25.0°C, respectively (the initial concentration of methyl-β-CD was 2.00×10^{-2} M).

As methyl-β-CD was not detected in the aqueous phase, the effect of methyl-β-CD can be interpreted in terms of 1:1 complex formation of *p*-nitrophenol with methyl-β-CD in the

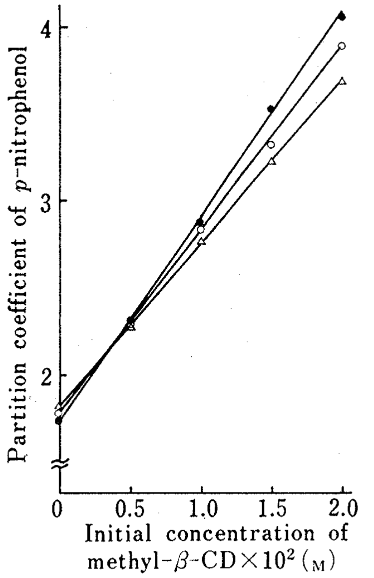

Fig. 1. Effect of Methyl-β-CD on the Partition Coefficients of *p*-Nitrophenol at 15.5°C, 20.0°C, and 25.0°C

Initial concentration of *p*-nitrophenol: 1.00×10^{-4} M.
●, at 15.5°C; ○, at 20.0°C; △, at 25.0°C.

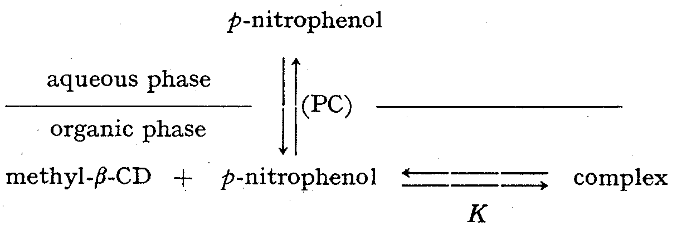

Chart 1

$CHCl_3$ phase as shown in the chart 1, where (PC) and K are the intrinsic partition coefficient of *p*-nitrophenol and the stability constant of the complex in the $CHCl_3$ phase, respectively.[8] Higuchi *et al.* calculated the stability constants of complexes formed in the aqueous phase by measuring the partition coefficients.[8] The stability constant of the complex formed in the $CHCl_3$ phase was calculated by a similar procedure. From the linear relationship between the partition coefficients and the initial concentration of methyl-β-CD, it was assumed that the stoichiometry of the complex between methyl-β-CD and *p*-nitrophenol in the $CHCl_3$ was 1: 1. *p*-Nitrophenol is present in the molecular form in the aqueous phase at pH 1.30, and self-association of *p*-nitrophenol does not seem to take place in either phase since the partition coefficients are independent of the initial concentration of *p*-nitrophenol. The following equations can be written:

$$(\mathrm{PC}) = \frac{[\mathrm{PNP}]_{\mathrm{org}}}{[\mathrm{PNP}]_{\mathrm{aq}}} \tag{1}$$

$$(\mathrm{PC})_{\mathrm{MCD}} = \frac{[\mathrm{PNP}]_{\mathrm{org}}+[\mathrm{Complex}]_{\mathrm{org}}}{[\mathrm{PNP}]_{\mathrm{aq}}} \tag{2}$$

$$K = \frac{[\mathrm{Complex}]_{\mathrm{org}}}{[\mathrm{MCD}]_{\mathrm{org}}[\mathrm{PNP}]_{\mathrm{org}}} \tag{3}$$

where $[\mathrm{PNP}]_{\mathrm{org}}$, $[\mathrm{MCD}]_{\mathrm{org}}$, and $[\mathrm{Complex}]_{\mathrm{org}}$ denote the concentrations of free *p*-nitrophenol, free methyl-β-CD, and the complex in the $CHCl_3$ phase, respectively. (PC), $(\mathrm{PC})_{\mathrm{MCD}}$, and K are the partition coefficients of *p*-nitrophenol without or with methyl-β-CD, and the stability constant of the complex, respectively. The initial concentration of *p*-nitrophenol and the initial concentration of methyl-β-CD are given by:

$$[\mathrm{PNP}]_{\mathrm{init}} = [\mathrm{PNP}]_{\mathrm{aq}} + [\mathrm{PNP}]_{\mathrm{org}} + [\mathrm{Complex}]_{\mathrm{org}} \tag{4}$$

$$[\mathrm{MCD}]_{\mathrm{init}} = [\mathrm{MCD}]_{\mathrm{org}} + [\mathrm{Complex}]_{\mathrm{org}} \tag{5}$$

from the equations (1)—(5), we may write

$$K = \frac{(\mathrm{PC})_{\mathrm{MCD}}-(\mathrm{PC})}{[[\mathrm{MCD}]_{\mathrm{init}}-\{(\mathrm{PC})_{\mathrm{MCD}}-(\mathrm{PC})\}[\mathrm{PNP}]_{\mathrm{init}}/\{1+(\mathrm{PC})_{\mathrm{MCD}}\}](\mathrm{PC})} \tag{6}$$

Table II shows the stability constants of the complexes obtained from equation (6) at 25.0°C. The stability constants are independent of the initial concentration of methyl-β-CD but depend slightly on the initial concentration of *p*-nitrophenol. The increase in the stability constants with increasing initial concentration of *p*-nitrophenol might be due to the formation of higher-order complexes, *e.g.*, $MCD(PNP)_2$, because methyl-β-CD has many ethereal oxygens which are presumably able to form hydrogen-bonded complexes with *p*-nitrophenol in the $CHCl_3$ phase, as will be shown later. In a dilute solution of *p*-nitrophenol, however, the 1: 1 complex seems to be formed predominantly. Equation (6) is convenient and useful to evaluate the stability constants in small amounts of samples.

As the effect of methyl-β-CD on the partition coefficients was influenced by the magnitude of the stability constants of the complexes, it is suggested that medicinals which have higher stability constants with methyl-β-CD increase the partition coefficients more effectively and *vice versa*.

Table II. Stability Constants (M^{-1}) of *p*-Nitrophenol–Methyl-β-CD Complexes in the $CHCl_3$ Phase at 25.0°C

Concentration of *p*-nitrophenol (M)[a]	Concentration of methyl-β-CD (M)[a]					
	5.00×10^{-3}	1.00×10^{-2}	1.50×10^{-2}	2.00×10^{-2}	2.50×10^{-2}	3.00×10^{-2}
1.00×10^{-4}	49±4[b]	52±3	51±2	51±2	50±1	49±1
6.00×10^{-4}	—	54±5	—	55±3	—	54±3
1.00×10^{-3}	—	55±3	—	56±1	—	54±1
2.00×10^{-3}	—	55±4	—	56±2	—	55±2
4.00×10^{-3}	—	59±5	—	58±4	—	57±3
1.00×10^{-2}	—	66±6	—	65±2	—	64±2

a) Initial concentration.
b) Standard deviation ($n=5$).

Figure 2 shows the effect of *n*-butyl ether instead of methyl-β-CD on the partition coefficients of *p*-nitrophenol at 25.0°C. *n*-Butyl ether has an ethereal oxygen, like methyl-β-CD, and is almost wholly present in the $CHCl_3$ phase after distribution.[9] The partition coefficients increase linearly with *n*-butyl ether concentration. This effect may be attributed to 1:1 hydrogen-bonded complex formation of *p*-nitrophenol with *n*-butyl ether in the $CHCl_3$ phase.[10] From the figure, it is clear that the slope of the *n*-butyl ether–*p*-nitrophenol system is smaller than that of the methyl-β-CD–*p*-nitrophenol system. The stability constant of the *n*-butyl ether–*p*-nitrophenol complex was calculated as 7 M^{-1}.

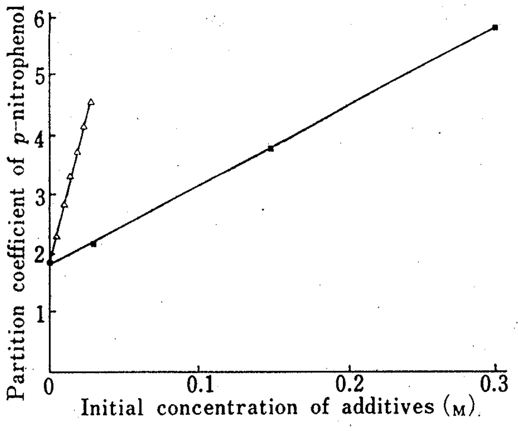

Fig. 2. Effect of *n*-Butyl Ether on the Partition Coefficients of *p*-Nitrophenol at 25.0°C

Initial concentration of *p*-nitrophenol: 1.00×10^{-4} M. △, Methyl-β-CD; ■, *n*-butyl ether.

Studies of the UV Spectra

Figure 3 shows the effect of (a) methyl-β-CD addition and (b) β-cyclodextrin addition on the UV spectra of *p*-nitrophenol in 0.05 N HCl at 25°C. With increasing concentration of cyclodextrins, the absorption maxima of *p*-nitrophenol (λ_{max}) shifted to longer wavelength and the absorbances decreased concomitantly with isosbestic points at 255 nm in (a) and at 254 nm in (b). The addition of α-methyl-D-(+)-glucoside or maltopentaose which is composed of linearly α-1,4-linked glucose units, caused no spectral changes of *p*-nitrophenol in the aqueous solution. In view of the effect of methyl-β-CD, which is very similar to that of β-cyclodextrin on the UV spectra, it is considered that methyl-β-CD includes a *p*-nitrophenol molecule in its cavity in the aqueous solution.

Figure 4 shows the UV spectra of *p*-nitrophenol with methyl-β-CD in $CHCl_3$ saturated with 0.05 N HCl at 25°C. The absorption intensities of *p*-nitrophenol changed and λ_{max} shifted to longer wavelength. Isosbestic points were seen at 303 and 251 nm. The addition of *n*-butyl ether to the *p*-nitrophenol $CHCl_3$ solution caused spectral changes as well, but the effect was not as clear, as shown in Fig. 4. It is apparent that the *p*-nitrophenol molecule undergoes hydrogen bonding with the methyl-β-CD molecule in the $CHCl_3$ solution, as does *n*-butyl ether. Furthermore, as methyl-β-CD forms inclusion complexes in organic solvents,[3a] it is concluded that methyl-β-CD forms an inclusion complex with *p*-nitrophenol in the $CHCl_3$ solution also.

Since the stoichiometries of the inclusion complexes were considered to be 1:1 from the results of the partitioning experiment, the stability constants were calculated by means of

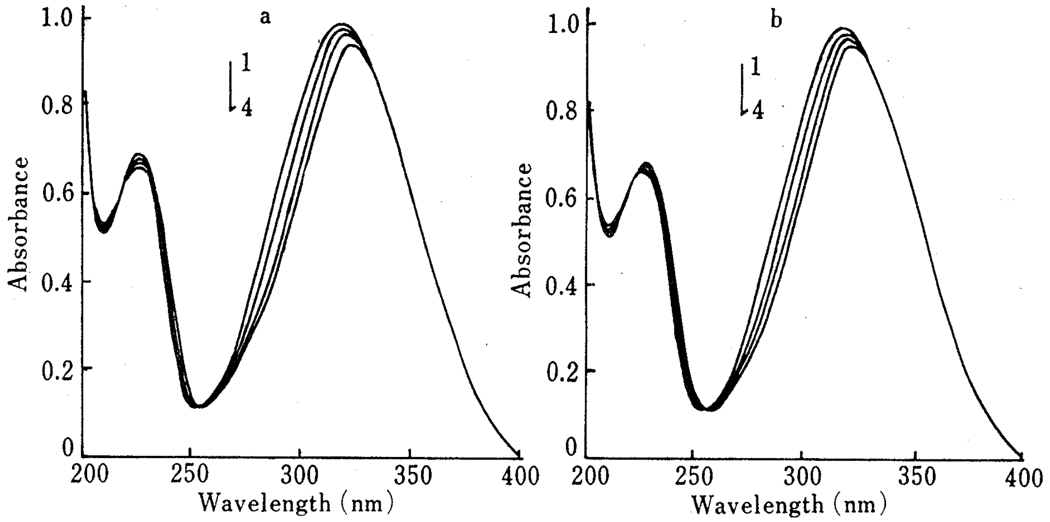

Fig. 3. Effect of Methyl-β-CD and β-Cyclodextrin on the UV Absorption Spectra of p-Nitrophenol in 0.05 N HCl at 25°C

Concentration of p-nitrophenol: 1.00×10^{-4} M.
(a) Concentration of methyl-β-CD: curve 1, 0; curve 2, 1.00×10^{-3}; curve 3, 4.00×10^{-3}; curve 4, 8.00×10^{-3} M.
(b) Concentration of β-cyclodextrin: curve 1, 0; curve 2, 1.00×10^{-3}; curve 3, 3.00×10^{-3}; curve 4, 6.00×10^{-3} M.

Scott's equation.11) The stability constants of the complexes are summarized in Table III. Shimizu *et al.* reported the stability constant of p-nitrophenol and β-cyclodextrin as 244 M^{-1},12) and this value is consistent with the value of 201 M^{-1} obtained in this report. The value for the p-nitrophenol and methyl-β-CD system in the $CHCl_3$ solution was close to that obtained from the partitioning experiment. The stability constant of the methyl-β-CD system in 0.05 N HCl was about 0.6 times that of the β-cyclodextrin system. In the $CHCl_3$ solution, the stability constant of the complex between p-nitrophenol and methyl-β-CD was about 0.6 times that in the aqueous solution. This may be attributed to differences of the main driving forces for complex formation. In the aqueous solution, hydrophobic interactions and hydrogen bonding seem to be mainly responsible for the complexation.13) On the other hand, in $CHCl_3$, hydrogen bonding plays a major role in the complexation.

PMR Studies of the Inclusion Complex

Figure 5 shows the effect of methyl-β-CD addition on the PMR spectra of p-nitrophenol in D_2O. Both the *ortho* and *meta* protons of p-nitrophenol were shifted to lower field, by 0.14 and 0.12 ppm, respectively, by the addition of methyl-β-CD. These shifts may be attributed to the diamagnetic anisotropy of particular bonds or regions of the host, van der Waals shifts, or steric perturbation.14) When maltopentaose was added, significant shifts were not induced, so it can be concluded that p-nitrophenol is included in the cavity of methyl-β-CD from the results of the PMR study as well as the UV study.15) Bergeron *et al.* reported that in the p-nitrophenolate–α-cyclodextrin system *ortho* and *meta* protons of p-nitrophenolate were shifted to lower field.14) On the other hand, in the p-nitrophenolate–β-cyclodextrin system no large shifts were observed upon complexation.14) They inferred that in the β-cyclodextrin systemthe contact between the guest and the host was not as close as in the complex of p-nitrophenolate with α-cyclodextrin. Therefore, from the proton shifts of p-nitrophenol in the methyl-β-CD system, it is considered that the p-nitrophenol molecule is in close proximity to the methyl-β-CD molecule. The properties of cyclodextrin, such as the cavity size and the conformation, may be changed by permethylation, and the disposition of p-nitrophenol in the methyl-β-CD system may thus be different from that in the β-cyclodextrin system. This is in accord with the difference between the stability constants of the methyl-β-CD system and the β-cyclodextrin system in 0.05 N HCl determined by the UV method.

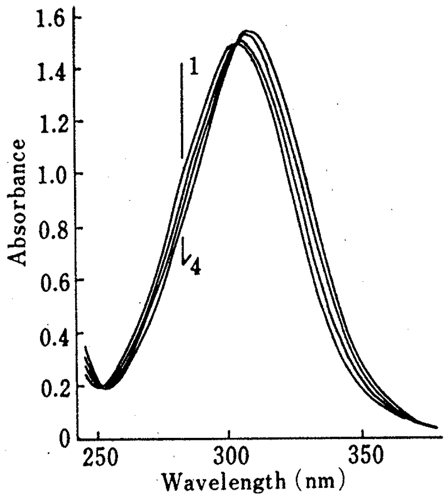

Fig. 4. Effect of Methyl-β-CD on the UV Absorption Spectra of p-Nitrophenol in $CHCl_3$ Solution saturated with 0.05 N HCl at 25°C

Concentration of p-nitrophenol: 1.50×10^{-4} M. Concentration of methyl-β-CD: curve 1, 0; curve 2, 5.00×10^{-3}; curve 3, 1.50×10^{-2}; curve 4, 2.50×10^{-2} M.

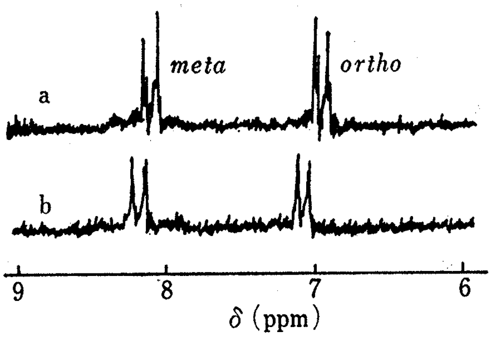

Fig. 5. Effect of Methyl-β-CD on the PMR Spectra of p-Nitrophenol in D_2O at 26.5°C

(a) p-Nitrophenol (6.4×10^{-2} M) alone.
(b) p-Nitrophenol (7.6×10^{-2} M)+methyl-β-CD (2.7×10^{-2} M).

TABLE III. Stability Constants (M^{-1}) of p-Nitrophenol–Cyclodextrin Complexes at 25°C (UV Method)

System	Solvent	Stability constant (M^{-1})
p-Nitrophenol–Methyl-β-CD	0.05 N HCl	120
p-Nitrophenol–β-Cyclodextrin	0.05 N HCl	201
p-Nitrophenol–Methyl-β-CD	$CHCl_3$ saturated with 0.05 N HCl	73

A spectral change of methyl-β-CD was induced by the addition of p-nitrophenol in D_2O. However, a detailed discussion cannot be given in this report, since H-2, H-3, H-4, H-5, and H-6 were not resolved clearly and overlap with methoxyl-proton signals.[3a,7] Further studies are in progress.

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