# Studies on the Interaction of Thiamines and Cyclodextrins

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**Abstract** ☐ Interactions between thiamine·HCl and its disulfide derivatives TTFD, TPD and $\alpha$-, $\beta$-cyclodextrins were investigated. By measuring the $^1$H-NMR, $^{13}$C-NMR chemical shifts, the assumption that cyclodextrin may form a inclusion complex with thiamines was supported qualitatively. To calculate the stability constants of them, anion exchange chromatography was applied. The simple, rapid HPLC method was proved to be pertinent thiamine/cyclodextrin system which was chemically unstable and less soluble.

**Keywords** ☐ Thiamine, Cyclodextrin, Complexes, $^1$H-NMR, $^{13}$C-NMR, Stability constants, Anion exchange chromatography.

Thiamine, being a water soluble compound with a quaternary nitrogen, is poorly absorbed into CNS[1] and poorly absorbed from the GI tract.[2] Thiamine passes through these barriers both in CNS and oral absorption, so it is actively absorbed. However, active absorption processes are suitable and/or easily inhibited.[3-4]

Thiamine undergoes a rather unusual second ionization to a thiolate ion, and derivatization of thiolate ion leads to many lipid soluble thiamine derivatives of the disulfide type, such as TPD (thiamine propyl disulfide). TTFD (thiamine tetrahydro furfuryl disulfide), diacyl type such as O.S-diacetyl thiamine, and O.S-and S-carbonate esters of thiamine such as O.S-diethoxy carbonyl thiamine.

However, the synthesis of these derivatives was not necessarily aimed at preferential GI or CNS absorption of thiamine, but was geared mainly to the possible use of these lipid soluble thiamine derivatives as stable food additives.[5] These compounds and their homologues do not possess a quaternarized nitrogen so allowing them to be passively absorbed from the GI tract. Each are quantitatively converted to thiamine once in the body.[6-7].

But, in most cases, these compounds are poorly soluble, relatively unstable and not sweet. Recently, the improved oral bioavailability of thiamine through dosing with various thiamine derivatives is well doing[8] and through pharmaceutical formulations also. Especially, inclusion complexes of various drugs with cyclodextrins have been successfully applied in pharmaceutical formulations to enhance the solubility,[9] chemical stability[10] and absorption characteristics[11] of the drugs.

One of the important characteristics of cyclodextrins is the formation of inclusion complexes with various compounds (guest molecule), in which guest compounds are included in the cavity of cyclodextrins (host molecule).[11]

The most direct evidence for the inclusion of a guest into the cyclodextrin cavity in solution was obtained by proton nuclear magnetic resonance ($^1$H-NMR) spectroscopy.[19] The determination of the stability constants (Kc) of inclusion complexes have been reported by several authors using the varity of techniques, such as solubility,[12] potentiometry,[13] polarography[14]

and spectroscopic methods.[15]

These methods, however, do not appear to be suitable for the chemically unstable compounds and for systems accompanying no spectral changes. In the previous paper,[16] cyclodextrin complexations have been successfully applied to rapid analysis of various compounds such as prostaglandins, barbiturates and phenothiazines by HPLC methods. Also, Ikeda et al.[17] showed that different Kc values were successfully applied in separating of prostaglandin isomers. In other cases, between the configurations of xylene isomers and pyridine derivatives could be distinguish using cyclodextrin resins by Gas Solid Chromatography.[18] It has then been shown that the retention times of the compounds decreased significantly by the addition of $\alpha$-, and $\beta$-cyclodextrins ($\alpha$-CD, $\beta$-CD) into aqueous mobile phase on anion exchange support, because of the formation of soluble complexes in the mobile phase.

In the present study, for the purpose of elucidating the stoichiometry of these complexes and the mode of interactions between thiamine·HCl & its disulfide derivatives and $\alpha$-, $\beta$-cyclodextrins, spectrophotometry was applied. But, thiamine·HCl & its disulfide derivatives had not remarkable spectral changes and had not induced Circular Dichroism when they were completed inclusion complexations in aqueous solution. Therefore, ^{1}H-NMR spectroscopy of $\alpha$-CD & $\beta$-CD in the absent/present of three thiamines were used as a proof of inclusion complexation.

And the retention times of thiamine·HCl, TTFD and TPD on the anion exchange support were quantitatively treated as a function of $\alpha$-CD & $\beta$-CD concentration in the mobile phase in order to obtained Kc values of these complexes. And with anticipations on the revelation of the mechanism responsible for inclusion complexation, ^{1}H and ^{13}C-NMR chemical shifts were investigated.

## EXPERIMENTAL METHODS

### Materials

Thiamine·HCl, thiamine tetrahydro furfuryl disulfide (TTFD) and thiamine propyl disulfide (TPD) were favored by Il Dong Pharm. Co. $\alpha$-, and $\beta$-cyclodextrins ($\alpha$ CD, $\beta$ CD) were purchased from Tokyo Kasei Kogyo Co., Ltd. and recrystallized from water and dried with $P_2O_5$ in vacuo. Deionized water was used for buffer. All other materials were of analytical reagent grade.

### Apparatus and Conditions of HPLC

Hitachi 638-50 liquid chromatogrape instrument and Hitachi 635-M multiple wavelength UV-detector were employed. 4.1g of Bondapak AX/Corasil ($37\sim50\mu$m) was delivered to (2.3 mm i.d. $\times$ 500mm long) glass column by dry packing method.

Deionized water, 0.1 N-NaOH and sodium phospate buffer were employed in order to column washing and column regeneration. The effluents were monitored with a UV-detection at
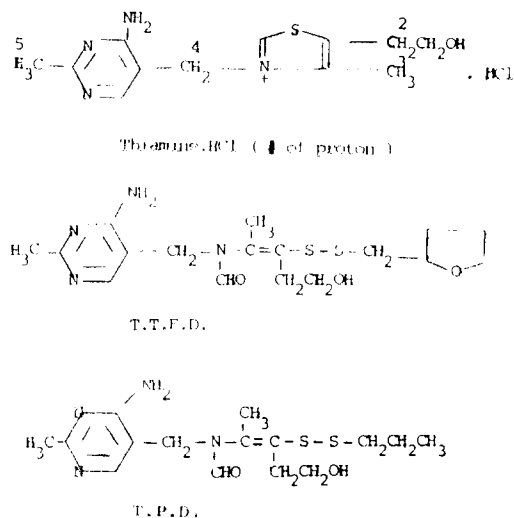


Fig. 1: Structure of thiamine·HCl, TTFD and TPD.

250nm and 280nm. The mobile phases were adjusted at pH 7.0 and ionic strength $\mu=0.2$ with 0.1N NaOH and 0.1N HCl. A stock solution of thiamine·HCl (1.2mg/ml), TTFD (1.6mg/ml) and TPD (1.5mg/ml) were prepared in ethanol. The column temperature was ambient ($25\pm2°C$) and $2\mu l$ aliquot of the sample was injected at a flow rate of 1.0ml/min. The retention times of the drugs in the absence and in the presence of excess amounts of $\alpha$-CD, $\beta$-CD (varied from 0.4 to $10\times10^{-3}$M) in the mobile phase were measured. The stability constant, Kc, was calculated as described previously.[16]

*$^1H$ NMR, $^{13}C$-NMR Studies*

$D_2O$ solutions of $\alpha$-CD (O.IM) and $\beta$ CD (O.IM) were prepared with different amounts of thiamine·HCl & it's D disulfide derivatives from O to 0.4 M. A few drops of DMSO was added for solubility.

$^1H$- and $^{13}C$-NMR spectra were obtained using Perkin Elmer R 32 spectrometer, operating at 90 MHz and Varian XL 100 spectrometer, operating at $^{13}C$-25.16 MHz in the pulsed Fourier Transform mode. Chemical shifts were measured in ppm downfield from extrernal TMS. $^{13}C$-NMR parameters were set in the following ranges: maximum frequency 200ppm, sample points 16 K, pulse interval 30°, Band pass filter TC(4), pulse interval 30 sec., wide band decoupling 3000 Hz, decoupling power 10 w. Resolution was $\pm0.04$ ppm temperature was 35 $\pm0.2°$ C and $^{13}C$-NMR spectra were proton-decoupl-ed in 12 mm tubes. All analytical data induced by inclusion complexation have been used for the assignment.

## RESULTS AND DISCUSSION

*Stability Constants Determined by HPLC Method*

It was observed that the retention times of weakly acidic or basic drugs decreased significantly by the addition of $\alpha$-CD or $\beta$-CD into the mobile phase on ion exchange supports.[16] Thiamine & it's disulfide derivatives used in this study had a linear relationship when they were applied to previous equation which designated assuming 1:1 stoichiometry, the retention behaviors of three thiamines & it's inclusion complexes within ion exchange support could be investigated. Also, plots of molar ratio of $\alpha$-CD, $\beta$-CD/thiamines vs. changes in chemical shifts of thiamine showed that a 1:1 complex is formed, which is discussed later related with $^1H$-NMR spectra.

It was shown that typical HPLC chromatograms of thiamine·HCl, TTFD and TPD on anion exchange support in the absence and in the presence of $\alpha$-CD and $\beta$-CD.

The aqueous mobile phase used was sodium phosphate buffer (pH=7.0, $\mu=0.2$), since phosphate anions do not interfere with inclusion complexation. A simple phosphate buffer gave relatively long retention times and broad peaks in three thiamines, but when $\alpha$-CD, $\beta$-CD were added to phosphate buffer, the retention times decreased significantly. plottings of Tobs (Sec.) versus (CD)m in each thiamine-CD system which is appeared in Fig. 2 showed that increase in $\alpha$-CD and $\beta$-CD concentration shortened the retention times of thiamine·HCl, TTFD and TPD respectively. Tobs is the retention time of thiamine at a given concentration of CD in the mobile phase, i.e. (CD)m. These indicated that increase in the solubilty of them by the binding to each CD.

The data in Fig. 2 treated according to following equation.[16]

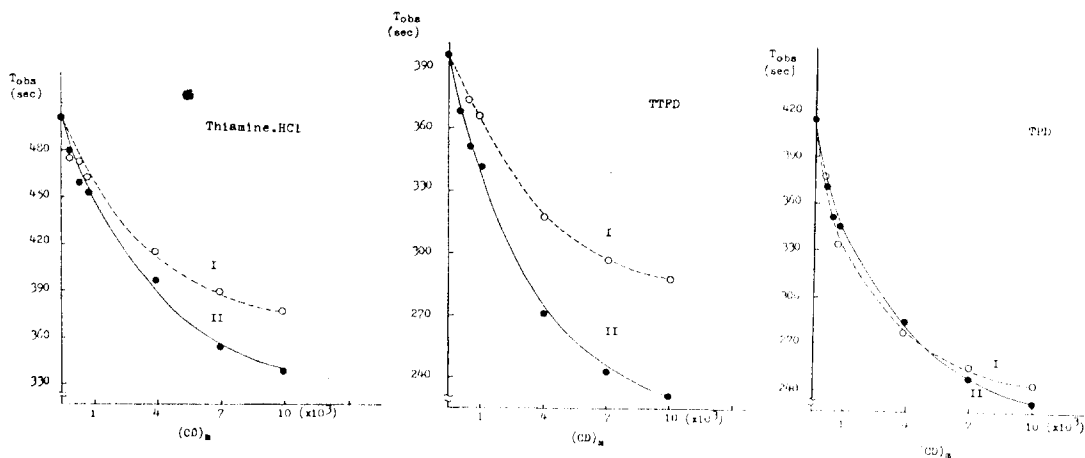$$\frac{(CD)m}{T-Tobs} = \frac{1}{T-Tc}(CD)m + \frac{1}{kc(T-Tc)}$$

**Fig. 2**: Observed retention times for thiamine with varying concentration of CD in the mobile phase (0.1M phosphate buffer, pH=7.0, $\mu=0.2$)
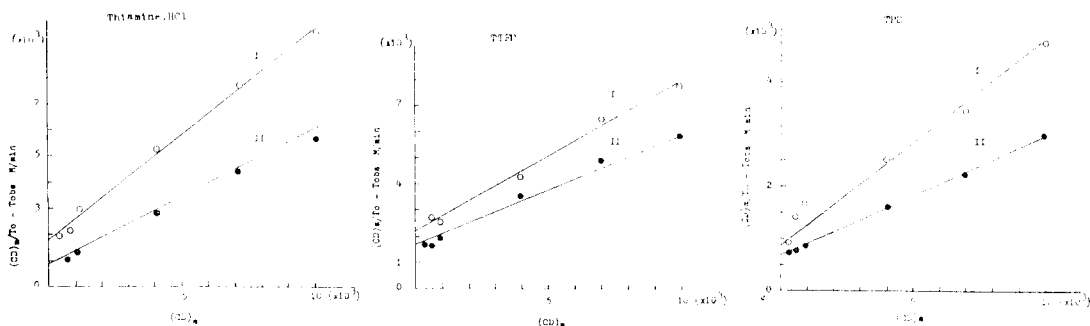
　　　1) $\beta$-CD system 　　　2) $\alpha$-CD system



**Fig. 3**: Determination of Kc from retention time data (Fig. 2) of thiamine-CD complexes according to eq. in ref. 16.

　　　1) $\beta$-CD system 　　　2) $\alpha$-CD system

T and Tc are retention times of thiamine itself, that of thiamine CD complex, respectively. Kc is the stability constant and (CD)m could be assumed to de equal to the added concentration of CD, i.e. CD)t, when concentration of CD is largely in excess compared to that of drugs in the mobile phase. As shown in Fig. 3, plottings of (CD)m/To-Tobs (M/min.) vs. (CD)m in each thiamine-CD system, were obtained with a linear relationships for both $\alpha$-CD and $\beta$-CD systems, verifying 1:1 stoichiometry in previous chart.[17] Then Kc values were calculated from

**Table I: Stability constants of inclusion complexes between thiamin·HCl, TTFD and TPD and $\alpha$-, $\beta$-CD.**

|              | with $\alpha$-CD | with $\beta$-CD |
|--------------|------------------|------------------|
| Thiamine·HCl | 204.81           | 176.21           |
| TTFD         | 387.98           | 359.84           |
| TPD          | 479.07           | 405.88           |

intercept and slope by a least square method. Table I summerized Kc values for inclusion complexes of thiamine·HCl, TTFD and TPD with $\alpha$-CD, $\beta$-CD. The advantages of the HPLC

method used here were that Kc values could be
rapidly obtained by simple procedure with
minimum quantity of the guest molecules even
when significant spectral changes are not obse-
rved by complexation. This method was proved
to be pertinent to inclusion complexes between
thiamine・HCl & it's disulfide derivatives and
α-CD, β-CD.

## ¹H-NMR Spectra

α-CD, β-CD are composed of 6 and 7, D-
glucopyranose residues respectively joined by
α-1. 4-linkages to produce a macrocyclic form.
Owing to its simple symmetry, the conformati-
onal analysis of CD is relatively simple, as it
closely resembles its constituent monosaccharides.
The chair conformation has been established for
the constituent glucose unit in CD.[20] And as
it has primary and secondary -OH crowding
in the opposite ends of its torus, H-3 and
H-5 directed toward its interiour, and H-1,
and H-4 located on its exteriour. In general, it
might be expected that if inclusion occurs in
the neighbourhood of protons located within or
near the cavity, H-3, H-5 and H-6 should be
strongly shielded by the ring current effect of
the aromatic ring of guest molecule. Alternati-
vely, if association takes place at the exteriour
of torus, H-1, H-2 and H-4 should de strongly
affected.

The typical ¹H-NMR spectrum of α-CD was
showed in Fig. 4. Although, the H-5 signal
of α-CD could not be directed observed by 90
MHz NMR, because it overlapped with the
H-3 signals in the spectral region of 4.5-4.2
ppm. It was clear that a new sharp signal ass-
igned to H-5 progressively shifted to upfield,
becoming seperated in the presence of increasing
amounts of thiamine・HCl. Thiamine・HCl i-
nduced ¹H-NMR chemical shifts of α-CD,
β-CD at various molar ratios of thiamine・HCl



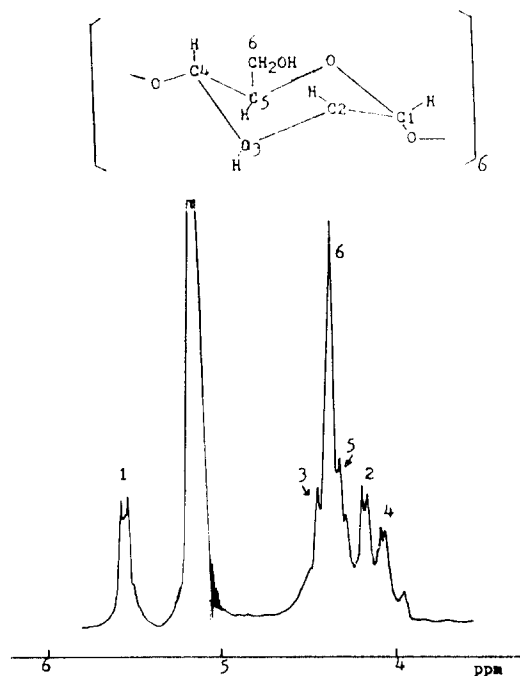**Fig. 4**: ¹H-NMR spectra of α-CD($1 \times 10^{-1}$M) inD₂O
solution.
Assinged by following ref. 19.

to each CD were showed an downfield shift,
while the H-5 protons showed upfield shift.
Plots of the molar ratio of thiamine・HCl/α-CD,
β-CD vs. the chemical shifts of each CD
protons(fig. 5) indicated that a 1:1 complex
was formed, though some ambiguity remains.
As no further substantial changes of the chem-
ical shifts took place above a molar ratio of
thiamine・HCl/α-CD, β-CD =1.0. The chemi-
cal shifts of the H-1, H-2 and H-4 protons
were marginal affected. These results also ind-
icate the inclusion of the hydrophobic functional
group of thiamine derivatives in the cavity of
CD. The upfield shift for the H-5 atom was
attributed to the anisotropic shielding effect of
the pyrimidine ring of thiamine derivatives,
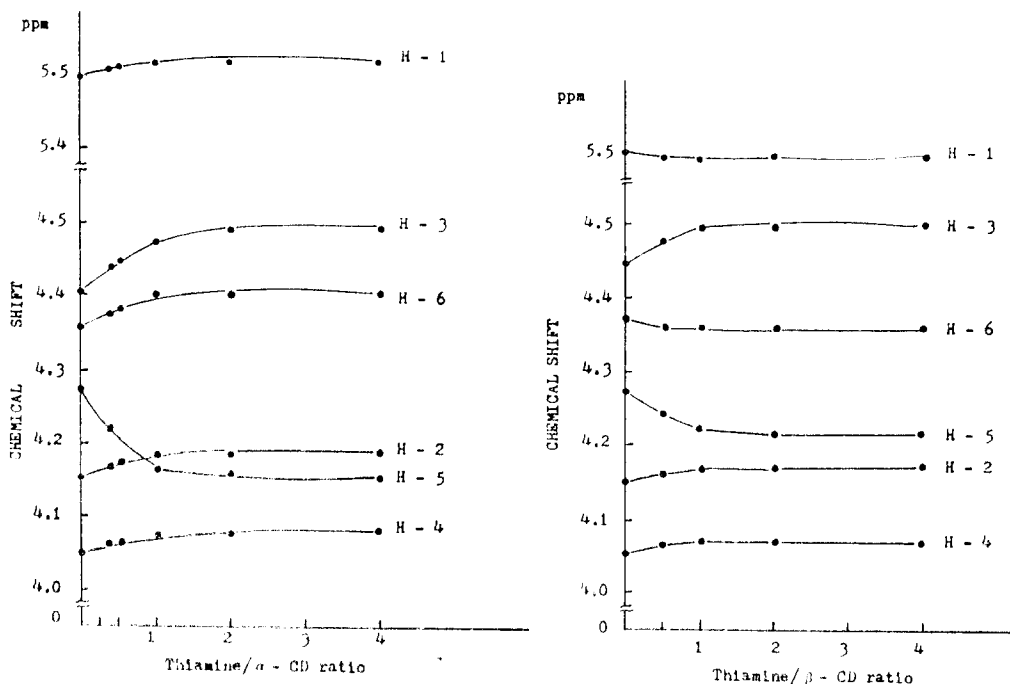which is included in the CD cavity. The dow-

**Fig. 5**: Variation of $^1$H chemical shifts of 0.1M $\alpha$-CD with increasing molar ratio of thiamine.
HCl to $\alpha$-CD, $\beta$-CD.

nfield shift for the H-3 atom was interpreted in terms of the van der Waals deshielding effect of the nitrogen atom of pyrimidine ring as well as the ring current effect of the aromatic ring.

The TTFD-induced and TPD-induced $^1$H NMR chemical shifts of $\alpha$-CD, $\beta$-CD were presented in tables II, III., respectively. It was

considered possible that the pyrimidine moity of each compound was induced in the cavity of $\alpha$-CD, $\beta$-CD ; H-3, H-5 and posssibly H-6 (located within the cavity of CD) would then be affected by deshielding effect and anisotropic shielding due to pyrimidine moity, whereas H-1, H-2 and H-4 (located outside the cavity) were relatively unaffected. Considering the lar-

**Table II**: $^1$H-NMR chemical shift of $\alpha$-CD in the presence or absence of TTFD and TPD.

| Proton | Chemical shift | | |
|---|---|---|---|
| | itself | with TTFD | with TPD |
| 1 | 5.55 | 5.55 | 5.55 |
| 2 | 4.15 | 4.19 | 4.17 |
| 3 | 4.41 | 4.48 | 4.51 |
| 4 | 4.05 | 4.09 | 4.06 |
| 5 | 4.26 | 4.34 | 4.16 |
| 6 | 4.36 | 4.38 | 4.38 |

**Table III**: $^1$N-NMR chemical shift of $\beta$-CD in the presence or absence of TTFD and TPD.

| Proton | Chemical shift | | |
|---|---|---|---|
| | itself | with TTFD | with TPD |
| 1 | 5.55 | 5.56 | 5.55 |
| 2 | 4.15 | 4.17 | 4.17 |
| 3 | 4.45 | 4.50 | 4.52 |
| 4 | 4.05 | 4.08 | 4.07 |
| 5 | 4.27 | 4.34 | 4.33 |
| 6 | 4.37 | 4.38 | 4.38 |

ger difference in relative magnitude of between H-5 and H-3 than other protons, it could be assumed that the association between three thiamines and $\alpha$-CD, $\beta$-CD may takes place by the approach of the thiamine molecule to the primary hydroxyl side of $\alpha$-CD, $\beta$-CD.

The effects of $\alpha$-CD, $\beta$-CD on the $^1$H-NMR spectra of thiamine·HCl in $D_2O$ solution were shown in Fig. 10a). 10b). All of the proton signals of thiamine shifted to downfield with increasing molar ratio of thiamine·HCl/ $\alpha$/CD, $\beta$-CD. The motion of pyrimidine ring was also assumed by downfield shifts of ring proton and H-5 proton of thiamine·HCl. Although, $NH_2$ proton signal was appeared in above 10ppm

without $\alpha$-CD, $\beta$CD, it wasn't shown in $^1$H-NMR spectra of thiamine-HCl-$\alpha$-CD, $\beta$-CD complexes.

It was assumed that the formation of hydrogen bridge with inner proton in the cavity of CD when inclusion complexation occurs made the peak broadening, even disappeared.

All of the proton signals of thiamine·HCl shifted to downfield wth increasing amounts of $\alpha$-CD, $\beta$-CD. Similar chemical shift changes have been observed for other drug systems.[21] This downfield shift of all other proton signals might be induced by diamagnetic anisotropy of particular bonds of $\alpha$-CD, $\beta$ CD[22] and van der Waals shifts.[23] The downfield shift of the
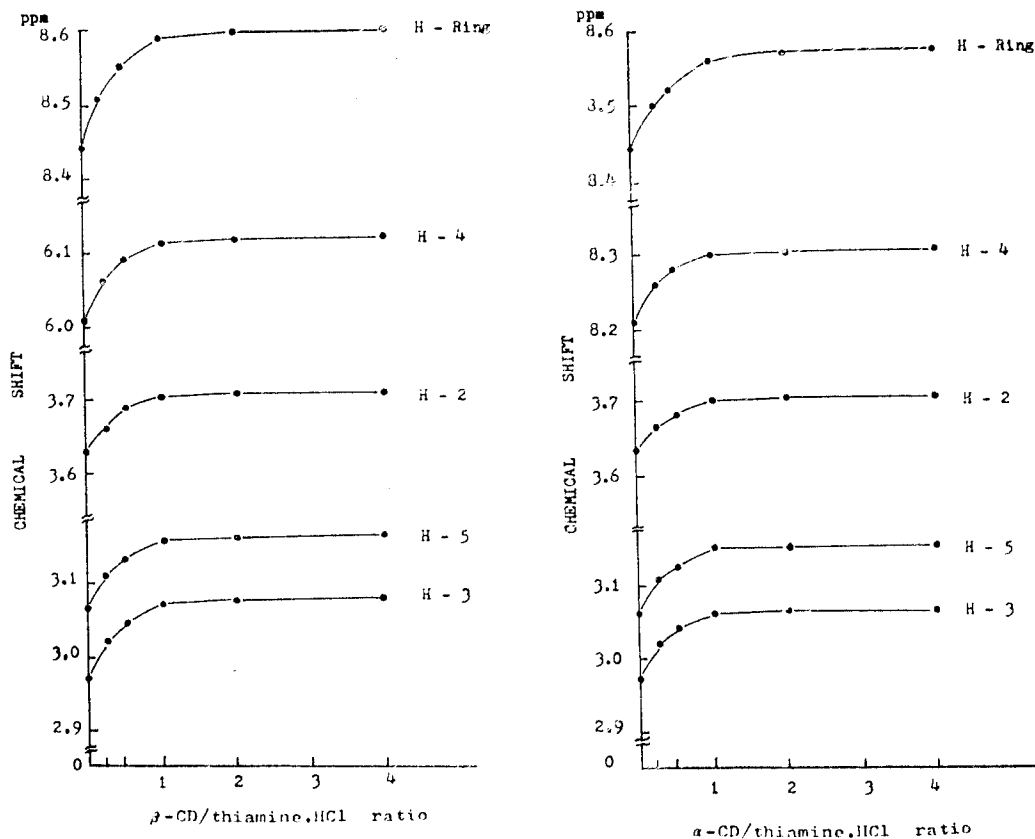


**Fig. 6**: Change of $^1$H chemical shifts of thiamine·HCl with various molar ratio of thiamine· HCl to $\alpha$-CD, $\beta$-CD assigned by following ref. 27.

side chain portion might be induced by steric perturbation.[24]

## ^{13}C-NMR Spectra

^{13}C-NMR spectroscopy is known to be a powerful technique for the investigation of intermolecular interactions.[25] The ^{13}C–NMR spectra of thiamine·HCl α CD and their complexes were presented in Fig. 7.

These are asssigned by ref. 19 and ref. 26. Table IV shows the effect of thiamine·HCl on the ^{13}C -NMR spectrum of α–CD in D$_2$O-solution. All signals of α CD showed shifts to downfield in the presence of thiamine·HCl except for C$_5$ and C$_3$. C$_1$ and C$_4$ of α–CD were affected more than other carbons. This effect may have resulted from a small conformational change of α CD, as C$_1$ and C$_4$ positions are mobile because α CD is α-1.4-linked. The primary alcohol group of α-CD (C$_6$) was also affected ; its internal rotation may decrease with the inclusion of thiamine·HCl. Since C$_3$ and C$_5$ groups are oriented at the center of the cavity, these carbons may be particularly susceptable to the shielding due to hydrophobic interaction.[23] These result apparently indicate that thiamine·HCl may interact with α–CD at interior of the cavity to form a relatively rigid complex.

The downfield shifts both in the ^{13}C-NMR

**Table IV**: ^{13}C-NMR chemical shift of α-CD in the presence or absence of thiamine·HCl

| Carbon | Chemical shift | | |
| --- | --- | --- | --- |
| | without thiamine·HCl | with thiamine·HCl | Δ∂ |
| 1 | 102.89 | 103.03 | - 0.14 |
| 2 | 74.62 | 73.53 | +0.09 |
| 3 | 73.37 | 73.42 | -0.05 |
| 4 | 82.77 | 82.96 | -0.19 |
| 5 | 72.93 | 73.02 | +0.09 |
| 6 | 61.65 | 61.70 | -0.05 |

**Table V**: ^{13}C-NMR chemical shift of thiamine·HCl in the presence or absence of α-CD.

| Carbon | Chemical shift (ppm) | | |
| --- | --- | --- | --- |
| | without α-CD | with α-CD | Δ∂ |
| 1 | 21.55 | 22.55 | −1.00 |
| 2, 3 | 158.27 | 164.66 | −6.39 |
| 4 | 103.08 | 107.13 | −4.05 |
| 5 | 140.80 | 146.19 | −5.39 |
| 6 | 38.67 | 51.34 | −12.67 |
| 7 | 158.07 | 164.46 | −6.39 |
| 8 | 138.53 | 144.09 | −5.56 |
| 9 | 132.51 | 137.77 | −5.26 |
| 10 | 11.95 | 12.60 | −0.65 |
| 11 | 29.39 | 30.65 | −1.26 |
| 12 | 49.22 | 61.66 | −12.44 |

spectra and in the ^{1}H-NMR spectra of the pyrimidine moity of the thiamine·HCl might be induced by a steric compression effect.[23] It is possible that when the pyrimidine moity of the thiamine·HCl is induced in the cavity of α–CD, the side chain may extrude from the cavity and may be in a sterically different situation. Table V summerized the effect of α-CD on the each carbon of thiamine·HCl. It showed this trend well.
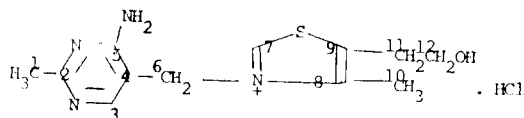


Thiamine·HCl (# of carbon)

## CONCLUSION

This study was initiated to ascertain that thiamine·HCl & its disulfide derivatives (TTFD, TPD) forms inclusion complexes with α-CD, β-CD and to examine the effect of cyclodextrins on the three thiamines. By measuring the ^{1}H-NMR, ^{13}C-NMR chemical shifts, this

assumption took for granted.

The H-3 protons of $\alpha$-CD, $\beta$-CD showed an downfield shift, while the H-5 protons of them showed upfield shift when they formed inclusion complexes with three thiamines. Whereas the chemical shifts of the H-1, H-2 and H-4 protons of $\alpha$-CD, $\beta$-CD were marginal affected. These results indicated the inclusion of thiamines in the cavity of each CD. All the proton signals of thiamine·HCl shifted down field with increasing molar ratio of thiamine·HCl/$\alpha$-CD, $\beta$-CD. Ring protons of thiamine·HCl had a downfield shift at the same condition, too. These results indicated that the pyrimidine moity of thiamine·HCl & its disulfide derivatives was located within the hydrophobic cavity of both $\alpha$-CD, $\beta$-CD due to the inclusion complexation. And the large downfield shifts of ring carbon were suitable for the speculation. Also, the upfield shift of $C_3$ and $C_5$ carbons of $\alpha$-CD spoke for the interaction between thiamine·HCl and $\alpha$-CD at the inner portion of $\alpha$-CD cavity. These actual facts led an experiment into the next step, to determine the stability constants of them.

The HPLC method to calculate the stability constant was true of inclusion complexes between thiamine·HCl, TTFD & TPD and $\alpha$-CD, $\beta$-CD. And they showed a 1 : 1 stoichiometry, since that they had a linear relationship when they were applied to the previous eq.,[16] which designated assuming 1 : 1 stoichiometry.

As previously reported,[20] volume of the guest molecule served as an important factor in inclusion complexation. The sizes of three thiamines were well fitted with $\alpha$-CD, which has smaller cavity size than that of $\beta$-CD in consequence of this study. It seems to be possible that $\alpha$-CD took form more stable complexes than $\beta$-CD with each thiamines. What's more, TPD-$\alpha$-CD

system had the largest stability constant than the others since TPD has the least bulky side chain. The bulky of thiamine·HCl side chain effected the less stable characteristics of inclusion complexes, whereas the less bulky side of TTFD was relatively stable.

In view of the results so far achieved, ion exchange chromatography is applicable to the determination of stability constants for thiamine·HCl, TTFD and TPD/$\alpha$-CD, $\beta$-CD system which had not remarkable spectral changes after complexation. And, there is every promise of qualitative analysis in three thiamines-cyclodextrins inclusion complxation with ^{1}H-NMR, ^{13}C-NMR chemical shifts, although they gave a rough quantitative data. But relexation times of both in ^{1}H and ^{13}C atoms produce information about molecular dynamics of them, they'll make a more detail investigation on the mechanism and driving forces of three thiamines/ $\alpha$-CD, $\beta$-CD complexation, like as ref. 21). And this approach will be continued later.

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