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Interaction of Pharmaceuticals with Betacyclodextrin I

Interaction with Sulfonamides

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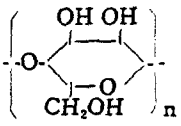
閔信弘 : Betacyclodextrin 과 醫藥品과의 相互 反應 I Sulfonamide와 Betayclodextrin 과의 相互 反應

Betacyclodextrin과 數種의 sulfonamide와의 相互反應을 solubility method로 考察하였다. 本實驗에서 使用한 betacyclodextrin의 濃度 범위內에서는 sulfonamide의 solubility증가와 betacyclodextrin의 濃度와의 關係가 函數關係에 있음을 나타내었다. 熱力學的인 解釋을 試圖하여 本反應의 formation constant, free energy, enthalpy 및 entropy를 槪測하였다.

The drug complexation has become a very important problem in pharmaceutical formulations and research with respect to the activity of drugs.

The phenomenon of complex formation as a means of drug solubilization and its possible influence on the stability has received considerable attention in recent times. Complexation by means of inclusion formation is believed to occur through that the host molecule which has hollow space enough to fit a prospective guest species can spatially enclose the guest molecule. Therefore, inclusion compound formation is mainly due to the peculoar stereochemical and geometrical features of the interaction species rather than their chemical characteristics. As inclusion formation does not form by means of ionic bonds, these inclusion compounds are often called "no bond" complexes.

This type of inclusion is typified by the cyclodextrins. The cyclodextrins are a series if homologous oligosaccharides obtained from the breakdown of starch by the action of *Bacillus macerans* amylase. They are homologous cyclic molecules composed of six or more alpha-D-glucopyranose units linked 1, 4 as amylose. Their chemical structure is



where n is 6, 7 or 8, knows as the alpha, beta, and gamma cyclodextrins, respectively. As illustrated above betacyclodextrin has cyclic structure and the relatively large open space within molecule(8Å). This

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steric configuration confers upon the compound the ability to form inclusion compound. Labile compounds such as unsaturated fatty acids and vitamin A[1] was stabilized by combination with the cyclodextrins.

Lach *et al.*[2-6] investigated the complexation of many organic compounds with cyclodextrins by use of the solubility method. Koizumi[7-8] also studied the inclusion compounds of betacyclodextrin with atropine, adrenaline and various pharmaceuticals.

Inhibition of hydrolysis by means of molecular complex formation between benzocaine and cyclodextrin was reported by Lach[9].

The purpose of this experiment is to investigate and compare the interaction tendencies of a series of sulfonamides with betacyclodextrin.

EXPERIMENTAL

Materials——Betacyclodextrin; sulfaguanidine, m.p.190—193°; sulfamerazine, m. p. 235—239°; sulfamethazine, m. p. 198—199°; sulfanilamide, m. p. 165—166°; sulfisoxazol, m. p. 193—194°; sulfisomidine, m. p. 242—243°; sulfamethizole, m. p. 208—209°; sulfadimetho xine, m. p. 201—203°; sulfamonomethoxine, m. p. 215—216°.

Apparatus——A constant temperature incubator with magnetic stirerr, 100ml capacity vials with stoppers, Beckman DU spectrophotometer, 1cm quartz cells.—

Preparation of the Betacyclodextrin——Betacyclodextrin was separated from cyclodextrin mixtures(Matsutani Chem. Co., Ltd.) by their differing solubilities of α,β,γ-cyclodextrins and purified by repeated crystallizations from water $[\alpha]_D^{25}=+160°$ (c, 1 in H_2O) Betacyalode xtrin was then dried in an oven at 80° and kept in desiccator until used. Solutions of the betacyclodextrin were freshly prepared in distilled water and concentrations of betacycodextrin employed was from 0.8818×10^{-3}M to 8.8183×10^{-3}M.

Solubility Method——The solubility method of Higuchi *et.al.*[10] was used to study inclulsion complex formation throughout this experiment. Excess quantities of the normal solubilities of the sulfonamides were weighed into 100ml bottles. Solutions of betacyclodextrin were accurately measured in and water added to make a volume of 50ml of solution in each bottle. The bottles were closed then agitated in a constant temperature incubator with magnetic stirrer for 72 hours at 20° and 30°, an interval sufficient for the attainment of equilibrium.

A series of control bottles in which contained distilled water rather then betacyclodextrin were also similarly prepared.

Method of Analysis——After equilibrium was completly reached the solution were filtered and aliquots of the filtrate diluted with distilled water and analyzed for total drug concentration by UV spectrophotometry. The wave lenght of the maximum absorption

of the compound was used in the analysis of the particular sulfonamide under investigation.

The betacyclodextrin showed absorption at the wavelengths and concentrations employed. Suitable blanks were perpared to correct for spectral contributions of betacyclodextrin.

RESULTS AND DISCUSSION

The effect of betacyclcdextrin on the amount of sulfonamides in aqueous solution in equilibrium with a definite quantity of sulfa drugs is tabulated in Table I—V and shown in Fig. 1—3.

Table I—Total solubility of sulfanilamide in water containing betacyclodextrin.

Betacyclodextrin added to system Concn. $\times 10^3$M	Total sulfanilamide at saturation Concn. $\times 10^2$M		
	15°	20°	30°
0	2.6009	3.3873	5.6858
0.8818	2.6730	3.4591	5.7584
1.7636	2.7461	3.5310	5.8310
2.6454	2.8147	3.6028	5.8915
3.5272	2.8914	3.6644	5.9762
4.4090	2.9639	3.7465	6.0488
5.2908	3.0244	3.8184	6.1214
6.1726	3.0969	3.9005	6.1818
7.0544	3.1813	3.9491	6.2665
7.9362	3.2520	4.0339	6.3270
8.8183	3.3147	4.1058	6.3512

Table II—Total solubilities of sulfamonomethoxine and sulfadimethoxine in water containing betacyclodextrin.

Betacyclodextrin added to system	Total sulfamonomethoxine at saturation. Concn. $\times 10^4$M		Total sulfadimethoxine at saturation. Concn. $\times 10^4$M	
Concn. $\times 10^3$M	20°	30°	20°	30°
0	1.239	1.713	0.694	0.980
0.8818	1.713	2.091	0.810	1.111
1.7636	2.116	2.594	0.903	1.215
2.6454	2.620	2.922	1.018	1.307
3.5272	3.048	3.246	1.111	1.458
4.4090	3.476	3.753	1.215	1.597
5.2908	3.829	4.131	1.319	1.678
6.1726	4.207	4.433	1.435	1.782
7.0544	4.685	5.114	1.527	1.898
7.9362	5.189	5.315	1.643	2.025
8.8183	5.744	5.819	1.736	2.129

Table III—Total solubilities of sulfamerazine and sulfamethazine in water contianing betacyclodextrin.

Betacyclodextrin added to system	Total sulfamerazine at saturation. Concn. $\times 10^4$M		Total sulfamethazine at saturation. Concn. $\times 10^3$M	
Concn. $\times 10^3$M	20°	30°	20°	30°
0	5.608	8.653	1.5569	2.0578
0.8818	6.302	9.347	1.7093	2.2102
1.7636	6.837	10.042	1.8618	2.3408
2.6454	7.531	10.683	2.0469	2.5042
3.5272	8.172	11.484	2.1775	2.6457
4.4090	8.813	12.018	2.3626	2.7763
5.2908	9.721	12.819	2.5259	2.9397
6.1726	10.255	13.353	2.6675	3.1030
7.0544	11.110	14.155	2.8417	3.2173
7.9362	11.965	14.956	2.9941	3.3752
8.8183	12.605	15.543	3.1738	3.5167

Table IV—Total solubilities of sulfamethizole and sulfisoxazole in water containing betacyclodextrin.

Betacyclodextrin added to system	Total sulfamethizole at saturation. Concn. $\times 10^3$M		Total sulfisoxazole at saturation. Concn. $\times 10^3$M	
Concn. $\times 10^3$M	20°	30°	20°	30°
0	1.5231	2.2303	0.5509	0.7855
0.8818	2.0563	2.7743	0.8060	1.0560
1.7636	2.6329	3.2639	1.0202	1.2804
2.6454	3.1551	3.9167	1.2345	1.4960
3.5272	3.6991	4.4390	1.4748	1.7804
4.4090	4.2649	4.9830	1.6171	2.0457
5.2908	4.7654	5.4399	1.8263	2.2344
6.1726	5.3746	6.1145	2.0609	2.4895
7.0544	5.8751	6.6585	2.2548	2.7395
7.9362	6.4409	7.0719	2.4538	2.9078
8.8183	6.8543	7.7465	2.6526	3.0405

Table V—Total solubilities of sulfaguanidine and sulfisomidine in water containing betacyclodextrin.

Betacyclodextrin added to system. Concn. $\times 10^3$M	Total sulfaguanidine at saturation. Concn. $\times 10^3$M		Total sulfisomidine at saturation. Concn. $\times 10^3$M	
	20°	30°	20°	30°
0	2.8833	5.6102	4.3112	5.5629
0.8818	3.3921	6.3408	4.6356	5.8874
1.7636	3.9141	6.6539	4.9371	6.2119
2.6454	4.4490	7.2932	5.2152	6.4900
3.5272	4.9056	7.6971	5.5629	6.8609
4.4090	5.5319	8.0891	5.9106	7.2318
5.2908	6.0538	8.7415	6.1424	7.4867
6.1726	6.5235	9.3677	6.3741	7.8344
7.0544	7.0454	9.7852	6.7218	8.1126
7.9362	7.6455	10.2158	7.0232	8.4834
8.8183	8.0630	10.8290	7.3245	8.7384

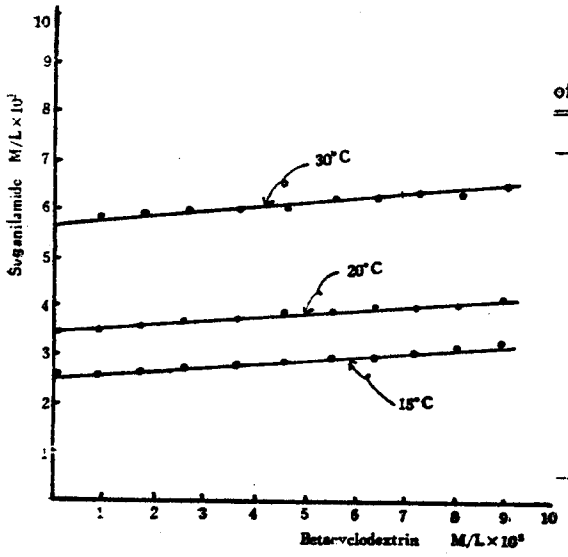

Fig. 1— Influence of betacyclodextrin on the solubility of sulfanilamide.

Table VI—Slopes of interaction isotherms of sulfonamides and beta cyclodextrin (30°C).

Drug	Slope
Sulfadimethoxine	0.01322
Sulfamonomethoxine	0.0475
Sulfamerazine	0.0783
Sulfamethazine	0.164
Sulfisoxazole	0.321
Sulfisomidine	0.375
Sulfaguanidine	0.60
Sulfamethizole	0.618
Sulfanilamide	0.835

A definite interaction was observed between all of the sulfonamides and betacyclodext rin. The linear interaction isotherms obtained, indicating a first-order dependence of th- interactions on the complexing agent concentration, show that the increase in the solubility of the drug is a function of the concentration of the betacyclodextrin. As seen in Fig. 1—3, all of the sulfa drugs studied in this experiments forms soluble complexes with the betacyclodextrin.

Where no plateau region is obtained, exact stoichiometry can't be calculated from these diagram because the concentration of free drug present in solution is an invariant. In this study no plateau regions observed, slopes of the interaction isotherms used a indications of relative complexing tendencies. However, comparison of slopes of interactions of no clear-cut stoichiometries can lead to errors in interpretation. The slopes of these isothe erms are evaluated by the method of least squares are shown in Table VI.

Data indicate the smallest molecule and most soluble sulfonamides show the highest slopes. Results of Lach *et.al.*[3] support this conclusion. A plot of log slope *vs.* log So(solu ility in absence of betacyclodextrin at 30°C), Fig. 4, shows fair regularity of this trend (correlation coefficient:- 0, 8559).

In case of sulfisoxazole and sulfamethizole their solubilities in water are small but heir reactivity with betacyclodextrin is very high. This phenomenon can be attributed to

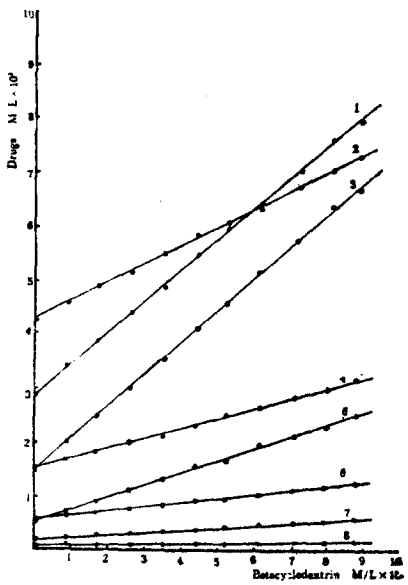

Fig. 2—Influence of betacyclodextrin on the solubilities of sulfa drugs. at 20°C Key: 1, sulfaguanidin; 2, sulfisomidine; 3, sulfamethi ole;4, sulfamethazine; 5, sulfisoxazole; 6, zsulfamerazine; 7, sulfamonomethoxine; 8, sulfadimethoxine.

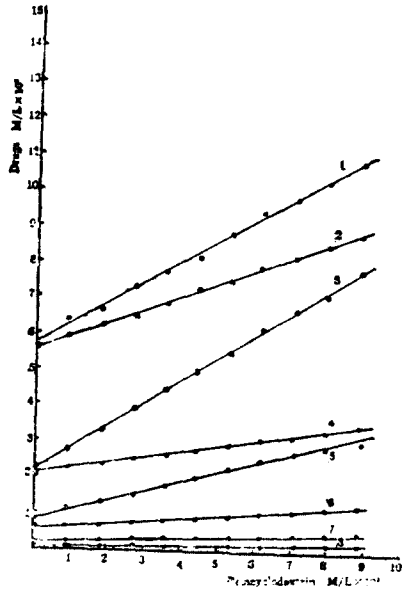

Fig. 3—Influence of betacyclodextrin on the solubilities of sulfa drugs at 30°C. Key: 1, sulfaguanidine; 2, sulfisomidine; 3, sulfamethi zole; 4, sulfamethazine; 5, sulfisoxazole; 6, sulfamerazine; 7, sulfamonomethoxine; 8, sulfadimethoxine.

their small molecular size and to the fact that they have 5-membered isoxazolyl and thiadiazolyl ring, respectively. The presence of reactive functional groups in sulfanilamide and sulfaguanidine capable of bonding with or being partially included by the betacyclodextrin, could be responsible for the observed interactions.

The comparison of the thermodynamic value is another method of the investigation to know the interaction tendencies. Formation constants at serveral temperatures, the heat of reaction, the entropy change and the free energy change have been approximated. As mentioned above, because of no clear-cut stoichiometry the formation constants and therm odynamic values were calculated by assuming that only monomolecular reaction took place between sulfonamide and betacyclodextrin. Thermodynamic functions were obtained from the following equations.

$$\Delta F = -RT \ln K \qquad \Delta H = d(\Delta F/T)/d(1/T) \qquad \Delta S = (\Delta H - \Delta F)/T$$

Fig. 5 shows the slope of the plot of log K against 1/T was supposed to be constant in the present temperature range.

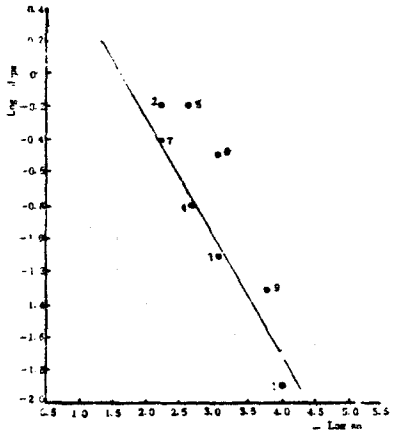

Fig. 4—Relationship of slope of interaction isotherms of drugs with the betacyclodextrin to initial solubility at 30°C. Key: 1, sulfadimethoxine; 2, sulfaguanidine; 3, sulfamerazine; 4, sulfamethazine; 5, sulfanilamide; 6, sulfisoxazole; 7, sulfisomidine; 8, sulfamethizole; 9, sulfamonomethoxine.

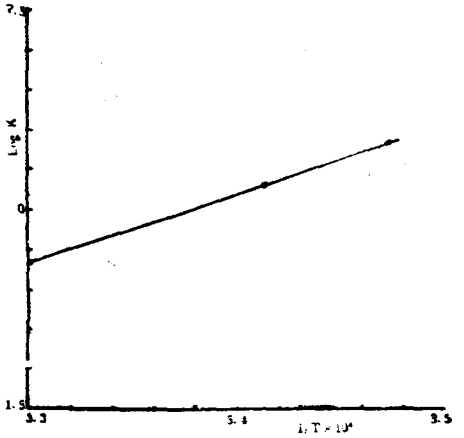

Fig. 5—Relationship between solubility of sulfanilamide and temperature.

Formation constants for this reaction are too large to determine precisely, so calculate approximately. It is evident that the great magnitude of formation constants indicate formation of complex of increased stability. As seen in Table VII, sulfisoxazole, sulfamethiz-

Table VII—Summary of equilibrium constants and thermodynamic values of the betacyclodextrin and sulfonamides interaction.

Complex	Temp.,	Equil. constant.	$-\Delta F$ Kcal/mole	$-\Delta H$ Kcal/mole	ΔS eu
	15	170	3.09	9.4	−22.4
Betacyclodextrin-	20	128	2.83	9.4	−22.4
Sulfanilamide	30	75	2.60	9.4	−22.4
Betacyclodextrin-	20	496	3.61	11.0	−25.2
Sulfaguanidine	30	262	3.35	11.0	−25.2
Betacyclodextrin-	20	175	3.00	4.3	−4.4
Sulfadimethoxine	30	137	2.96	4.3	−4.4
Betacyclodextrin-	20	429	3.53	7.4	−13.2
Sulfamonomethoxine	30	280	3.39	7.4	−13.2
Betacyclodextrin-	20	124	2.81	3.3	−1.6
Sulfisomidine	30	103	2.79	3.3	−1.6
Betacyclodextrin-	20	614	3.74	4.4	−2.3
Sulfisoxazole	30	479	3.72	4.4	−2.3
Betacyclodextrin-	20	146	2.90	7.0	−13.9
Sulfamerazine	30	98	2.76	7.0	−13.9
Betacyclodextrin-	20	141	2.88	6.6	−12.6
Sulfamethazine	30	97	2.76	6.6	−12.7
Betacyclodextrin-	20	1057	4.05	6.5	−8.4
Sulfamethizole	30	730	3.97	6.5	−8.4

-ole, sulfamonomethoxine and sulfaguanidine have quite high formation constants compared to those other sulfonamides. It is well known that a complex is often formed between solubilizing agent and organic substance accompanying the increase of solubility. In such a complex formation, generally, enthalpy decrease in the amount of several Kcal/mole, and entropy also decreases. The results obtained above were the same with respect this point.

The large negative free energies of formation also indicate that the fit between the betacyclodextrin and the various sulfa drugs must be quite favorable.

The negative entropy change means the decrease of the freedom of molecule movement due to form a larger complex molecule.

Besides the solubilities and molecular sizes of sulfonamides we assume that other force such as hydrogen bonding play a significant role in this reaction. As we know the betac yclodextrin has many hydroxyl and carboxyl groups in acqueous solution.

CONCLUSION

Sulfonamides and betacyclodextrin are shown to interact in acqueous solution. The data indicate that the smallest and most soluble drugs in water showed the greater inclusion formation with betacyclodextrin. The thermodynamic functions such as formation constant, enthalpy change, free energy change and entropy change have been approximated. The mechanism of this interaction can't be completely interpreted but inclusion formation s chiefly dominant and in turn the hydrogen bonding

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