[Chem. Pharm. Bull. 24(9)2094—2101(1976)]

UDC 547.68.02 : 548.33

Effect of Additives on the Polymorphic Transformation of Chlortetracycline Hydrochloride Crystals

Shozo Miyazaki,[1a] Masahiro Nakano, and Takaichi Arita[1]

Faculty of Pharmaceutical Science, Hokkaido University[1]

(Received November 21, 1975)

The effect of various additives on the polymorphic transformation of the more energetic chlortetracycline hydrochloride (CTC-HCl) β form to the water stable α form in aqueous suspensions was studied by infrared spectrophotometric method. This transformation was found to be retarded by the addition of some pharmaceutical additives such as sodium carboxymethylcellulose (CMC-Na), pectin, gelatin, and acacia; which have been commonly coadministered with drugs. The attempt was made to elucidate possible mechanisms of this effect. The results suggest that the effect of CMC-Na or pectin on transformation rates may be mainly due to the increased viscosity. On the other hand, the adsorption of gelatin or acacia on drug particles may be one of several factors which retard the transformation.

The effect of these additives on the blood levels of the CTC-HCl β form in suspensions was also tested using the rat as the test animal. Higher blood levels were observed when the CTC-HCl β form was administered with CMC-Na or pectin than those in its absence, probably because the additives retarded the transformation. Administration of the CTC-HCl β form with gelatin or acacia resulted in slightly lower or almost identical blood levels compared to control values. It is suggested that gelatin or acacia may interfere with intestinal absorption of the drug.

When polymorphism is present, various crystal modifications of solid drug can exhibit drastically different physical characteristics. Most important of these characteristics from a standpoint of drug bioavailability is different dissolution characteristics such as solubility and dissolution rate. As a result of different dissolution characteristics, the polymorphic form may also differ with respect to bioavailability. Since the higher energy crystal form of drugs exhibits higher activity and the transformation of the unstable form to more stable form produces a less soluble form of the drug, maintenance of the physical stability of the high energy polymorphic form of drugs becomes very important.

Polymorphic transformations have been extensively studied, but studies on the retardation of the transformation are relatively few.[2-8] It has been found that additives such as macromolecules,[2,3,7,8] surfactant,[5,8] and structurally related compounds to the polymorphic drug[4,6,8] prevent the undesirable polymorphic changes in aqueous suspensions.

In our previous paper,[9] the chlortetracycline hydrochloride (CTC–HCl) β form which has a higher solubility and greater bioavailability than the α form,[10] was found to be transformed to the water stable α form in aqueous suspensions in a short period of time by infrared (IR) spectrophotometric method. In the solid state, however, stabilization of the CTC–HCl β form may be achieved by abstracting moisture from its environment.

1) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo*; *a*) Present address: *Faculty of Pharmaceutical Sciences, Josai University, Keyakidai 1-1, Sakado, Saitama.*
2) J.D. Mullins and T.J. Macek, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 245 (1960).
3) E. Shefter and T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **52**, 781 (1963).
4) H.Y. Saad and W.I. Higuchi, *J. Pharm. Sci.*, **54**, 1303 (1965).
5) J.E. Carless and A.A. Foster, *J. Pharm. Pharmac.*, **18**, 697 (1966).
6) J.E. Carless, M.A. Moustafa, and H.D.C. Rapson, *J. Pharm. Pharmac.*, **20**, 639 (1968).
7) L.J. Ravin, E.G. Shami, and E. Rattie, *J. Pharm. Sci.*, **59**, 1291 (1970).
8) A.R. Ebian, M.A. Moustafa, S.A. Khalil, and M.M. Motawi, *J. Pharm. Pharmac.*, **25**, 13 (1973).
9) S. Miyazaki, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **24**, 1832 (1976).
10) S. Miyazaki, T. Arita, R. Hori, and K. Ito, *Chem. Pharm. Bull.* (Tokyo), **22**, 638 (1974).

The object of this investigation was to study the effect of additives on the polymorphic transformation of the more energetic crystal form (β form) of CTC–HCl in aqueous suspensions. The IR spectrophotometric method previously described[9] was used to measure the rates of such transformations. In addition, studies were undertaken to determine the effect of additives on the blood levels of the CTC–HCl β form using the rat as the test animal.

Experimental

Materials——The CTC-HCl α and β forms were prepared as described previously.[10] The CTC-HCl β form used in this study was a mixture containing 8—10% w/w of the α form in the β form. Additives used were; methylcellulose, 4000 cps[11]; gelatin[11]; sodium carboxymethylcellulose[12]; polyvinylpyrrolidone, K-90[13]; polyvinylalcohol[12]; albumin from egg[12]; acacia, J.P.[14]; pectin from citrus[11]; soluble starch[12]; dextrin[15]; sodium alginate, 300 cps[12]; sucrose[11]; polysorbate 80[12]; and sodium lauryl sulfate[16].

Measurement of Rates of Transformation——A same method as that described previously[9] was used for the determination of transformation behaviors in aqueous suspensions at 20°. Suspensions of the CTC-HCl β form at 6% w/v concentration, each containing various concentrations of additives, were prepared. The suspensions were left in a constant temperature bath without agitation. Samples were taken at various time intervals, filtered and the concentration of the β form in the solid phase was determined by IR spectrophotometric method as described before.[9]

Procedure for Dissolution Studies——Dissolution behaviors of crystalline powder were determined as previously reported[10] at 20° in distilled water and aqueous solutions containing additives.

Solubility Determinations——The solubility of CTC-HCl (α form) were determined at 20° in water and aqueous solutions containing various concentrations of additives, by the method similar to that described previously.[9]

Measurement of Viscosity——The relative viscosity measurements were made with an Ostwald viscometer with water at 20°.

Procedure for Absorption Studies——Male Wistar rats, weighing 180—220 g, were fasted for 20—24 hr before the experiment. One ml aqueous suspensions at 6% w/v concentration, each containing a known amount of additives, were administered orally to rats by a stomach tube. The animals were sacrificed by decaptitation and blood samples were analysed following Kohn's method.[17]

Results and Discussion

Effect of Additives on Polymorphic Transformation

The relative rate of transformation of the CTC–HCl β form was evaluated in various concentrations of additive solutions by IR spectrophotometric method. A list of additives tested and their effects on the transformation rate at 20° are given in Table I, where the transformation rates are expressed as the time required for 50% of the β form to be transformed, and in Figs. 1 and 2.

The use of surfactants to retard the transformation was suggested by Higuchi.[18] Mullins and Macek[2] found that methylcellulose and polyvinylpyrrolidone decreased the rate of polymorphic transformation of novobiocin. The result in this study showed that these substances were ineffective in retarding the overall transformation process of the CTC–HCl β form. On the other hand, Shefter and Higuchi[3] found the 2.0% acacia and 2.5% gelatin decreased the relative rates of nucleation and growth of the stable form of theophylline.

Some agents such as sodium carboxymethylcellulose (CMC-Na), pectin, gelatin, and acacia were found to alter the rates of the transformation, as shown in Figs. 1 and 2.

11) Wako Pure Chemical Industries.
12) Koso Chemical Co.
13) Daiichi Pure Chemical Co.
14) Iwaki Seiyaku Co.
15) Nakarai Chemical Co.
16) Kanto Chemical Co.
17) K.W. Kohn, *Anal. Chem.*, **23**, 862 (1961).
18) T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 657 (1958).

CMC–Na was found in this study to be more effective than any other additives observed in retarding the transformation. The effect of CMC–Na on the transformation behavior was dependent on its concentration, resulting in the greater effect at high concentrations, as shown in Fig. 1(A). Table I shows the pH of CMC–Na solutions to be slightly increased, comparing with that of distilled water. Pectin produced similar effect and decreased the rate of transformation of the β form. The additive tended to decrease the pH of the medium.

As shown in Fig. 2(A), gelatin decreased the rate of transformation with the 1 and 2% concentrations. In addition, the rate-retarding effect of acacia was observed with the 2 and 4% concentrations. The pH of solutions was slightly increased by addition of gelatin and decreased by acacia.

TABLE I. Effect of Additives on the Transformation of the Chlortetracycline Hydrochloride β Form in Aqueous Suspensions[a] at 20°

Additive	Concentration (w/v %)	pH of solution	$T_{50\%}$[b] (min)
No additive (distilled water)	—	5.6—9	1.6
Methylcellulose	0.2	5.42	1.7
	0.5	5.12	2.2
	0.8	5.30	4.3
Sodium carboxymethylcellulose	0.2	6.23	6.4
	0.5	6.35	8.4
	0.8	6.40	9.6
Polyvinylpyrrolidone	1.0	6.13	2.0
Polyvinylalcohol	2.5	6.10	2.7
Gelatin	1.0	6.20	6.2
	2.0	6.15	5.8
Alubumin	0.5	6.52	2.0
	2.0	6.82	3.3
Acacia	2.0	4.82	5.3
	4.0	4.61	4.6
Pectin	0.5	3.71	3.6
	1.0	3.42	6.7
Starch	2.0	6.32	3.4
	4.0	6.28	2.0
Dextrin	2.0	4.01	2.6
Sodium alginate	0.2	6.15	2.5
Sucrose	5.0	5.67	2.2
Polysorbate 80[c]	0.05	5.90	2.7
Sodium lauryl sulfate	0.05	5.31	2.3

a) 6.0% suspension of the chlortetracycline hydrochloride β form prepared in distilled water.
b) $T_{50\%}$ is the time necessary for the transformation of 50% of the β form.
c) % v/v

It is certain that some adjuvant will be applied to retard the transformation of the CTC–HCl β form in aqueous suspensions. No agent, however, provided adequate protection against the transformation for really significant period of time in these experimental conditions employed. Since the pH of solutions and temperature were found to be very important factors influencing the rates of transformation in aqueous suspensions,[9] their effects on the transformation in this system were investigated.

The effect of CMC–Na at the 0.2% concentration was compared in aqueous and buffered suspensions; the buffered suspension was prepared by suspending the β form in a 0.2% solution of CMC–Na in pH 5.30 phosphate buffer. As can be seen from Fig. 3, there was some stabilizing action in the buffered suspensions. The CTC–HCl β form suspension prepared in pH 5.30 buffer exhibited a longer half-life than the corresponding suspension prepared in water at 20°. This was due to the higher final pH of the buffered suspension.

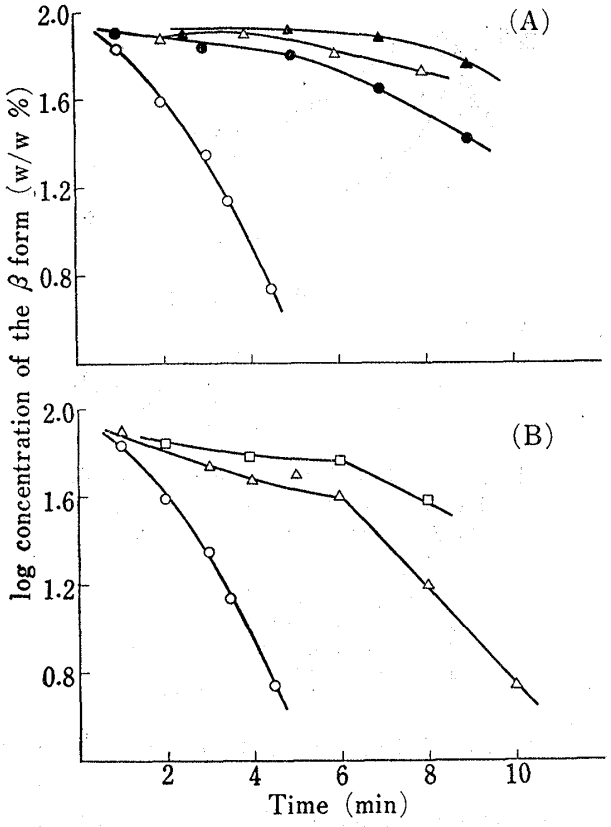

Fig. 1. Effect of Additives on the Transformation of the Chlortetracycline Hydrochloride β Form in Aqueous Suspensions at 20°

(A): —○—, no additive; —●—, 0.2% CMC-Na; —△—, 0.5% CMC-Na; —▲—, 0.8% CMC-Na
(B): —○—, no additive; —△—, 0.5% pectin; —□—, 1% pectin

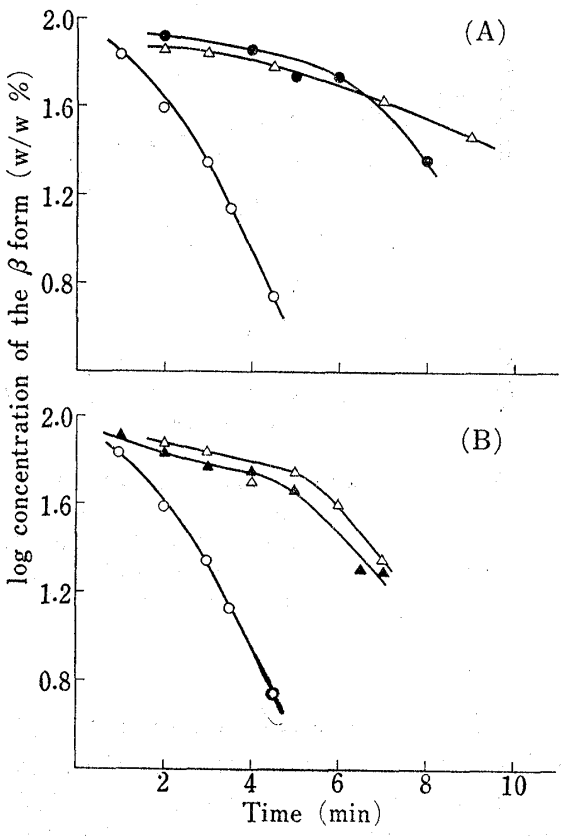

Fig. 2. Same as Fig. 1

(A): —○—, no additive; —●—, 1% gelatin; —△—, 2% gelatin
(B): —○—, no additive; —△—, 2% acacia; —▲—, 4% acacia

Furthermore, the buffered suspension kept at 5° exerted a pronounced stabilizing action; $T_{50\%}>2$ days.

These results suggest that the transformation will be drastically inhibited by adjusting the pH of solution and/or the temperature of storage.

Studies were carried out with CMC–Na, acacia, and gelatin to determine their effects on the dissolution behavior of the CTC–HCl β form in suspensions. It was impossible to study CMC–Na at higher concentrations and pectin because of the difficulty of the filtration. The result is shown in Fig. 4, where the concentration of the drug in solution are plotted as a function of time. In order to facilitate a comparison of the dissolution behavior in the presence of additives, the figure also includes the dissolution curves of the α and β forms in distilled water at 20°. The distinct difference in the dissolution behavior was observed between the two crystal forms in water, the CTC–HCl β form yielding a concentration supersaturated with respects to the α form in an initial stage of dissolution. However, the decrease in concentration in solution with time after the peak solubility was observed for the β form within a short time, as being due to nucleation and growth of the more stable crystal form.[3,19]

The dissolution process was apparently altered in the presence of additives tested. The more slow decline of the concentration in solution in the presence of the additives resulted from the retardation of transformation. In particular, CMC–Na had a significant effect on the rate of the stable form formation.

19) A.L. Thakkar and N.A. Hall, *J. Pharm. Sci.*, **58**, 68 (1969).

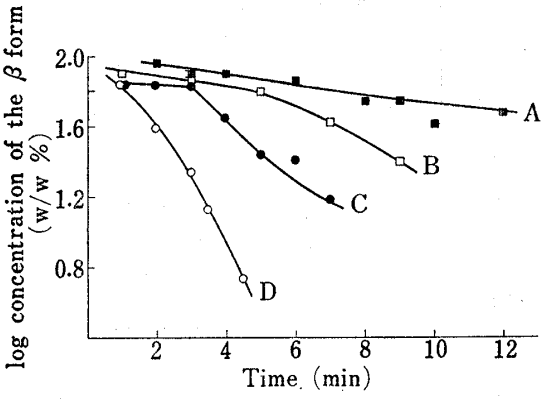

Fig. 3. Transformation of the Chlortetracycline Hydrochloride β Form Suspensions at 20° in: A, 0.2% CMC–Na in pH 5.30 Buffer. B, 0.2% CMC–Na in Water. C, pH 5.30 Buffer Alone. D, Water Alone.

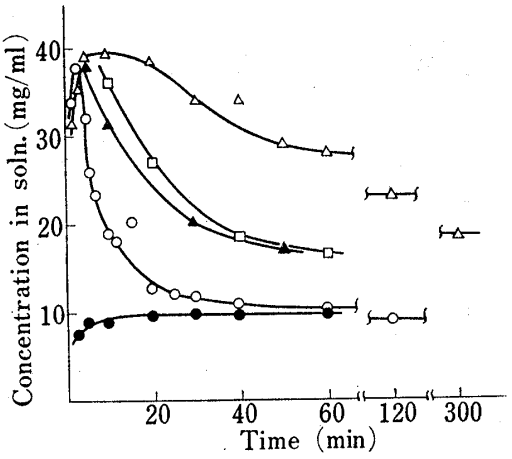

Fig. 4. Dissolution Curves of the Chlortetracycline Hydrochloride α and β Forms in the Presence and Absence of Additives at 20°

—○—: β form in water
—●—: α form in water
—△—: β form in 0.1% CMC–Na solution
—▲—: β form in 2% gelatin solution
—□—: β form in 4% acacia solution

The attempt was also made to elucidate the mechanisms of action of the macromolecular substances for retarding the transformation.

Solubility determinations were designed to test the presence of (or absence of) any appreciable interaction between CTC–HCl and additives at the concentrations used in these studies. The study could not be carried out with CMC–Na at higher concentrations due to the difficulty of the filtration. As shown in Table II, the solubility was decreased by the addition of acacia and gelatin, and to a lesser degree by that of pectin, while slight decreases were observed in CMC–Na solutions. The decrease in CTC–HCl solubility in the presence of gelatin and acacia may be due to the adsorption of the additives on the CTC–HCl particles.[20]

This agrees with qualitative results obtained from microscopical observation of the accompanying changes in crystal shape. The residue remaining after equilibration (overnight at 20°) of an excess sample of the CTC–HCl β form with additive solutions was collected for microscopic examinations. Photomicrographs of crystals obtained with 4% gelatin and 4% acacia are shown in Fig. 5. The transformation of the β form (needles) into the α form (platelets) in aqueous suspensions was accompanied by the change in the crystal habit, as shown in Fig. 5(A). In the solutions of both CMC–Na and pectin the crystal exhibited no external sign of the change in habit. The difference in the crystal habit could be detected in both gelatin and acacia solutions. Identification of the formed crystals was determined by X-ray powder diffraction using a Rigaku Denki D-9C X-ray Diffractometer. This result indicated that the crystals were fully the CTC–HCl α form.

These results suggest that the adsorption of gelatin and acacia on CTC–HCl particles during the transformation may be responsible for the marked delayed transformation.[21,22]

The results of relative viscosity measurements at different concentrations of additives are shown in Table III. In all cases, the viscosity appears to increase with increasing concentrations. CMC–Na and pectin, however, had a much higher viscosity than the others. This suggests that in CMC–Na and pectin solutions viscosity behavior was more important than the others.

20) R.N. Nasipuri and S.A.H. Khalil, *J. Pharm. Sci.*, **62**, 473 (1973).
21) F.V. Williams and R.A. Ruehrwein, *J. Am. Chem. Soc.*, **79**, 4898 (1957).
22) D.J. Allen, G. Milosovich, and A.M. Mattocks, *J. Pharm. Sci.*, **54**. 383 (1965).

Table II. Solubility of Chlortetracycline Hydrochloride in the Presence of Additives at 20°

Additive	Concentration (w/v %)	Solubility (mg/ml)
No additive (distilled water)	—	9.83
Sodium carboxymethylcellulose	0.01	9.29
	0.05	9.19
	0.1	9.29
Pectin	0.5	8.33
	1.0	7.79
Gelatin	1.0	7.48
	2.0	5.45
Acacia	2.0	6.96
	4.0	6.32

Table III. Relative Viscosity of Additive Solutions at 20°

Additive	Concentration (w/v %)	Relative viscosity
No additive (distilled water)	—	1.0
Sodium carboxymethylcellulose	0.2	6.5
	0.5	20.1
	0.8	44.5
Pectin	0.5	3.8
	1.0	12.2
Gelatin	1.0	1.2
	2.0	1.7
Acacia	2.0	2.4
	4.0	3.2

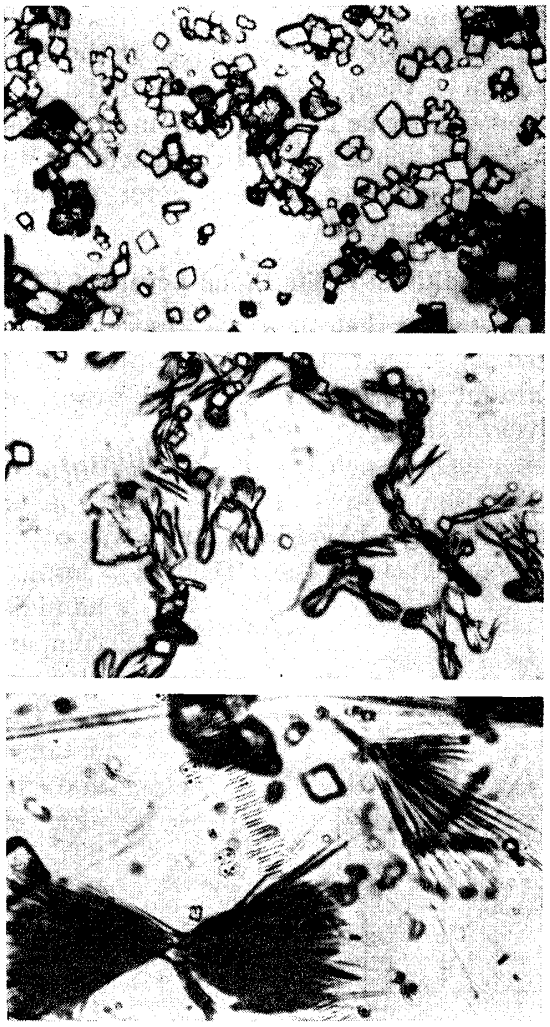

Fig. 5. Effect of Additives on the Habit of the Chlortetracycline Hydrochloride α Form Crystals grown in the Aqueous Suspensions of the β Form (×600)

(A): no additive (B): 4% gelatin (C): 4% acacia

Polymorphic transformation in aqueous suspensions may be retarded by increasing the viscosity of the medium or by addition of substances that are capable of adsorbing on the crystals.[18] There is the possibility that retarding the transformation of the CTC–HCl β form depends on the following factors: the viscosity or pH of solution, interaction of CTC–HCl with additives, adsorption of macromolecules on the drug particle, and so on. A few observation provided some insight into the mechanism of retardation the transformation in the presence of additives.

The experimentally observed higher viscosity of CMC–Na and pectin solutions than others indicates that the effect of this group of additives may be attributed to the increased viscosity which retards the diffusion controlled process involved in solution phase transformation.[9] In highly viscous solutions, the molecular mobility of the drug would be severely decreased. This, in turn, slows down the dissolution, nuclei formation, and crystal growth on the nuclei formed.

$$\text{solid } \beta \text{ form} \xrightarrow{\text{dissolution}} \text{solute molecules} \xrightarrow{\text{nucleation}} \text{nuclei} \xrightarrow{\text{crystal growth}} \text{solid } \alpha \text{ form}$$

On the other hand, it seemed that interaction of CTC–HCl with additives is one of several factors which retarded the transformation in the presence of gelatin and acacia, resulting

in the decrease in solubility. The effect of these additives which is evident from Fig. 5 is preferential adsorption of additives at the nuclei formed and/or growing crystal surface of the stable form. Allen, *et al.*[22] found that the effect of surface active agents in retarding the growth of the needle habit of sodium urate could be one of adsorption. A similar mechanism was suggested for the inhibited dissolution[23] and crystallization.[24] Further studies of the simultaneous influences of these factors should be made in order to get a complete understanding of the mechanism.

Effect of Additives on the Blood Levels of CTC after Administration of the CTC-HCl β Form

It is known that the drug absorption is affected by pharmaceutical additives.[25-28] It has been suggested that certain excipient materials may prevent the polymorphic transformation in aqueous suspensions.[2-8] However, no attention has been paid to the effect of the additives on the drug absorption.

Some agents such as CMC–Na, pectin, gelatin, and acacia were found to retard the overall transformation process of the CTC–HCl β form, which are commonly coadministered with various drugs. A blood level study was conducted in rats to determine whether or not they affect blood levels of the CTC–HCl β form. Blood concentrations of CTC–HCl after oral administration of the β form with additives were compared to control levels produced by the drug given alone.

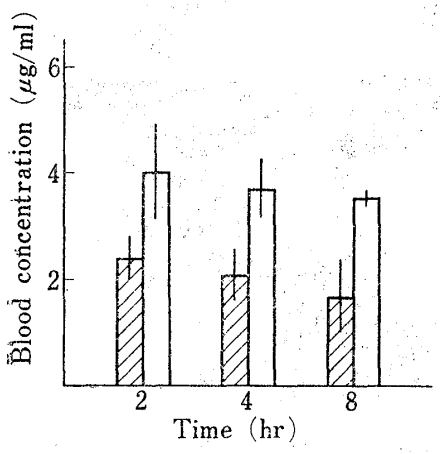

Fig. 6. Blood Levels after Oral Administration of the α and β Forms of Chlortetracycline Hydrochloride to Rats

▨: α form □: β form
The 60 mg of sample dispersed in 1 ml of water was given by a stomach tube. Values are given as the mean±S.E. of four experiments.

In the previous paper,[10] blood plasma levels obtained in rabbits after intraduodenal administration and cumulative amounts excreted in human subjects after oral administration were compared for the CTC–HCl α and β forms. The results indicated that the β form was more efficiently absorbed from the gastrointestinal tract of rabbits and human subjects than the α form. In this study, blood concentrations of CTC–HCl were determined first after oral administration of the α and β forms as suspension in water to rats, in order to use the rat as the test animal.

As indicated in Fig. 6, the administration of the CTC–HCl β form gave higher concentrations in blood than those after the administration of the α form. The similar result with that of previous paper[10] was also noted after oral administration to rats and the result of this comparison suggests that in rats CTC–HCl β form is better absorbed than the α form.

Figures 7 and 8 show the blood concentrations of CTC–HCl obtained following oral administration of the β form to rats in the presence and absence of the additives at the solution concentration of interest. As shown in Fig. 7, it is apparent that, in the first 4 hour, CTC–HCl concentrations in the presence of CMC–Na were higher than those in its absence. The data obtained in this study are in accord with the dissolution data (Fig. 4). The increase in CTC–HCl blood levels at 2 and 4 hours in the presence of CMC–Na may be explained by the transformation retarding effect, thereby increasing the amount of CTC–HCl available in the absorp-

23) J. Piccolo and R. Tawashi, *J. Pharm. Sci.*, **59**, 57 (1970).
24) A. Otsuka, H. Sunada, and M. Bo, *Yakuzaigaku*, **31**, 97 (1971).
25) P. Singh, J.K. Guillory, T.D. Sokoloski, L.Z. Benet, and V.N. Bhatia, *J. Pharm. Sci.*, **55**, 63 (1966).
26) H. Seager, *J. Pharm. Pharmac.*, **20**, 968 (1968).
27) R. Tawashi and J. Piccolo, *J. Pharm. Sci.*, **61**, 1857 (1972).
28) M.J. Akers, J.L. Lach, and L.J. Fisher, *J. Pharm. Sci.*, **62**, 391 (1973).

tion. When the pectin was administered in conjugation with dose of the CTC–HCl β form, higher blood levels of the drug were also obtained, probably because the additives retarded the transformation.

It is reasonable to expect that these additives do not affect the absorption of CTC–HCl. Greenspan, *et al.*[29] reported that CMC–Na did not adsorb CTC–HCl *in vitro*, and did not interfere with the intestinal absorption.

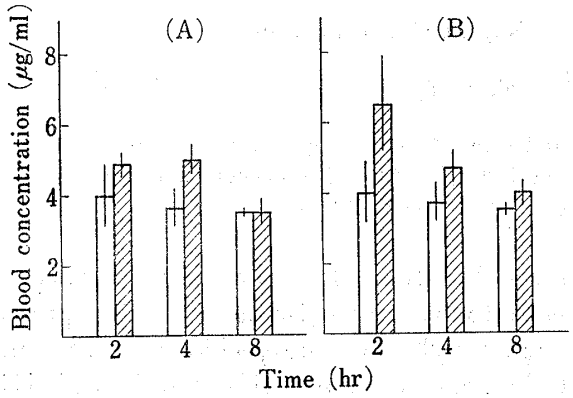

Fig. 7. Effect of 0.2% Sodium Carboxymethylcellnlose (A) and 1% Pectin (B) on Blood Levels after Oral Administration of the Chlortetracycline Hydrochloride β Form to Rats

□: drug alone ▨: in the presence of additive
The 60 mg sample dispersed in 1 ml of additive solutions was administered by a stomach tube. Values are give as the mean±S.E. of four experiments.

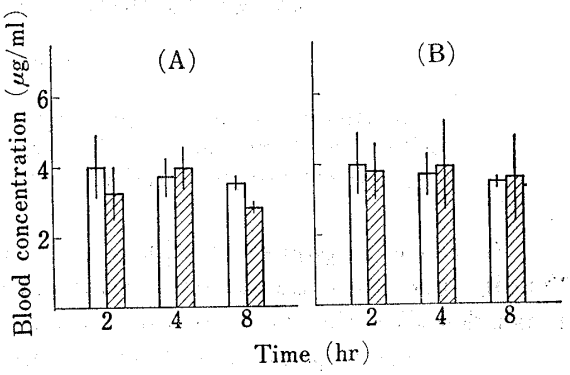

Fig. 8. Effect of 2% Gelatin (A) and 4% Acacia (B) on Blood Levels after Oral Administration of the Chlortetracycline Hydrochloride β Form to Rats

□: drug alone ▨: in the presence of additive
The 60 mg of sample dispersed in 1 ml of additive solutions was administered by a stomach tube. Values are given as the mean±S.E. of three experiments in the presence of both additives.

On the other hand, it is evident from the results presented in Fig. 8 that the combination of the CTC–HCl β form with gelatin resulted in slightly lower blood levels of the drug compared to those resulting from adminsitration of the drug alone except at 4 hour. In addition, no difference in the CTC–HCl blood levels were observed after oral administration of the β form when the drug was administered with the acacia. On the basis of dissolution study, some differences in the blood levels would be expected by the CTC–HCl β form administration with the gelatin and acacia. However, the administration of the CTC–HCl β form with the gelatin and acacia resulted in slightly lower or almost identical blood levels compared to control levels. This result suggests that these additives may affect the intestinal absorption of CTC–HCl. The possible influence by gelatin or acacia on the gastrointestinal absorption of CTC–HCl from the α form suspension or the solution is worthy of further investigation. Intraduodenal administration of CTC–HCl solution with gelatin or acacia to rats resulted in significantly lower blood levels of the drug compared to those resulting from the administration of the drug solution alone.[30]

From a standpoint of drug bioavailability, these findings indicate that CMC–Na and pectin could be used to retard the polymorphic transformation in this system. On the other hand, special precautions should be taken against possible retardation when gelatin or acacia are used, because the blood concentration of CTC after the administration of the CTC–HCl β form may be affected by these additives.

29) R. Greenspan, H. Maclean, A. Milzer, and H. Necheles, *Am. J. Digest. Dis.*, **18**, 35 (1951).
30) S. Miyazaki, M. Nakano, and T. Arita, unpublished data.