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Polymorphic Transformation of Chlortetracycline Hydrochloride Crystals Studied by Infrared Spectrophotometric Method[1]

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A quantitative method was described which utilized infrared spectroscopy for the determination of chlortetracycline hydrochloride (CTC–HCl) crystalline forms in mixtures. Data were included which demonstrated that the method could be utilized to follow the rate of transformation taking place in the biologically available crystalline form (β form).

Transformation of the crystalline form under different conditions such as solid state and aqueous suspension was investigated. The solid CTC–HCl β form was shown to change to the more stable α form in high humidities, over 75% relative humidity (R.H.) at 20°. The rate of transformation was found to be proportional to the humidity. On the other hand, under 65% R.H. no polymorphic transformation was observed for 40 days at 20°. Transformation of the β form was also not detected by heating at 70°. In aqueous suspensions, rapid solution phase transformation was observed from the CTC–HCl β form to the water-stable α form. The rate of transformation in the suspensions was found to be temperature and pH dependent.

Results obtained from these transformation studies suggested that water and water vapor were important factors in the transformation of the CTC–HCl β form.

In the previous work,[3] two crystal forms (α and β forms) of chlortetracycline hydrochloride (CTC–HCl) were prepared and their dissolution and absorption characteristics were studied. From the dissolution studies with crystalline powder, the CTC–HCl β form which has a higher solubility and greater bioavailability than the α form was found to be transformed to the α form in a relatively short period. Furthermore, the two crystal forms were compared for their behaviors on exposure to humidities. The data obtained indicated that the CTC–HCl α form was stable while the β form was hygroscopic and was converted to the α form under high humidities such as 84% or 95% relative humidities (R.H.).

More recently, much attention has been given to the possible utilization of more energetic crystal forms of drugs in pharmaceutical preparations. Since the greater bioavailability of the metastable modification may be affected by the polymorphic transformation, it has become desirable to determine rates of transformation to the stable form and to find means of retarding this transformation. An example of such a rate study is that by Moustafa, *et al.*,[4] who determined the kinetics of interconversion of sulfamethoxydiazine from Form II to Forms I

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4) *a*) M.A. Moustafa, S.A. Khalil, A.R. Ebian, and M.M. Motawi, *J. Pharm. Pharmac.*, **24**, 921 (1972); *b*) A.R. Ebian, M.A. Moustafa, S.A. Khalil, and M.M. Motawi, *ibid.*, **25**, 13 (1973).

or III and studied the effect of additives on the rate of transformation. A number of other studies on polymorphic transformation have been described.[5-12]

The polymorphic transformation has been investigated by various method; infrared (IR) attenuated total reflectance method,[5] microscopic method,[6] Coulter Counter method,[7] X-ray diffraction method,[8] solubility method,[9] differential scanning calorimetric method,[10] and dilatometric method.[11] An IR spectrophotometric method has been used extensively in the past for the study of polymorphism. However, the use of this method in measuring rates of the transformation was not fully investigated except in a few reports.[4,12]

In the present paper, a simple but convenient method using IR spectroscopy is presented for the quantitative determination of the CTC–HCl β form in the presence of the α form. It is the purpose of this report to use this procedure to follow the kinetics of transformation of the CTC–HCl β form to the α form in suspension or solid states.

Experimental

Materials——Anhydrous CTC–HCl used was supplied by Lederle (JAPAN), LTD. The α and β forms of CTC–HCl were prepared as described previously.[3a] All other materials were of J. P. VIII or reagent grade.

IR Spectrophotometric Method——IR spectra were recorded on a JASCO IR-S spectrophotometer. All samples were prepared as Nujol mulls. The IR spectra of α form, β form, and 1: 1 mixture of α and β forms between 2800 cm^{-1} and 3600 cm^{-1} were shown in Fig. 1. A mixture of α and β forms was determined by using absorbance at 3380 cm^{-1} and 3280 cm^{-1} as key bands. The absorbance of the key bands was measured by the base line method.[13] The base line chosen is shown in Fig. 1.

The base line absorbance (Ab) is obtained from the equation

$$Ab = \log (I_0/I)$$

where I_0 is the distance from zero line to the base line, and I the distance from the zero line to the top of the absorbance peak. The absorbance ratio Ab 3380 cm^{-1}/Ab 3280 cm^{-1} was measured for the mixture of the α and β forms. The β form content was calculated from the measured absorbance ratio by referring to a calibration curve as that in Fig. 2, obtained using reference samples at different concentration in Nujol mull.

Measurement of Rates of Transformation——a) Transformation in the Solid State: About 200 mg samples of the β form were spread on watch glasses and placed in a desiccator adjusted to constant relative humidities (65, 75, 84, and 100%) by saturated salt solutions[14] at $20 \pm 2°$. Samples were taken at various time intervals and assayed for the β form as described above.

b) Transformation in Suspension: The rates of transformation were studied by preparing 6% w/v suspension of the β form in aqueous solutions. One milliliter portion of solvents previously kept at the experimental temperature was added rapidly into individual 10 ml Erlenmeyer flasks, which contained 60 ± 1 mg of the sample, and they were left in a constant temperature bath without agitation. The portions of residual solid materials were taken at various time intervals by separating from the solvent by filtration. The resulting precipitate was washed with a small amount of cold acetone and dried *in vacuo* at room temperature. The concentration of the β form in the residue was determined as described above.

The CTC–HCl β form used in the transformation studies was a 8—10% w/w mixture of the α form in the β form.

5) W.I. Higuchi, W.E. Hamlin, and S.C. Mehta, *J. Pharm. Sci.*, **58**, 1145 (1969).
6) *a*) J.P. Mullins and T.J. Macek, *J. Am. Pharm. Assoc., Sci. Ed.*, **49**, 245 (1960); *b*) S.-L. Lin and L. Lachman, *J. Pharm. Sci.*, **58**, 377 (1969).
7) *a*) J.E. Carless, M.A. Moustafa, and H.D.C. Rapson, *J. Pharm. Pharmac.*, **20**, 630 (1968); *b*) J.T. Pearson and G. Varney, *ibid.*, **21**, Suppl., 60S (1969).
8) *a*) E. Schefter and G. Kmack, *J. Pharm. Sci.*, **56**, 1028 (1967); *b*) E. Shefter, H.-L. Fung, and O. Mok, *ibid.*, **62**, 791 (1973).
9) *a*) E. Shefter and T. Higuchi, *J. Pharm. Sci.*, **52**, 781 (1963); *b*) S.-L. Lin, *ibid.*, **61**, 1423 (1972).
10) *a*) M.A. Moustafa and J.E. Carless, *J. Pharm. Pharmac.*, **21**, 359 (1969); *b*) E.G. Shami, P.D. Bernardo, E.S. Rattie, and L.J. Ravin, *J. Pharm. Sci.*, **61**, 1319 (1972).
11) A.J. Aguiar, *J. Pharm. Sci.*, **58**, 963 (1969).
12) M.A. Moustafa, S.A. Khalil, A.R. Ebian, and M.M. Motawi, *J. Pharm. Sci.*, **63**, 1103 (1974).
13) M. Maruyama, N. Hayashi, and M. Kishi, *Takamine Kenkyusho Nempo*, **13**, 176 (1961).
14) H.M. Spencer, *International Critical Table*, **1**, 67 (1962).

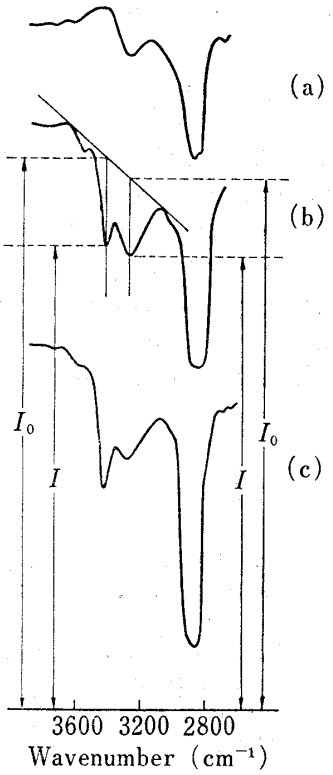

Fig. 1. Method for Determining the Base-Line Absorbance

(a) CTC-HCl α form
(b) 1: 1 mixture of CTC-HCl α and β forms
(c) CTC-HCl β form

Solubility Measurement——Solubility of CTC–HCl was determined in distilled water at different temperatures and in acetate buffers at different pH values. Aqueous suspension containing excess amounts of samples (α form) was shaken mechanically overnight. The filtrate was then assayed following the method described by Levine, *et al.*,[15] using a Hitachi Model 139 spectrophotometer. A filter (Millipore) pore size of 0.45 μ was used.

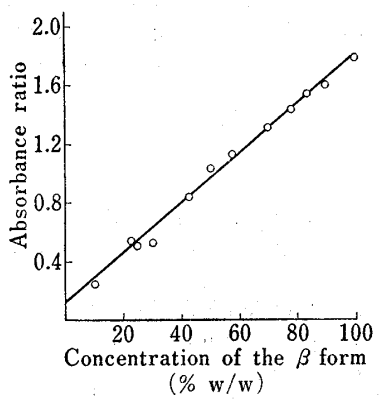

Fig. 2. Calibration Curve for Determination of the Chlortetracycline Hydrochloride β Form in the the Presence of the α Form Using the Ratio Ab 3380 cm^{-1}/Ab 3280 cm^{-1}

Results and Discussion

Application of IR Spectrophotometric Method for the Study of Polymorphic Transformation

An IR spectrophotometric method was investigated for the determination of the ratio of the CTC–HCl α form to the β form in the mixture and for the measurement of rates of polymorphic transformation. The method of measurement, as shown in Fig. 1, was found to give reproducible results.

Figure 2 illustrates a linear relationship between the concentration of the CTC–HCl β form and absorbance ratio of 3380 cm^{-1} to 3280 cm^{-1}. This relationship was utilized to quantitate the amount of the β form in samples. Accordingly, by using this calibration curve, analysis of unknown samples could be made. To verify this, mixtures of the CTC–HCl α and β forms were prepared and determined. The amount of the β form in the mixture varied from about 40 to 80%. The result of this experiment is shown in Table I, in which agreements between the theoretical values and the experimental values were reasonable with an error of $\pm$5%. This may be acceptable in view of the difficulty encountered in quantitative determinations by IR spectra run on solid samples.[4a] Thus, this method of determination was found to be applicable for evaluation of the amount of the CTC–HCl β form in the mixture and useful when rates of transformation are being studied.

Transformation in the Solid State

The transformation of the CTC–HCl β form under different relative humidities was studied by IR spectrophotometric method. The result is shown in Fig. 3, where the concentra-

15) J. Levine, E.A. Garlock, Jr., and H. Fishbach, *J. Am. Pharm. Assoc. Sci. Ed.*, **38**, 473 (1949).

Table I. Determination of the Chlortetracyline Hydrochloride β Form in the Presence of the α Form by IR Spectrophotometric Method

Mixture	CTC-HCl β form (%)		Error (%)
	Theoretical	Found	
A	83.1	78.2	−4.9
		83.9	+0.8
B	57.9	54.5	−3.4
		59.8	+1.9
C	42.4	42.6	+0.2
		44.5	+2.1

tion of the CTC–HCl β form are plotted as a function of time. The rate of transformation was extremely rapid under 100% R.H. and the complete transformation was observed to occur within a day at 20°. In addition, the CTC–HCl β form gradually changed during storage to the α form when exposed to 75% or 84% R.H. Increase in relative humidities from 74 to 100% R.H. increased the rate of transformation approximately 10 times. On the other hand, under 65% R.H. no polymorphic transformation was observed when the CTC–HCl β form was stored for 40 days at 20°.

These results suggest that water vapor is an important factor in the transformation of the CTC–HCl β form in the solid state. Since some moisture is always present under pharmaceutical storage conditions, special precautions should be taken to maintain the solid state in the β form crystal.

Result of the present investigation that the transformation is mainly initiated by moisture absorption is in agreement with that of the previous work[3b] (reproduced in Fig. 4, with the result of the same experiment under 75% R.H.).

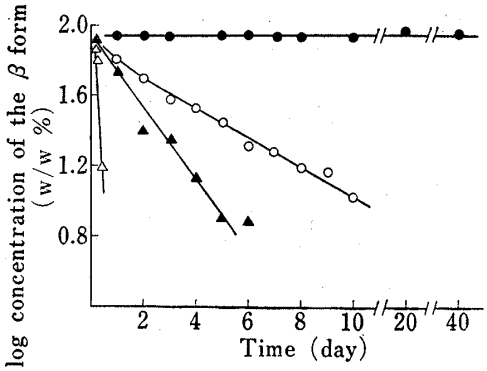

Fig. 3. Transformation of the Chlorotetracycline Hydrochloride β Form to the α Form in Solid State under Different Relative Humidities at 20°

—●—: 65% R.H. —▲—: 84% R.H.
—○—: 75% R.H. —△—: 100% R.H.

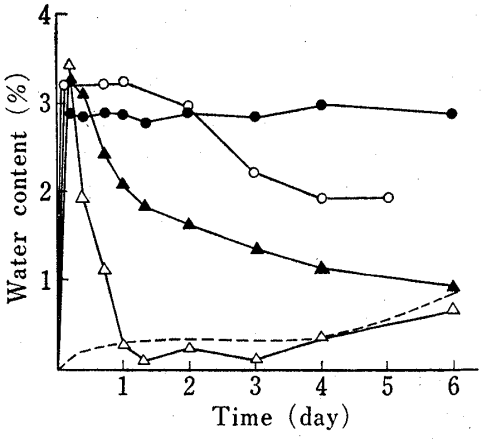

Fig. 4. Change of Water Content of the Chlortetracycline Hydrochloride β Form under Different Relative Humidities at 20°

—●—: 65% R.H. —▲—: 84% R.H.
—○—: 75% R.H. —△—: 95% R.H.
The dotted line indicates the change of water content of the CTC–HCl α form under 95% R.H. at 20°.

Since it was not known whether the CTC–HCl β form would transform if no moisture was present, an experiment was run in the absence of water vapour. Sample of the β form was stored in a desiccator over SiO_2 at room temperature. The result indicated that the solid β form was stable under anhydrous condition, over a period of 2 years. Thus, stabilization

of the CTC–HCl β form may easily be achieved in the solid state by simply abstracting moisture from its environment.

Possible transformation of the CTC–HCl β form by heating was also studied. To study the rate of this transformation, about 200 mg samples of the β form were spread on watch glasses and placed in an oven at constant temperature. The result is shown in Table II. Studies carried out at 70° showed that there was no significant transformation of the β form to the α form beyond an experimental error by heating the sample over a period of a month.

Table II. Transformation of the Chlortetracycline Hydrochloride β Form to the α Form by Heating at 70°

Time (day)	β form content (%)	Time (day)	β form content (%)
Initial	90.3	10	95.5
5	89.5	30	88.3
7	87.3		

Transformation in Aqueous Suspension

The CTC–HCl β form is readily transformed into the stable form in aqueous suspension at 37°.[3] In the present study, the change of the β form to the α form in aqueous suspension was examined by IR spectrophotometric method at various temperatures. These results are shown in Fig. 5; each curve is drawn through points obtained from at least two experimental runs. The results were satisfactorily reproducible. Suspension in water produced a much faster transformation of the β form to the stable form in water than the transformation in the solid state as described above. Indeed, the transformation half-life ($T_{50\%}$), expressed as the time required for 50% of the β form to be transformed, was 1.6 min for the suspension at 20°. The transformation was not accompanied by the induction period and started immediately after the addition of the solvent. The rate of transformation was found to be temperature dependent, refrigeration at 5° markedly slowing rate of transformation. It is therefore important that the CTC–HCl β form suspension is kept in a cool place. In addition, studies were undertaken to determine the effect of pH on the transformation in suspension. These studies were made at pH values of 2.8, 3.8, 4.9, and 6.0 in 0.2M acetate buffer solutions at 20°. The result is shown in Fig. 6. It was found that in higher pH regions the rate of transformation

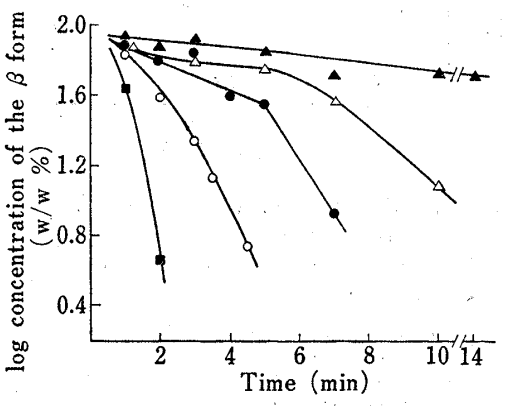

Fig. 5. Transformation of the Chlortetracycline Hydrochloride β Form to the α Form in Aqueous Suspensions at Different Temperatures

—▲—: 5° —○—: 20°
—△—: 10° —■—: 28°
—●—: 15°

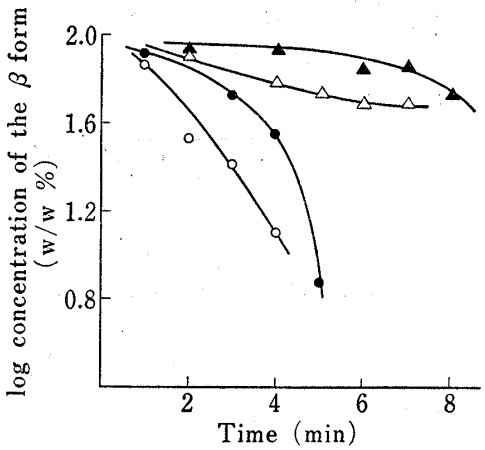

Fig. 6. Transformation of the Chlortetracycline Hydrochloride β Form to the α Form in Aqueous Suspensions at Different pH Values at 20°

—○—: pH 2.8 —△—: pH 4.9
—●—: pH 3.8 —▲—: pH 6.0

was markedly retarded. It is apparent from these results that pH is a very important factor influencing the rate of transformation in aqueous suspensions. At pH 6.0 and higher, however, there is the possibility of the conversion from the hydrochloride to the free base and no attempt was made to inquire further into this point.

The reversion in aqueous suspensions may involve direct solid-to-solid transformation to some extent, but it appears that a considerable amount of the CTC–HCl β form is transformed through solution phase; the more soluble form will dissolve and the less soluble will grow.[16] It is demonstrated by the fact that in acetone and ether, this transformation proceeded very slowly, due to the very slight solubility of CTC–HCl.[17]

Present results indicate that the rate of crystalline transformation in aqueous suspension is very rapid under the experimental conditions employed. This can be explained by relatively high solubility of CTC–HCl in aqueous solutions and greater difference in solubility of the two crystalline forms.[16]

The CTC–HCl β form used in the transformation studies was a mixture containing 8—10% w/w of the less-soluble α form in the more-soluble β form, the former serving as nuclei for the growing phase. On the other hand, the transformation of the 100% β form in aqueous suspension was also found to be completed in a very short time; $T_{50\%}$ is about 1.5 min at 20°. This indicates that the rapid transformation was not due to the presence of the nuclei of the stable form.

It is considered that the observed change in the transformation rates may be due to a change in the solubility of CTC–HCl, which is influenced by the temperature and pH of the aqueous portion. This is confirmed by the solubility data over the range of temperature and pH studied, as shown in Table III and IV. These results suggest that the transformation progresses more rapidly if the higher the solubilities.[16]

Table III. Solubility Data and Transformation Rate in Distilled Water at Different Temperature

Temperature (°C)	Solubility (mg/ml)	$T_{50\%}$ [a] (min)
5	7.61	>14
10	8.51	5.8
15	9.41	3.2
20	9.83	1.6
28	11.06	0.9

a) $T_{50\%}$ is the time necessary for the transformation of 50% of the β form

Table IV. Solubility Data and Transformation Rate in Different pH Values at 20°

pH	Solubility (mg/ml)	$T_{50\%}$ [a] (min)
2.82	10.51	1.8
3.78	6.19	3.3
4.92	2.84	6.0
6.00	1.29	8.4

a) $T_{50\%}$ is the time necessary for the transformation of 50% of the β form.

16) J. Haleblian and W. McCrone, *J. Pharm. Sci.*, **58**, 911 (1969).
17) P.J. Weiss, M.L. Andrew, and W.W. Wright, *Antibiotic and Chemotherapy*, **7**, 374 (1957).

According to the results of the present study, it may be concluded that there is little danger of transformation of the CTC–HCl β form in solid dosage form by simply abstracting moisture from its environment. In aqueous suspensions, however, rapid solution phase transformation was observed from the β form to the α form. Unless this fact is taken into account in the formulation of a suspension dosage form, the drug will be transformed to the less soluble crystal form and the higher solubility and greater bioavailability of the metastable modification may be affected by the polymorphic transformation. A search has recently been conducted for additives that would significantly retard or even prevent the transformation of the CTC–HCl β form in aqueous suspensions.