[Chem. Pharm. Bull. 23(6)1197—1204(1975)]

UDC 547.91.03 : 615.33.011.4

A Comparison of Solubility Characteristics of Free Bases and Hydrochloride Salts of Tetracycline Antibiotics in Hydrochloric Acid Solutions[1]

Shozo Miyazaki, Masahiro Nakano, and Takaichi Arita

Faculty of Pharmaceutical Sciences, Hokkaido University[2]

(Received September 5, 1974)

The pH-solubility profiles of tetracycline antibiotics in HCl–sodium acetate buffer solution were obtained at 37°. Solubility *vs.* pH curves of chlortetracycline hydrochloride (CTC–HCl) passes through a maximum at approximately 2.8 whereas solubility of tetracycline hydrochloride (TC–HCl) increases with decrease in pH. The similar solubility vs. pH curve was also obtained for demethylchlortetracycline hydrochloride (DMCT–HCl) and methacycline (MOTC–HCl). From the studies on the salts effect on the solubility of the tetracycline antibiotics, the drop at more acidic pH value in pH-solubility profiles of CTC–HCl, DMCT–HCl, and MOTC–HCl was found to be due to the common ion suppression of the solubility product equilibrium.

The dissolution behavior of the free base and the hydrochloride of tetracyclines was examined in dilute hydrochloric acid solutions, whose pH ranges from 1.2 to 2.1. The results indicated that the free base of CTC, DMCT, and MOTC was more soluble than the corresponding hydrochloride at the gastric pH values (pH 1.2—1.4 for CTC and DMCT and pH 1.2—2.1 for MOTC). On the other hand, TC–HCl exhibited a greater solubility than the base in pH 1.2 since the solubility of TC–HCl was little affected by addition of common ion (Cl^-).

Many important drugs are weak acids or bases. Salts of acidic or basic drugs have solubility characteristics different from those of acids or bases and therefore tend to show different bioavailability. Sodium or pottassium salts of weak acids dissolve much more rapidly than the corresponding free acids regardless of the pH of the dissolution medium. The same is usually true of the hydrochloride salts or other strong acid salts of weak bases. It was demonstrated that sodium salts of weak acids dissolve much more rapidly than the weak acids themselves, regardless of the initial pH of the solvent.[3]

The salt formation, however, does not necessarily result in an enhancement of the dissolution rate and the bioavailability. For instance, the aluminum salt of aspirin dissolves so slowly in gastrointestinal fluids that absorption of the drug is incomplete. The formation of a water-insoluble aluminum compound on the surface of the solid retards the dissolution.[4] Solvang and Finholt[5] reported that phenobarbital tablets prepared from the free acid had a much higher dissolution rate than tablets from the sodium salt in acidic media. They attributed this observation to the fact that tablets containing the free acid disintegrated rapidly in acidic media, whereas tablets containing the sodium salt, which disintegrates rapidly in water, did not disintegrate in the acidic media, but only swelled and dissolved slowly from the surface. Lin, *et al.*[6] pointed out that the free base of the experimental antihypertensive compound, Su-17770, exhibited a greater dissolution rate than the monohydrochloride salt in 0.1N HCl and this was attributed to the common ion effect.

1) A part of this work was presented at 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.
2) Location: *Kita-12, Nishi-6, Kita-ku, Sapporo.*
3) E. Nelson, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 297 (1958).
4) G. Levy and B.A. Sahli, *J. Pharm. Sci.*, **51**, 58, 294 (1962).
5) S. Solvang and P. Finholt, *J. Pharm. Sci.*, **59**, 49 (1970).
6) S.-L. Lin, L. Lachman, C.J. Swartz, and C.F. Huebner, *J. Pharm. Sci.*, **61**, 1418 (1972).

The tetracyclines are amphoteric antibiotics, forming acid and basic salts which are readily soluble in water. Their hydrochloride salts are originally used clinically. In the previous work,[7] during the dissolution studies of chlortetracycline (CTC) base, a decrease in the concentration of CTC in solution was observed in the simulated gastric fluid at pH 1.2 due to the conversion from the free base to the hydrochloride salt. Further experiments were conducted to compare the dissolution behavior between the two species. The data obtained showed that CTC base was much more soluble than the hydrochloride in the acidic medium.[8] These results suggest that CTC base may show greater bioavailability as compared with the hydrochloride salt when administered orally.

The purpose of the present investigation was to seek the reason why CTC base is more soluble than the hydrochloride in the acidic medium. In addition, the relative dissolution behavior of the base and the hydrochloride salt was examined in dilute hydrochloric acid solutions in greater detail. Furthermore, similar investigation was carried out with demethylchlortetracycline (DMCT), methacycline (MOTC), and tetracycline (TC).

Experimental

Materials——The hydrochloride salt of chlortetracycline was prepared by recrystallization of the hydrochloride[9] from distilled water. The free base of this antibiotics was obtained from methanol by the method described previously.[7] Demethylchlortetracycline base,[9] tetracycline hydrochloride,[9] and methacycline hydrochloride[10] were used without further purification. Methacycline base was prepared by dissolving the hydrochloride in water and then adjusting pH to 5 with aqueous ammonia. The resulting precipitate was filtered, washed with water, and dried *in vacuo* at 50—60°. Demethylchlortetracycline hydrochloride was obtained in water by the method of Origoni and Winterbottom.[11] Tetracycline base was prepared by recrystallization of the free base[10] from water by the method described previously.[7]

Table I. Analyses of the Composition of Tetracycline Antibiotics

Tetracyclines	mp[a] (decomp.)	Formula	Analysis (%) Calcd. (Found) C	H	N	Cl	H_2O (%) Calcd.	Found
CTC[b]	163—165°	$C_{22}H_{23}O_8N_2Cl$-3/2H_2O	52.17 (51.89)	5.14 (5.03)	5.53 (5.43)	5.34 c)	5.3	5.4[d]
CTC-HCl[b]	227—228°	$C_{22}H_{23}O_8N_2Cl$-HCl	51.27 (51.05)	4.70 (4.51)	5.44 (5.31)	13.76 c)	0	0.7[d]
DMCT	174—175°	$C_{21}H_{21}O_8N_2Cl$-3/2H_2O	51.27 (51.04)	4.88 (4.97)	5.70 (5.66)	7.21 (6.54)	5.5	6.1[a]
DMCT-HCl[e]	212—213°	$C_{21}H_{21}O_8N_2Cl$-HCl-H_2O	48.57 (48.63)	4.62 (4.40)	5.40 (5.45)	13.65 (13.88)	3.5	2.2[a]
MOTC[e]	164—166°	$C_{22}H_{22}O_8N_2$-H_2O	57.41 (57.63)	5.22 (4.95)	6.09 (6.07)	0 0	3.9	5.1[a]
MOTC-HCl	237—238°	$C_{22}H_{22}O_8N_2$-HCl	55.21 (55.18)	4.81 (4.91)	5.86 (5.74)	7.41 (7.35)	0	0[a]
TC[b]	170—172°	$C_{22}H_{24}O_8N_2$-3H_2O	53.01 (53.22)	6.08 (5.81)	5.62 (5.54)	0 c)	10.8	9.8[d]
TC-HCl	212—220°	$C_{22}H_{24}O_8N_2$-HCl	54.94 (54.52)	5.25 (5.18)	5.83 (5.73)	7.37 (7.50)	0	0[a]

a) Thermogravimetry and Differential Scanning Calorimetry
b) Samples were dried *in vacuo* at room temperature over SiO_2.
c) not determined
d) Karl Fisher method
e) dried *in vacuo* at 50—60° over P_2O_5

7) S. Miyazaki, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **23**, 552 (1975).
8) S. Miyazaki, M. Nakano, and T. Arita, unpublished data.
9) Supplied by Lederle (JAPAN). LTD.
10) Supplied by Chas. Pfizer Co.
11) V.E. Origoni and R. Winterbottom, U.S. Patent 3023239 Feb. 27, 1962, Appl. Dec. 9 (1959) [*C.A.*, **56**, 14407*i* (1962)].

The size of the crystals in the dissolution studies was not controlled. The melting point (decomp.) and the results of elemental analysis and measurement of water contents of these antibiotics are shown in Table I.

Solubility Determinations——Solubility of tetracycline antibiotics at various pH's were determined in HCl–sodium acetate buffer solution at 37°. Excess amounts of samples were suspended in 2 ml of the buffer solutions. These suspensions were shaken mechanically overnight and the filtered aliquots with Millipore filter (0.20 μ) were spectrophotometrically assayed in 0.1 N sulfuric acid. The wavelengths chosen for spectral analyses were: CTC, 265 mμ; DMCT, 265 mμ; MOTC, 243 mμ; and TC, 270 mμ. All absorbance measurements were made with a Hitachi Type 139 spectrophotometer. The pH of the aliquot was measured with a microglass–electrode (Type HG-9005, Tōa Electronics LTD.).

Procedure for Dissolution Studies——Dissolution behavior of crystalline powder was determined at 37° as previously described.[12] The experiments were performed at pH 1.2 (0.1 N), 1.4, 1.6, and 2.1 (0.01 N) in dilute hydrochloric acid. One milliliter portion of the solvent previously kept at 37° was added into individual 10 ml Erlenmeyer flasks, which were immersed in a constant temperature bath and contained an excess amount of samples, and they were immediately started to be mechanically shaken horizontally at a rate of 60±2 strokes/min. Sample solutions were filtered through a Millipore filter (0.20 μ).

Dissolution behavior of compressed disk of CTC base and the hydrochloride was also studied according to the method previously described.[12] The disks, 1.0 cm in diameter, were prepared by compressing 100 mg of samples in a potassium bromide die. The die was evacuated and a compressional force of 500 kg/cm^2 was applied. The disk sticked to glass tube was introduced into the beaker containing 50 ml of 0.1 N HCl (pH 1.2) at 37°, being stirred by magnetic stirring bar at a high speed. Sample solutions were pipetted out periodically by a pipette with a cotton-filter.

In both cases, the sample solution was analyzed spectrophotometrically as just described.

Result and Discussion

pH-Solubility Profile

Since tetracycline antibiotics are ampholytes, an increase in solubility is usually observed at more acidic and alkaline pH values, with minimum solubility at relatively neutral pH values.[13] The aqueous solubility of TC is about 10 times greater at pH 1—3 than at pH 5—6.[14] On the other hand, Ermakova, *et al.*[15] reported that the solubility *versus* pH curves of CTC passed through a maximum at 2.5—2.6. However, no details are given of this phenomenon.

Initially, the pH-solubility profile of CTC-HCl in HCl-sodium acetate buffer solution at 37° was determined since it may provide with the reason why the CTC base was more soluble than the hydrochloride in the acidic medium. Similar determinations were made with DMCT-HCl, MOTC-HCl, and TC-HCl. The results of this study are shown in Fig. 1. The solubility of TC-HCl, which increased with the decrease of pH, shows a usual pH-profile within experimental pH values. The pH-solubility profile of CTC-HCl was not so simple as that of TC-HCl but shows a solubility maximum at approximately pH 2.8. This result is in agreement with that of Ermakova, *et al.*[15] Similar solubility maxima were observed for DMCT-HCl and MOTC-HCl in the pH-solubility profile at pH 2.3 and 3.8, respectively.

The melting point (decomp.) and the result of elemental analysis of solid phases isolated from the medium after equilibrium at 37° are shown in Table II. In the case of CTC-HCl, DMCT-HCl, and MOTC-HCl, the free base was the solid phase in equilibrium with solution above the maximum point and the hydrochloride salt was the solid phase below this point. These results suggest that the final drop at more acidic pH values in the pH-solubility profile is not due to the formation of complex salt species.

Stability of samples was investigated by recording their ultraviolet (UV) absorption spectra as function of time in the pH 1.0 HCl-sodium acetate buffer solution. The UV spectra of all the hydrochlorides exhibited no significant change in the buffer solution over a period

12) S. Miyazaki, T. Arita, R. Hori, and K. Ito, *Chem. Pharm. Bull.* (Tokyo), **22**, 638 (1974).
13) P.P. Regna and I.A. Solomons, *Ann. N.Y. Acad. Sci.*, **53**, 229 (1950).
14) W.H. Barr, J. Adir, and L. Garrettson, *Clin. Pharmacol. Ther.*, **12**, 779 (1971).
15) N.M. Ermakova, B.P. Bruns, and V.B. Korchagin, *Med. Prom. S.S.S.R.*, **14**, 51 (1960) [*C.A.*, **55**, 3006*a* (1961)].

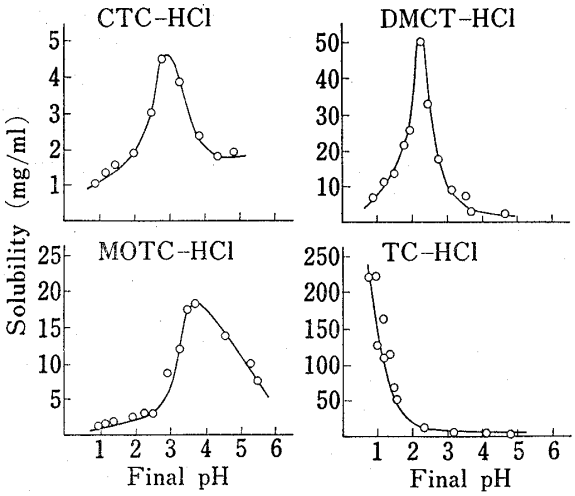

Fig. 1. The pH-Solubility Profiles of the Hydrochlorides of Tetracycline Antibiotics in HCl–CH_3COONa Buffer Solution at 37°

All data are expressed in terms of the free base equivalent.

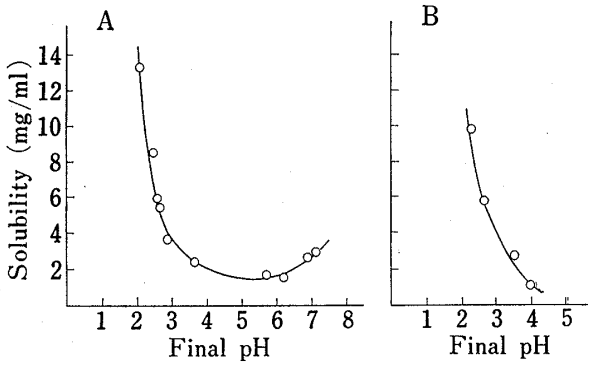

Fig. 2. The pH-Solubility Profiles of Chlortetracycline Hydrochloride in 0.1M Phosphate Buffer at 37° and 0.2M Acetate Buffer at 20°

All data are expressed in terms of the free base equivalent.
A: 0.1M H_3PO_4–Na_2HPO_4 buffer (37°)
B: 0.2M CH_3COOH–CH_3COONa buffer (20°)

Table II. Analyses of the Composition of Solid Phases in Equilibrium with HCl-Sodium Acetate Buffer Solution

Final pH	mp[a] (decomp.)	Formula	Analysis (%) Found[b] (Calcd.)[c] C	H	N	Cl
1.92	229°	CTC–HCl	51.03	4.80	5.46	13.73
3.42	162°	CTC-5/2H_2O	50.87 (50.38)	5.05 (5.34)	5.40 (5.34)	7.31 (7.16)
1.95	211°	DMCT–HCl–H_2O	48.89	4.46	5.18	13.34
2.44	173°	DMCT-3/2H_2O	51.04	4.86	5.67	7.81
2.94	[d]	MOTC–HCl	54.52	4.81	5.77	7.76
4.57	[d]	MOTC–3/2H_2O	56.71 (56.28)	5.25 (5.06)	5.56 (5.97)	0.93 (0)
0.90	217°	TC–HCl	54.71	5.22	5.70	7.79
1.65	170°	TC–H_2O	57.10 (57.14)	5.58 (5.68)	5.90 (6.00)	0.82 (0)

a) Thermogravimetry and Differential Scanning Calorimetry
b) Samples were washed with a small amount of cold water and dried at room temperature *in vacuo* prior to analyses.
c) See calculated values in Table I.
d) not determined

of 24 hrs. To check further stability of CTC-HCl, the pH-solubility profile was determined at 20°. The result gave a similar pH-solubility curve to that obtained at 37° (Fig. 1). Thus it may be concluded that the degradation of tetracycline antibiotics during actual solubility studies was negligible.

The influence of the composition of buffer solution on the solubility of CTC-HCl was also examined. The data were obtained for 0.1M H_3PO_4–Na_2HPO_4 buffer solution at 37° and 0.2M acetate buffer solution at 20°. As shown in Fig. 2, there was no drop in solubility in the acidic pH range in both buffer solutions.

In view of the fact that in HCl-sodium acetate buffer solution, a decrease in solubility below pH 2.8 for CTC-HCl was found and CTC base was more soluble than the hydrochloride in pH 1.2 simulated gastric fluid composed of HCl and NaCl, it is suggested that the maximum

solubility at acidic pH values is due to the common ion effect. This possibility is based on the results obtained by Dittert, *et al.*[16] in their study on the pH-solubility profile for triamterene in the presence of hydrochloric acid. They reported that the drop at more acidic pH values in the pH-solubility profile was due to common ion suppression of the solubility product equilibrium. The pH-solubility profile of DMCT-HCl and MOTC-HCl may also be explained in a similar effect.

Effect of Salts on the Solubility of Tetracycline Antibiotics

The experimental support of the supposition that a drop at more acidic pH values in pH-solubility profile of CTC-HCl, DMCT-HCl, and MOTC-HCl may be attributed to the common ion suppression of the solubility product equilibrium is presented in Fig. 3, 4, and Table III.

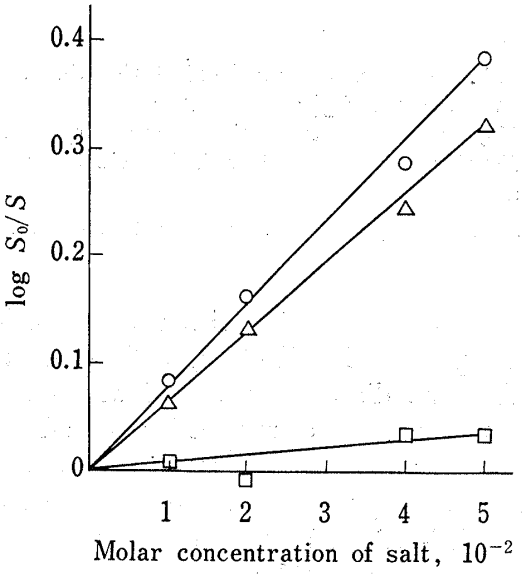

Fig. 3. Setschenow Plots for Chlortetracycline Hydrochloride in Salt Solutions at 37°

key: ○: NH_4Cl, △: NaCl, and □: Na_2SO_4

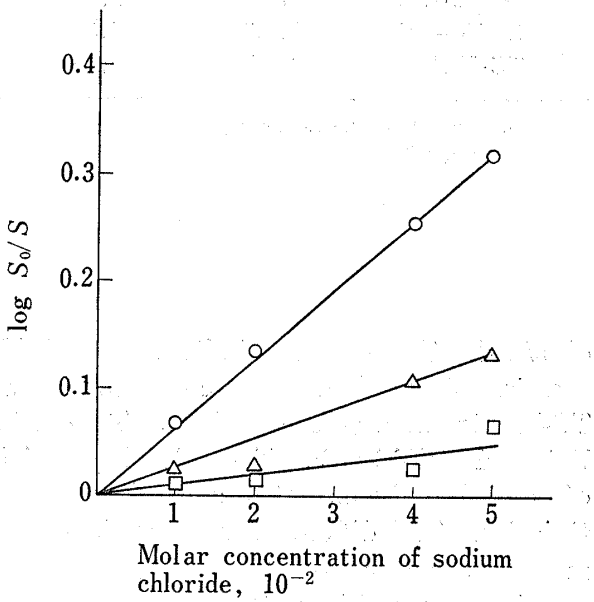

Fig. 4. Setschenow Plots for Several Tetracycline Antibiotics in Sodium Chloride Solutions at 37°

key: ○: MOTC-HCl, △: DMCT-HCl, and □: TC-HCl

Aqueous solutions of sodium chloride, ammonium chloride, and sodium sulfate were prepared in concentrations up to 0.05 M, and the equilibrium solubility of the hydrochlorides of tetracycline antibiotics in these solutions was studied at 37°. The most widely used empirical equation for determining the extent of salt effect is that of Setschenow[17]

$$\log S_0/S = kC$$

where S and S_0 are the solubility of the nonelectrolyte in solvent in the presence and absence of salt, respectively; C is the molar concentration of the salt solution. The overall salting-out constant, k, can be derived from the slope of a plot of log S_0/S *versus* C.

Setschenow plots for CTC-HCl in the presence of various salts are shown in Fig. 3. The ratio S_0/S of CTC-HCl increases progressively as the concentration of NaCl is increased, suggesting that NaCl depresses the solubility of CTC-HCl. A similar effect was noted with increase in concentration of NH_4Cl. The presence of Na_2SO_4, on the other hand, appears to have little influence on the solubility of CTC-HCl. In addition, solubility of the free base was little affected by the addition of NaCl, as shown in Table III which lists salting-out constants

16) L.W. Dittert, T. Higuchi, and D.R. Reese, *J. Pharm. Sci.*, **53**, 1325 (1964).
17) J. Setschenow, *Z. Phys. Chem.*, **4**, 117 (1889).

Table III. Salting-out Constant of Tetracyclines at 37°

Tetracyclines	Salt	Salting-out constant, $k^{a)}$
CTC–HCl	NaCl	6.52
CTC–HCl	NH_4Cl	7.68
CTC–HCl	Na_2SO_4	0.74
CTC-base	NaCl	0.23
DMCT–HCl	NaCl	2.68
MOTC–HCl	NaCl	6.36
TC–HCl	NaCl	0.85

a) derived from the slope of a plot of log S_0/S *versus* C

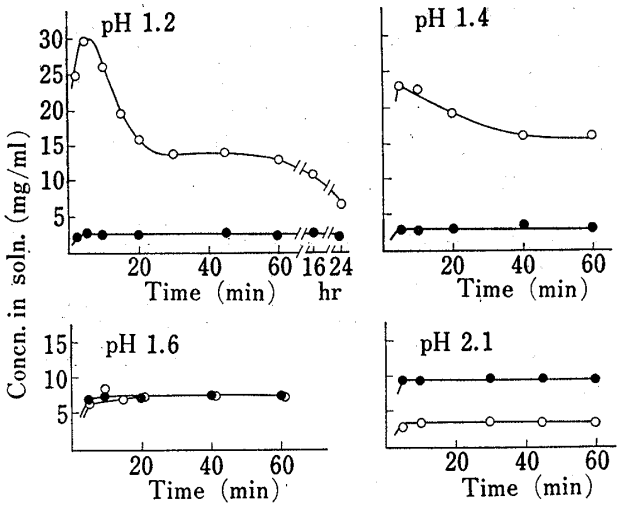

Fig. 5. Dissolution and Precipitation Curves of the Free Base (○) and the Hydrochloride Salt (●) of Chlortetracycline from Crystalline Powder in Dilute Hydrochloric Acid Solutions at 37°

All data are expressed in terms of the free base equivalent.

obtained. These results indicate that CTC-HCl is salted-out of salts with a common ion, that is, chloride ion. Figure 4 shows the Setschenow plots of DMCT–HCl, MOTC-HCl, and TC-HCl in the presence of NaCl. The salting-out effect was distinctly observed with DMCT-HCl and MOTC-HCl. The solubility of TC-HCl is also slightly decreased by the addition of NaCl, but the extent of salt effect was found to be much less than those of the other antibiotics. The difference between TC-HCl and other tetracyclines in salting-out behavior may arise partly from the fact that the solubility of TC-HCl in HCl-sodium acetate buffer solution is much higher than that of others. However, further consideration may be required to prove this point.

Thus, it is apparent that addition of salts containing chloride ion to the solutions of CTC-HCl, DMCT-HCl, and MOTC-HCl generally results in considerable salting-out effect (Table III). The significant decrease in the solubility of these antibiotics at the more acidic pH values (Fig. 1) can be rationalized on the basis of the common ion suppression of the solubility product equilibrium.

Dissolution Behavior in Hydrochloric Acid Solutions

The dissolution behavior of the base and the hydrochloride of tetracycline antibiotics was examined in hydrochloric acid solutions in detail since the behavior in hydrochloric acid solutions is more relevant to the bioavailability after oral administration. The dissolution curves of the free base and the hydrochloride of CTC in dilute hydrochloric acid solutions, whose pH ranges from 1.2 to 2.1, is shown in Fig. 5. Each curve is drawn through points obtained during at least two experimental runs. The distinct difference in dissolution behavior was observed between the two species at pH 1.2, the free base yielding much greater concentrations than the hydrochloride. For example, at 5 minutes the concentration of CTC in solution was more than 10 times higher for the base than the hydrochloride. However, a decrease in the concentration of the free base from approximately 30 mg/ml to a lower value was observed during actual dissolution studies due to the conversion from the free base to the hydrochloride. The confirmation of the conversion was provided by elemental analysis and thermal analysis of the solid phase isolated from the medium after 24 hr. A similar variation in the dissolution behavior was found at pH 1.4. On the other hand, at pH 1.6

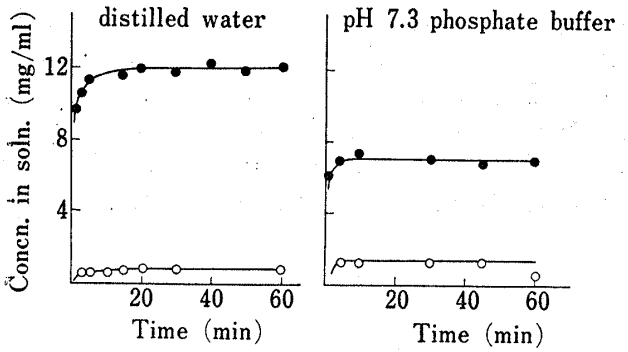

Fig. 6. Dissolution Curves of the Free Base (○) and Hydrochloride Salt (●) of Chlortetracycline from Crystalline powder in Distilled Water and pH 7.3 Phosphate Buffer at 37°

All data are expressed in terms of the free base equivalent.

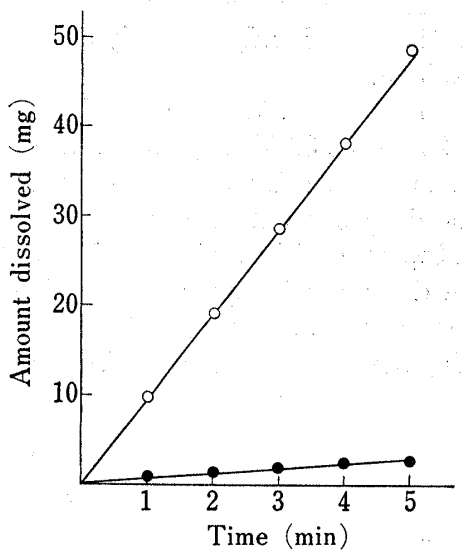

Fig. 7. Dissolution Curves of the Free Base (○) and the Hydrochloride Salt (●) of Chlortetracycline from Compressed Disk in pH 1.2 Hydrochloric Acid Solution at 37°

All data are expressed in terms of the free base equivalent. Points are the mean values of two experiments.

the free base and the hydrochloride was made equally soluble; the order was reversed when pH of the dissolution medium was raised still to 2.1.

Similar data obtained in the study conducted in water and pH 7.3 phosphate buffer solution are shown in Fig. 6. The hydrochloride is soluble in distilled water or pH 7.3 phosphate buffer solution whereas CTC base is poorly soluble in these media.

In order to ascertain the dissolution behavior in an initial stage, the dissolution curves of the free base and the hydrochloride of CTC obtained from compressed disks in pH 1.2 hydrochloric acid solution are illustrated in Fig. 7. As would be expected, the dissolution of the free base from compressed disks was shown to be much faster than that of the hydrochloride. The dissolution rates of the base and the hydrochloride were 9.55 and 0.75 mg/min/cm^2, respectively.

The free base of DMCT also exhibited greater solubility than the hydrochloride in pH 1.2 and 1.4 hydrochloric acid solutions. Figure 8 shows that maximum concentration of DMCT achieved in solution with the base is 2 times the solubility of the hydrochloride at pH 1.2.

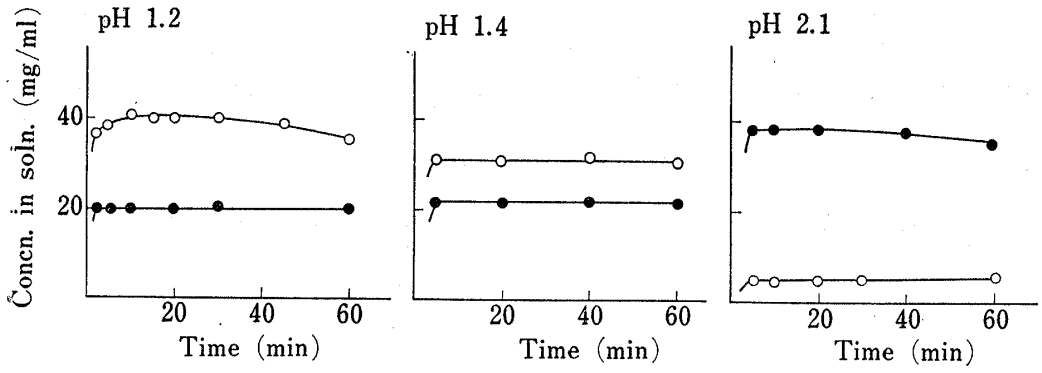

Fig. 8. Dissolution Curves of the Free Base (○) and the Hydrochloride Salt (●) of Demethylchlortetracycline from Crystalline Powder in Dilute Hydrochloric Acid Solutions at 37°

All data are expressed in terms of the free base equivalent.

On the other hand, the study at pH 2.1 shows DMCT-HCl to have much higher solubility than the base.

Marsh and Weiss[18] listed the solubility of MOTC hydrochloride and base in 0.1N HCl at 20° to be 3.352 mg/ml and more than 20 mg/ml, respectively. As shown in Fig. 9, MOTC base is more soluble than the hydrochloride in pH 1.2 hydrochloric acid solution (0.1N HCl). The drug concentration in solution remained at approximately 140 mg/ml for about 30 min and was then followed by gradual decreases of the drug concentration in solution. In addition, the difference in apparent solubility of these two forms was distinctly observed at pH 1.6. Even at pH 2.1 the solubility of the base was slightly greater than that of the hydrochloride salt, although the magnitude of difference was less than that at pH 1.2 or 1.6.

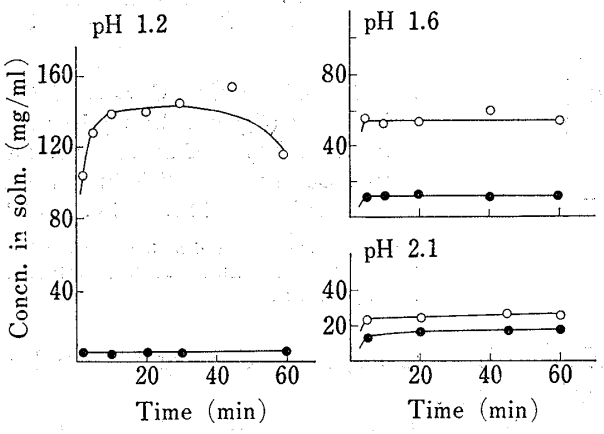

Fig. 9. Dissolution Curves of the Free Base (○) and the Hydrochloride Salt (●) of Methacycline from Crystalline Powder in Dilute Hydrochloric Acid Solutions at 37°

All data are expressed in terms of the free base equivalent.

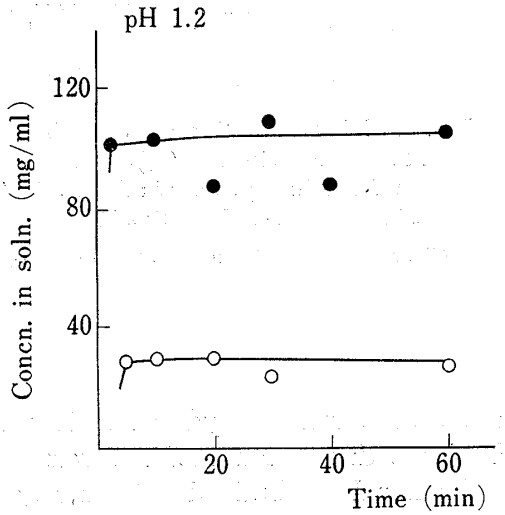

Fig. 10. Dissolution Curves of the Free Base (○) and the Hydrochloride Salt (●) of Tetracycline from Crystalline Powder in pH 1.2 Hydrochloric Acid Solution at 37°

All data are expressed in terms of the free base equivalent.

Figure 10 illustrates the dissolution curves of TC base and the hydrochloride in pH 1.2 hydrochloric acid solution at 37°. The hydrochloride exhibits a greater solubility than the free base. This greater solubility of the hydrochloride compared with the base is consistent with the results of dissolution rate studies[19] in the simulated gastric fluid.

From these results, it was confirmed that the free bases of CTC, DMCT, and MOTC are more soluble than the hydrochloride salts at the gastric pH values (pH 1.2—1.4 for CTC and DMCT, and pH 1.2—2.1 for MOTC) due to the common ion suppression of the solubility product equilibrium. Since the drug is usually administered orally, it is of interest to compare absorption and excretion of these tetracyclines when administered orally as the hydrochloride and as the base. Results obtained in animals and humans will be published.[20]

Acknowledgement The authors are grateful to Miss Makiko Tabata for her assistance in the experimental work.

18) J.R. Marsh and P.J. Weiss, *J. Ass. Offic. Anal. Chem.*, **50**, 457 (1967).
19) E. Nelson, *J. Am. Pharm. Assoc. Sci. Ed.*, **48**, 96 (1959).
20) S. Miyazaki, M. Nakano, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), "accepted."