Effect of Pharmaceutical Excipients on the Stability of Trichlormethiazide Tablets under Humid Conditions

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Received May 1, 2009; accepted September 11, 2009; published online September 24, 2009

The stability of trichlormethiazide (TCM) and the drug in the nine products available on the market (the original tablet (B) and 8 generic tablets (G1—G8)) were investigated under humid conditions. TCM was non-hygroscopic and was not degraded under humid conditions. Drug degradation in aqueous ethanol was accelerated with increased water concentration, and the drug stability in buffer solution was improved with decreased pH. TCM decomposition was not detected in each unwrapped tablet at low relative humidity. However, rapid degradation was observed for products G1 and G2, while product B and G7 showed higher stability at high relative humidity. The stability of products G1 and G2 decreased with increasing humidity. The same results were observed for the tablets in press-through packages (PTP), but the degradation rate was much slower than tablets without PTP packages. These results suggested that the adsorbed moisture by excipients cause TCM degradation. Various pharmaceutical excipients are added to TCM tablets and these vary between different pharmaceutical companies. Intact drug and pharmaceutical excipients, including lactose, microcrystalline cellulose, corn starch, hydroxypropylcellulose (HPC), low substituted HPC (L-HPC), calcium stearate, and light anhydrous silicic acid, were mixed, and the sample mixtures were stored in humid conditions. It was found that the TCM content decreased significantly in a binary mixture of TCM/HPC 1 : 1.

Key words trichlormethiazide; stability; excipient; hydrolysis; adsorbed water

Trichlormethiazide ((3*RS*)-6-chloro-3-dichloromethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide) (TCM) is a thiazide diuretic drug that is used in the treatment of hypertension. TCM has a steady hypotensive effect in long-term therapy in combination with β-receptor blocking agent and angiotensin converting enzyme inhibitor. Different types of 2 mg TCM-containing tablets are available from 10 pharmaceutical companies in Japan.

The hydrolysis of hydrochlorothiazide and two other hydrochlorothiazides was reported over a pH range 1 to 13 and gave a bell-shaped pH rate profile.[1,2] Yamana *et al.* also reported on the decomposition of hydrochlorothiazide in solutions with various pH values.[3,4] The stability of TCM incorporated into gelatin gels was studied, and the hydrolysis rate of TCM in the gels was found to depend on the amount of free water available for the reaction.[5]

As reported previously, the stability of active ingredients can be affected by pharmaceutical excipients. Aspirin degradation in solid dispersion with urea or povidone was accelerated by the adsorbed water onto the two carriers.[6] The loss of ascorbic acid increased progressively with increasing moisture content in a mixture of silica gel and ascorbic acid stored for 3 weeks at 45 °C.[7] The extent of hydrolyzed hydrochlorothiazide was decreased by the addition of aminodisulfamide.[8] The hydrolysis of TCM in silk fibroin gel prepared in various sugar solutions (such as ribose, fructose, mannose, and glucose solutions) was studied. It was revealed that the hydrolysis rate of TCM decreased in the following order: ribose>fructose>mannose>glucose and the hydrolysis rate constant decreased with an increase in number of the equatorial OH groups.[9] However, TCM in solid state has not been studied in detail, although the dosage form of the drug is marketed as tablet in Japan. The tablets are removed from press through package (PTP) and divided into two or four parts to titrate dose of the drug by prescription. The documentation on stability in the package insert for tablets available in Japan differs among pharmaceutical companies.

The purpose of the present investigation was to evaluate the stability of TCM in a solid state with excipients and tablet products in detail under various storage conditions to establish a reasonable stabilization design. The information obtained from these stability tests will be useful for the stabilization of dosage forms.

Experimental

Materials Bulk trichlormethiazide (TCM) was obtained from Iwaki Seiyaku Co., Ltd. and used without further purification. Silicon dioxide (Wako Pure Chemical Industries, Ltd.), calcium stearate (Wako Pure Chemical Industries, Ltd.), hydrated silicon dioxide, light anhydrous silicic acid, hydroxypropyl starch (Freund Corporation), synthetic aluminum silicate (Takeda Pharmaceutical Company Ltd.), magnesium aluminometasilicate (Fuji Chemical Industry Co., Ltd.), corn starch (Kanto Chemical Co., Inc.), lactose (DMV International), microcrystalline cellulose (Asahi Kasei Chemicals Co.), talc (Nacalai Tesque, Inc.), carmellose, carmellose calcium (Gotoku Chemical Co., Ltd.), hydroxypropylcellulose (Shin-Etsu Chemical Co., Ltd.), and low substituted hydroxypropylcellulose (Nippon Soda Co., Ltd.) were used as pharmaceutical excipients. The commercial solvents for HPLC analysis, dehydrated ethanol, methyl *p*-hydroxybenzoate as an internal standard, and other chemicals were of reagent grade. The original (B) and 8 generic products (G1—G8) of commercial TCM tablets were purchased.

Stability of TCM Solution TCM (100 mg) was dissolved in 100 ml of ethanol containing 0—30% (v/v) water. An aliquot of the solution (4 ml) was placed in a glass vial and sealed hermetically. The samples were stored at 35, 45, 50, or 60 °C in a constant temperature oven. The kinetics studies of TCM degradation were determined in the following aqueous solution (ionic strength adjusted to 0.5 M with sodium chloride): hydrochloric acid (pH 0.8—2.0), sodium acetate (pH 3.6—4.5), and sodium dihydrogenphosphate (pH 5.3) buffers. Drug ethanol solution (0.2 ml of 10 mg/ml) was added to 9.8 ml of each buffer solution, and the solutions were stored at 50 °C in a shaking constant-temperature water bath.

Stability of TCM in a Solid State TCM (10 mg) was weighed accurately into a small cup with a diameter of 30 mm and the cup was stored in

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an airtight box with silica gel or a saturated solution of an inorganic salt to control the relative humidity (RH) at 50 °C. The inorganic salts were $MgCl_2 \cdot 6H_2O$ (30% RH), NaCl (75% RH), and KNO_3 (85% RH).

The mixed powder of TCM and each excipient (20 mg; 1 : 1), 9 TCM tablets with and without PTP, divided tablets, and pulverized tablet powders were also stored in the same airtight box. The tablets were divided into half tablets by using the tablet cutter (HCL #7347, Health Care Logistics INC.) and pulverized into the fine powder using a pestle and mortar.

Assay Procedures for TCM The assay for the TCM content in the samples after storage was carried out using an HPLC system (Waters Co.) equipped with Pump 515 and Dual Absorbance Detector 2487 (256 nm, Waters Co.); a prepacked column (SunFire™ C8 5 μm, 150 mm×4.5 mm i.d., Waters Co.) was operated at room temperature at a flow rate of 1.0 ml/min. The mobile phase was composed of acetonitrile : 0.1% (v/v) phosphoric acid (1 : 3). A solution of methyl *p*-hydroxybenzoate in the mixed solution of acetonitrile and water (9 : 1) was used as an internal standard. After storage, an internal standard solution was added to the sample and stirred until the sample completely dissolved. The concentration of unchanged TCM was determined chromatographically using a stock solution. The mean of values determined in triplicate was used.

Water Vapor Sorption Isotherm Measurement The water vapor sorption isotherm of various excipients and the pulverized powders of commercial tablets were determined at 50 °C using a dynamic vapor sorption system (DVS) (Surface Measurement Systems Ltd.), which provides extremely accurate gravimetric moisture sorption with a control of relative humidity and temperature.

NMR and MS Spectrometry NMR spectra in DMSO-d_6 were recorded using a Varian VXR (500 MHz) with TMS as internal standard. Mass spectra were measured using a Hitachi M-80.

Data Analysis The remaining of each generic tablet after storage was compared with that of the brand tablet using the Dunnet's test. Statistical analysis for regression slope and correlation coefficient was performed by using comparison of two regression parameters and the Pearson correlation coefficient.

Results and Discussion

Effect of Water Content in Ethanol on the TCM Degradation It is described in the Japanese Pharmacopeia, Fifteenth Edition that TCM is slightly soluble in 95% ethanol and practically insoluble in water. In the present study, the effect of water on TCM degradation was investigated in ethanol by altering the water concentration. Figure 1 shows semi-logarithmic plots of the degradation profiles of TCM in ethanol with various water concentrations at 50 °C. Straight lines were obtained for each water concentration, suggesting that degradation of TCM in aqueous ethanol followed first-order kinetics. There was little change in the residual amount of TCM in dehydrated ethanol and 97.2% of intact TCM remained after storage for 48 h. On the other hand, TCM degraded quickly in ethanol containing water, and the amount of TCM decreased to approximately 11.2% with a water concentration of 30% (v/v) after the same storage time. These results indicated that TCM is unstable in aqueous ethanol.

The degradation rate constants calculated from the slopes of the regression lines in Fig. 1 are plotted against water concentration at various temperatures in Fig. 2. The degradation rate constant increased with increasing water concentration at every temperature. The value increased significantly at higher temperature compared with low temperature, indicating that TCM degradation attributed to water was affected by temperature.

The effect of the addition of water on the degradation of TCM is shown as Arrhenius plots in Fig. 3. The plots showed good linearity for all water concentrations at 35—60 °C, and no significant difference among the activation energy values was observed, and the average activation energy

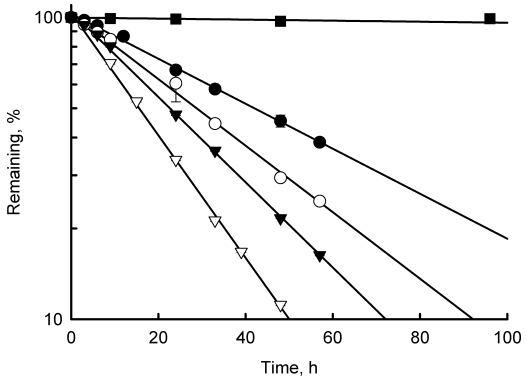

Fig. 1. Effect of Water Concentration on Percent Remaining of TCM in Ethanol at 50 °C

Water concentration, % (v/v): ■, 0 ●, 10 ○, 15 ▼, 20 ▽, 30.

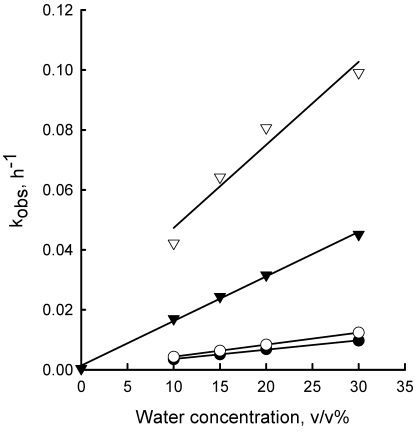

Fig. 2. Effect of Water Concentration on the TCM Degradation Rate Constants (k_{obs}) in Ethanol at Various Temperatures

Water concentration, % (v/v): ●, 10 ○, 15 ▼, 20 ▽, 30.

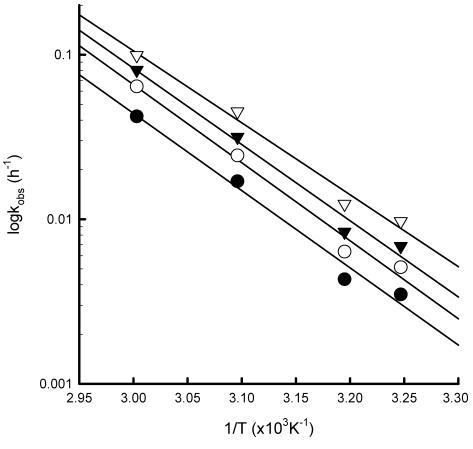

Fig. 3. Arrhenius Plots for the TCM Degradation in Ethanol Containing Various Concentrations of Water

Temperature (°C): ●, 35 ○, 40 ▼, 50 ▽, 60.

was 88.3 kJ/mol similar to the hydrolysis of penicillin[10] and cefadroxil.[11]

Effect of pH on TCM Degradation in Buffer Solution There are many previous reports on the effect of pH on drug degradation in solution. Hydrochlorothiazide stability of a benzothiadiazine analog like TCM was studied by Mollica *et al.*[2] and Yamana *et al.*[4] Mollica *et al.*[2] presented a bell-shaped pH-rate profile for hydrolysis of the drug. The pH

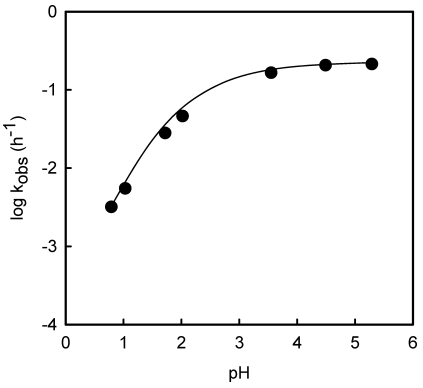

Fig. 4. The Effect of pH on the Degradation of TCM in Various Buffer Solutions at 50 °C and Ionic Strength 0.5

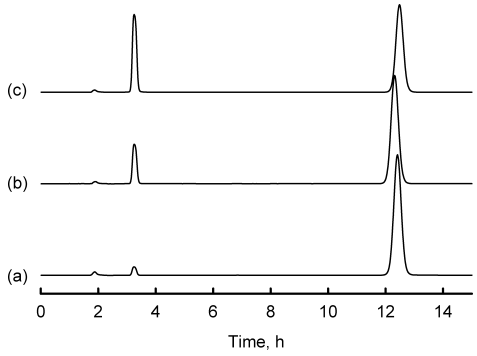

Fig. 5. Changes in HPLC Chromatogram of TCM in Aqueous Ethanol at 50 °C

Storage time: (a) 0 h, (b) 12 h, (c) 24 h.

profile curve of TCM (Fig. 4) shows that with increasing pH value, the degradation rate constants increased and that the constants were the same value approximately at a pH of more than 3.5, as also seen for hydrochlorothiazide. These data estimated that an imine intermediate exists in the hydrolysis reaction, and a carbinolamine was formed by water or hydroxide as with hydrochlorothiazide. The effect of pH on the TCM degradation was not investigated in a pH of more than 5.3, because TCM precipitated at this pH value.

Figure 5 shows the chromatograms resulting from the HPLC analysis of samples stored in water–ethanol (10% v/v) at 50 °C. The peak of the degradation product after storage was detected at 3.3 min in addition to the TCM peak at 12.5 min, and the peak intensity of the degradation product increased with increasing storage time. The molecular formula was determined to be $C_6H_8ClN_3O_4S_2$ from HR-EI-MS of the degradation product isolated by preparative HPLC, showing a molecular ion peak at *m/z* 284.9632 (Calcd for $C_6H_8ClN_3O_4S_2$, 284.9645). The ^{1}NMR spectrum (DMSO-d_6) showed two aromatic protons, as a singlet at δ 6.97 and 8.16, and 6 amine protons as a broad singlet at δ 6.61, 7.34, and 7.49. These data indicated that TCM was hydrolyzed by water to give 4-amino-6-chlorobenzene-1,3-disulfonamide (Chart 1) as with the hydrolysis of hydrochlorothiazide.

Effect of Humidity on the Stability of TCM in the Solid State TCM is used in a tablet dosage form in Japan. Figure 6 shows the remaining (%)TCM after storage in relative humidities between 0% and 85% after 2 weeks, and water sorption (%) at each relative humidity was measured using a dynamic vapor sorption system. Solid state TCM was extremely stable in all humid condition. This was attributed to the fact that the amount of water absorbed by solid-state TCM was extremely low under humid conditions, and it was practically insoluble in water. In fact, water sorption (%) of the TCM was lower than 0.10% at 85% RH (Fig. 6).

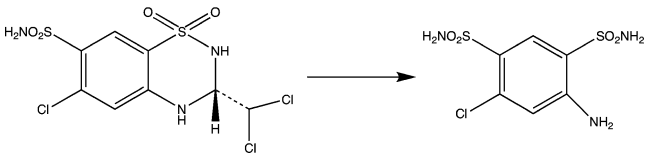

Chart 1. Reaction Mechanism of TCM in Aqueous Ethanol

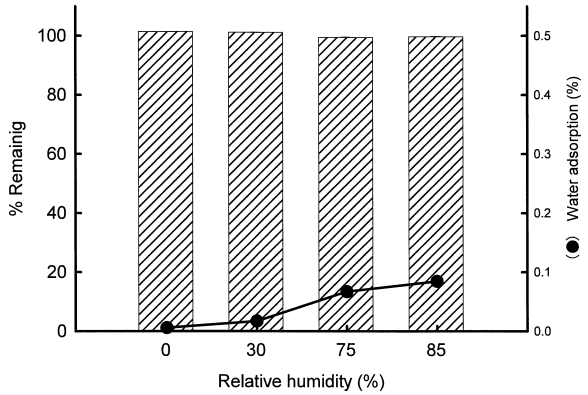

Fig. 6. Stability of Intact TCM under Various Humid Conditions at 50 °C and Water Sorption (%) of TCM Measured at Each Relative Humidity by Dynamic Vapor Sorption System

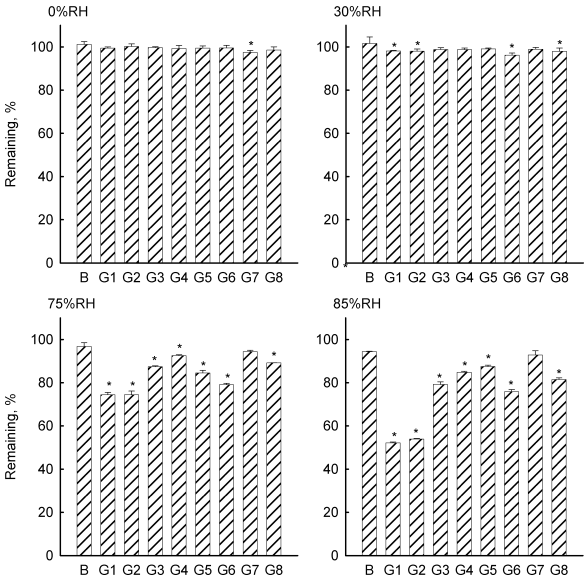

Fig. 7. Stability of TCM Tablets without PTP at Various Relative Humidities and 50 °C

* $p<0.05$ compared with B.

Effect of Relative Humidity on the Stability of TCM Tablets without PTP The B and generic drug (G1—G8) contain 2 mg of TCM with lactose and other excipients. HPC was added with G1, G2, G3, G4 and G6. Figure 7 shows the remaining (%)TCM in 9 tablets without PTP after storage for 2 weeks under various humidities at 50 °C. All products showed no change in the remaining (%)TCM at 0% and 30%

Table 1. TCM Remaining in Tablets with or without PTP, Divided Tablets, and Pulverized Powder after 2 Weeks Storage at 85% RH and 50 °C

Products	PTP (+)	PTP (−)	Divided tablet	Pulverized powder	Adsorbed water
B	94.2±1.0	94.4±0.1	92.1±1.4	89.6±1.4	6.13
G1	84.0±0.2	52.1±0.4	53.0±0.3	50.1±0.6	8.34
G2	89.9±6.9	53.9±0.3	52.3±0.2	48.2±1.1	8.81
G3	91.0±0.5	79.2±1.2	79.0±0.3	72.7±0.8	2.70
G4	93.8±1.4	84.8±0.5	85.7±0.7	82.0±0.7	10.24
G5	95.3±0.4	87.5±0.7	82.1±1.1	82.9±0.9	6.02
G6	95.3±1.5	75.8±1.0	75.5±1.7	74.5±0.5	4.75
G7	96.4±0.7	92.8±2.0	90.2±0.8	89.7±1.0	9.70
G8	89.3±0.4	81.5±0.8	81.9±1.4	63.9±2.3	11.11

RH. On the other hand, TCM degradation was observed in all tablets after stored at 75% and 85% RH after storage for 2 weeks and the remaining TCM was decreased significantly compared with B in all generic tablets except for G7. In particular, the TCM content was declined in G1 and G2 to 50% after 2 weeks stored at 85% RH. The water sorption in the each pulverized powder of the tablets was measured using DVS at 50 °C and 85% RH (Table 1). The TCM remaining (%) in tablets with and without PTP, divided tablets, and in pulverized powder after 2 weeks storage at 85% RH and 50 °C is summarized in Table 1. The adsorbed amount of water was not the same value among the 9 products. The stability of TCM can be improved by use of PTP. The remaining (%) of drug in undivided tablets without PTP was equal to that of divided tablets. The remaining values were much lower in the pulverized powders of tablets, because the area for water absorption was increased by grinding. Although the TCM content in the solid state was scarcely changed at high relative humidity, as described above, TCM in tablets was degraded at high relative humidity, indicating that the degradation may be attributed to the water adsorbed by excipients contained in the tablets.

Effect of Additives on TCM Stability under High Humidity Conditions A lot of different excipients are used in brand and generic tablets; the excipients containing in each tablet differ according to the manufacturer. The brand tablet contains seven kinds of additives, whereas more additives were used in generic products. Castello and Nattocks[12] reported that the coloration with aging in tablets containing lactose (I) and amine salts (II) was due to the reaction of I with the free amine base liberated from II by an alkali lubricant, such as magnesium stearate. Gold and Campbell[13] reported that different talcs of USP grade varied significantly in their effects on the stability of aspirin in tablets. Thus, drug stability could be affected by various additives. Equal mixtures of TCM and the excipient contained in brand and generic tablets were stored at 85% RH and 50 °C for 2 weeks (Table 2). TCM degraded with 8 out of 15 additives, *i.e.*, hydroxypropylcellulose, synthetic aluminum silicate, magnesium aluminometasilicate, hydrated silicon dioxide, low substituted hydroxypropylcellulose, corn starch, light anhydrous silicic acid, and microcrystalline cellulose. In particular, the degradation % of TCM increased to close to 15.9% with hydroxypropylcellulose. The more additives adsorbed water, the more the drug stability decreased.[14] The water adsorption (%) of 15 additives at 85% RH measured by DVS is shown in Table 2. Water was not adsorbed on talc, lactose, or silicon dioxide, while the adsorption of water was observed with other additives, and magnesium aluminometasilicate, synthetic aluminum silicate, and hydrated silicon dioxide had high moisture contents. The absorbed water content was 16.3% on hydroxypropylcellulose, which that showed the highest degradation % of TCM. These findings suggested that TCM degradation could not be estimated directly from the water content adsorbed on additives.

Table 2. Degradation (%) of TCM in the Presence of Various Additives, Water Adsorption (%) of Additives on 85% RH and pH of Additive Solution

Additive	Degradation, %	Water adsorption, %	pH
Hydroxypropylcellulose (HPC)	15.9	16.3	5.3
Synthetic aluminum silicate	3.5	30.5	6.8
Magnesium aluminometasilicate	3.5	33.0	7.8
Hydrated silicon dioxide	3.3	28.2	3.7
Low substituted HPC	2.9	20.0	5.7
Corn starch	2.9	19.4	4.8
Light anhydrous silicic acid	2.1	14.0	6.8
Microcrystalline cellulose	0.9	10.3	6.1
Carmellose calcium	0.0	23.9	4.6
Hydroxypropyl starch	0.0	20.1	6.6
Carmellose	0.0	19.2	3.7
Calcium stearate	0.0	2.6	7.1
Talc	0.0	0.2	7.5
Lactose	0.0	0.1	4.5
Silicon dioxide	0.0	0.1	5.9

The pH values of 5% (w/v) water solution or suspension of additive were measured, and the pH values of hydroxypropylcellulose, synthetic aluminum silicate, magnesium aluminometasilicate, and hydrated silicon dioxide were 5.3, 6.8, 7.8, and 3.7, respectively (Table 2). These findings suggested that the pH of the mixture with adsorbed moisture was inconsistent with TCM degradation (%), *i.e.*, 15.9% of TCM degraded with hydroxypropylcellulose compared with 3.5% with magnesium aluminometasilicate. The hydrolysis rate of nitrazepam was controlled by the N adsorption energy of the excipients, such as microcrystalline cellulose, corn starch, mannitol, and saccharose.[15] The aspirin degradation rate in binary mixtures with cellulose was affected by the brand of cellulose, and microcrystalline cellulose gave a higher degradation with aspirin compared with microfine cellulose, because the microcrystalline cellulose-sorbed water was not tightly bound to the excipient.[16] Hydroxypropylcellulose gave a higher degradation rate for TCM than low substituted hydroxypropylcellulose. Yoshioka *et al.* demonstrated that the hydrolysis rate of trichlormethiazide in gels was found to depend on the amount of free water available for the reaction.[5] It was estimated that the amount of free water on hydroxypropylcellulose was higher than that on low substituted hydroxypropylcellulose from their report.

The Effect of Hydroxypropylcellulose on TCM Stability in Tablets In Fig. 8, the degradation rate constant of TCM *vs.* water adsorption is plotted for tablets with or without hydroxypropylcellulose. A straight line was formed, indicating that the degradation rate constant was tightly related to water adsorption. The slope of the regression line for tablets containing hydroxypropylcellulose was approximately fourfold higher in tablets without hydroxypropylcellulose and significant differences in slope were found between with and without hydroxypropylcellulose. This result indicated that

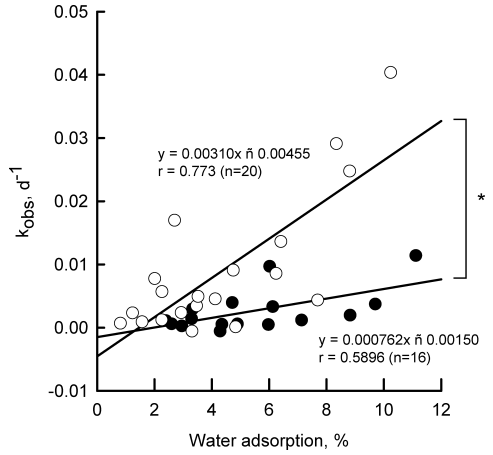

Fig. 8. Relationship between TCM Degradation Rate Constants and Water Adsorption at Various Humidities and 50 °C

○, HPC(+) ●, HPC(−). * $p<0.05$.

TCM stability in tablets was decreased by hydroxypropylcellulose under humid conditions.

Conclusions

TCM in a solid state was stable under humid conditions, but TCM in tablets were not stable under the same conditions. TCM degradation was accelerated by the addition of hydroxypropylcellulose. Various factors that may affect the TCM stability in tablets can be considered such as adsorption water, amount of free water, pH and so on.

These findings suggested that the excipient should be selected carefully in formulation design, because the TCM content decreased as a result of water adsorbed on the pharmaceutical excipients.

Acknowledgement The authors are grateful to Freund Corporation, Gotoku Chemical Co., LTD., Nippon Soda Co., Ltd., and Shin-Etsu Chemical Co., Ltd. for providing the pharmaceutical excipients.

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