Drug Development and Industrial Pharmacy, 26(10), 1091–1097 (2000)

RESEARCH PAPER

New Oral Dosage Form for Elderly Patients. III. Stability of Trichlormethiazide in Silk Fibroin Gel and Various Sugar Solutions

Takehisa Hanawa,[1,*] Ryohko Maeda,[2] Emi Muramatsu,[1]
Masahiko Suzuki,[1] Masayasu Sugihara,[3]
and Shin'ichiro Nakajima[1]

[1] *Pharmaceutical Department, Yamanashi Medical University, Yamanashi, Japan*
[2] *Pharmaceutical Department, Tokyo Women's Medical College, Tokyo, Japan*
[3] *Faculty of Pharmaceutical Sciences, Toho University, Chiba, Japan*

ABSTRACT

The hydrolysis of trichlormethiazide (TCM) in silk fibroin gel (SFG) prepared in various sugar solutions (such as ribose, fructose, mannose, and glucose solutions) was determined. The hydrolysis rate of TCM differed with the variety of sugars utilized in this study; that is, it decreased in the following order: ribose > fructose > mannose > glucose. To investigate the relationship between the hydrolysis rate of TCM and the physicochemical properties of the sugar molecule, the amount of unfrozen water of sugar molecules was calculated from differential scanning calorimetry (DSC). The amount of unfrozen water increased with an increase in the number of the equatorial OH groups n(e–*OH) per sugar molecule that are able to hydrate favorably to the surrounding water molecules. The hydrolysis rate constant decreased with increase in* n(e–*OH); glucose, having a large* n(e–*OH) in this study could effectively stabilize TCM.*

Key Words: *Differential scanning calorimetry; Hydrolysis; Silk fibroin gel; Trichlormethiazide.*

* To whom correspondence should be addressed. Department of Pharmacy, Yamanashi Medical University, 1110 Tamaho, Yamanashi 409-3898, Japan. Telephone: +81-55-273-1111, ext. 3201. Fax: +81-55-273-6672. E-mail: thana@res.yamanashi-med.ac.jp

Copyright © 2000 by Marcel Dekker, Inc. www.dekker.com

INTRODUCTION

It has been reported that jellylike preparations are available for the elderly in an oral dosage form (1) because of its easy handling and swallowing. These jellylike preparations have been prepared with various materials, such as sodium caseinate (2), glycerogelatin (3), and dried gelatin gel powder (4). Recently, silk fibroin (SF) has been used in various research fields, such as the medical (5), biomaterial (6), and food additive (7) fields, because of its unique physicochemical properties and its safety for use by humans. SF forms the principal constituent of the scleroproteins. Liang and Hirabayashi (8) demonstrated that SF can be stabilized in a β-structure by intermolecular hydrogen bonds between the polypeptide chains. We have already reported that silk fibroin gel (SFG) could be prepared from an SF aqueous solution at room temperature (20°C ± 5°C) (9,10). The rate of gelation was sufficiently accelerated by the addition of glycerol to the SF solution. The glycerol content also affected the rate of gelation of the SF solution. Furthermore, the gelation time of SFG prepared in sugar (e.g., ribose, fructose, mannose, and glucose) solutions were also investigated. The gelation time was affected by the kinds of sugars added (11). In practical use, however, the presence of water in the SFG, from a chemical point of view, seems to be unfavorable for the use of SFG as an oral dosage form.

In this study, we selected trichlormethiazide (TCM) as a model drug; it is well known to be hydrolyzed in aqueous solution by apparent first-order kinetics (12), and we initially investigated the stability of TCM in SFGs prepared in various sugar solutions. Furthermore, the relationship between the stability of TCM and the physicochemical properties of sugar solutions was also investigated by differential scanning calorimetry (DSC).

EXPERIMENTAL

Materials

Silk thread was purchased from Kayamachi (Kyoto, Japan). Trichlormethiazide was purchased from Sigma Company (St. Louis, MO). Ribose, fructose, mannose, and glucose were reagent grade and used without further purification.

Preparation of Silk Fibroin Powder

Silk thread was refined as previously reported (9). The purified silk thread was dissolved in a 50% (w/v) calcium chloride solution at 100°C and dialyzed using cellulose tube for 3 days under a distilled water stream. The solution was lyophilized with a freeze drier (Eyela FD81, Tokyo, Japan), and the SF powder was obtained.

Preparation of Silk Fibroin Gel

To prepare the SF gel, 1 g of SF was dissolved in 20 ml of various sugar solutions (0–50%) at room temperature (20°C ± 5°C), and the pH of the mixture was adjusted to 4.00 with 1 M citric acid.

Thermal Analysis

The thermal behavior of various samples was measured by DSC using a Mettler TA 300 (Greifensee, Switzerland) at a heating rate of 1°C/min in a range from −40°C to 30°C. About 10.0 mg of the liquid sample or SFG were weighed and sealed in the sample pan for the liquid sample (40 μl).

Stability Studies and Analytical Method

Stability of Trichlormethiazide in Aqueous Sugar Solutions

A definite volume (100 μl) of TCM methanolic solution (50 mg TCM/ml MeOH) was added to 10 ml of aqueous sugar solution (0–50%) and incubated at 70°C. A 1-ml portion of the fluid was removed at suitable intervals, and the amount of remaining TCM was measured with high-performance liquid chromatography (HPLC).

Stability of Trichlormethiazide in Silk Fibroin Gel

A definite volume (100 μl) of TCM methanolic solution (50 mg TCM/ml MeOH) was added to 10 ml of SF solution. After gelation, the SFG was stored at 20°C. After storing for suitable intervals, the amount of TCM remaining in the SFG was measured as mentioned above.

High-Performance Liquid Chromatography Conditions

HPLC measurements were carried out using a Shimadzu LC-3A (Kyoto, Japan) under the following conditions: Zorbax ODS column (406 mm i.d., 15 cm length); acetonitrile : 10 mM acetic acid 1 : 3 mobile phase; 1.5 ml/min flow rate.

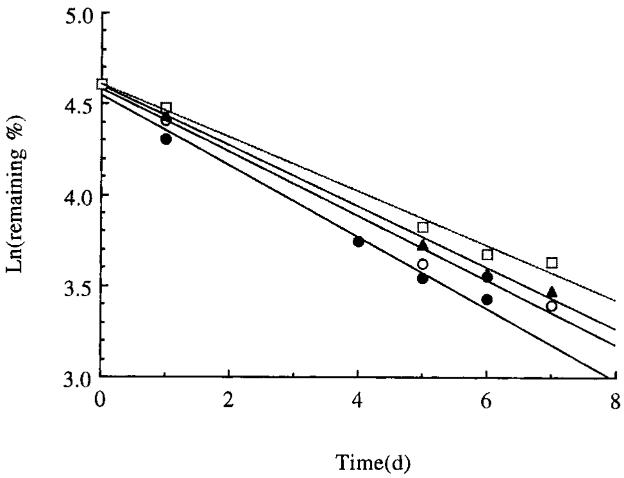

Figure 1. Hydrolysis of TCM in SFG prepared in various sugar solutions: ●, ribose; ○, fructose; ▲, mannose; □, glucose.

RESULTS AND DISCUSSION

Stability of Trichlormethiazide in Silk Fibroin Gel and Various Sugar Solutions

TCM is known to be hydrolyzed in aqueous solution by apparent first-order kinetics (12). Therefore, we used TCM as the model drug and investigated its kinetics of hydrolysis in SFGs prepared in various sugar solutions. Figure 1 shows the hydrolysis of TCM in SFGs prepared

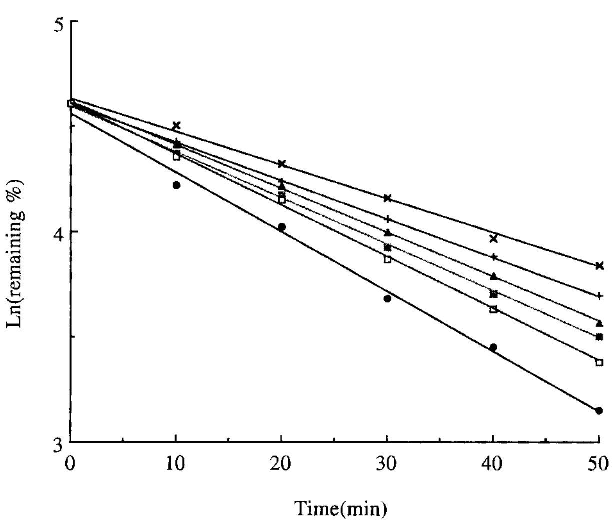

Figure 2. Hydrolysis of TCM in fructose solution at 70°C: ●, distilled water; □, 10% fructose solution; ■, 20%; ▲, 30%; +, 40%; ×, 50%.

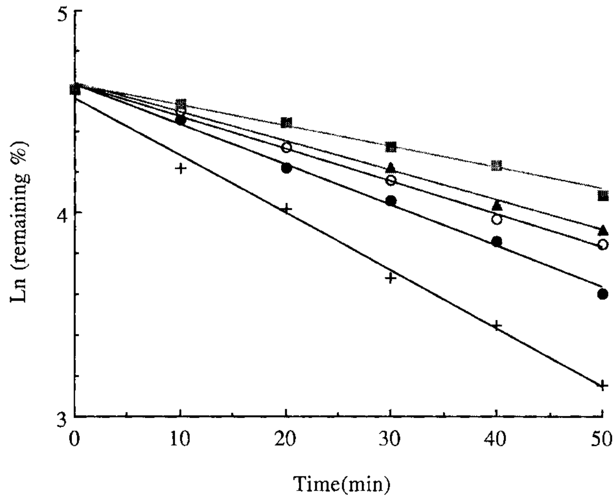

Figure 3. Hydrolysis of TCM in various sugar solutions at 70°C: +, distilled water: ●, ribose; ○, fructose; ▲, mannose; ■, glucose.

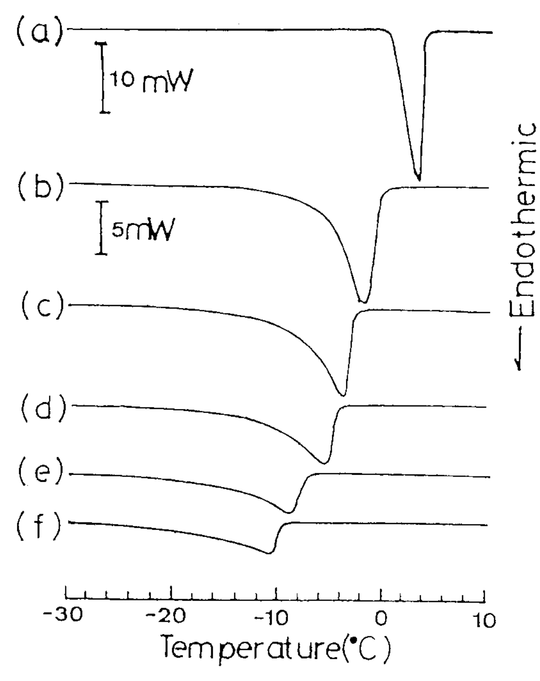

Figure 4. DSC curves of distilled water and 10–50 w/w% glucose aqueous solutions: (a) distilled water; (b) 10% glucose solution; (c) 20% glucose solution; (d) 30% glucose solution; (e) 40% glucose solution; (f) 50% glucose solution.

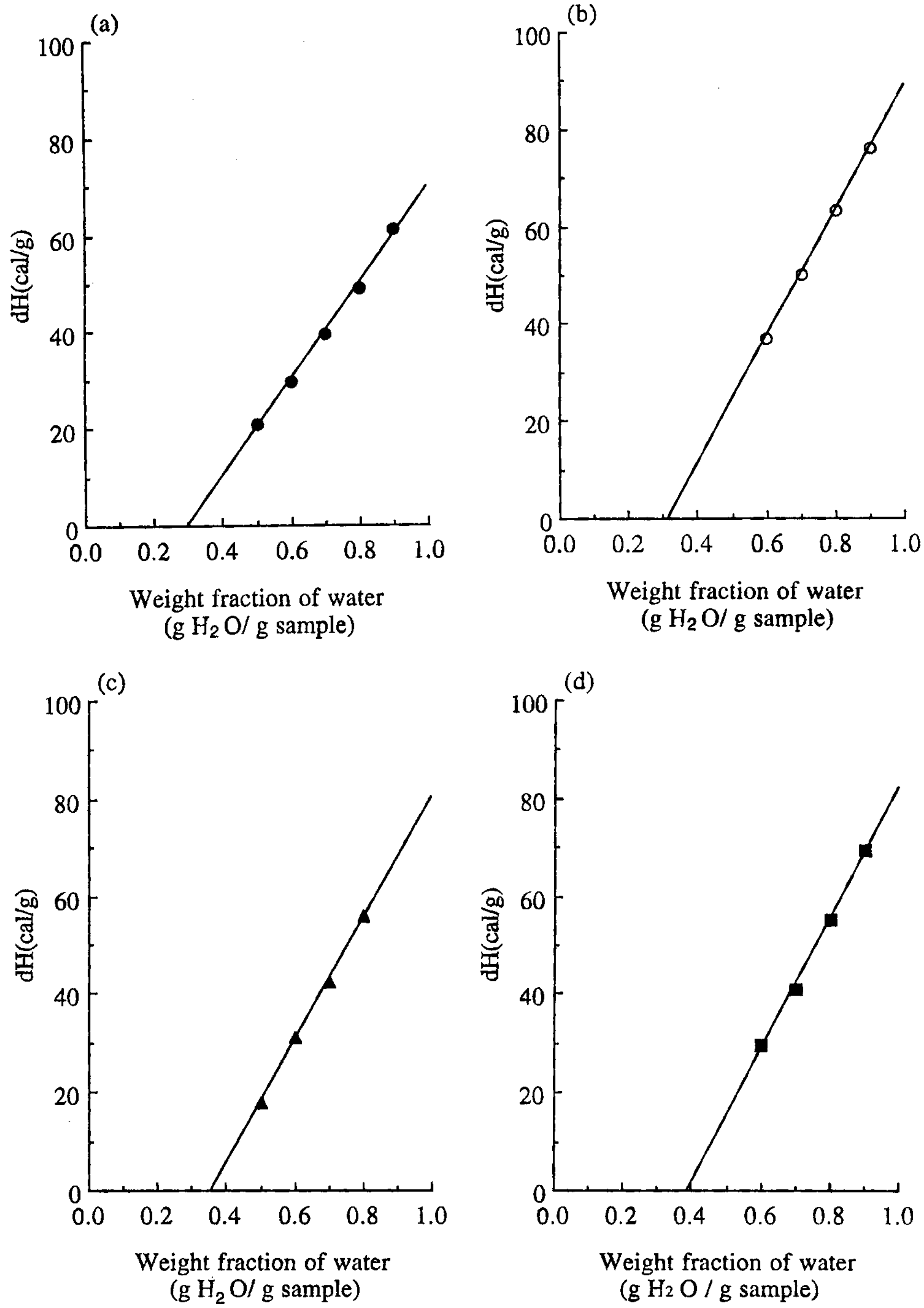

Figure 5. Relationship between the water content and the melting enthalpies of various sugar solutions: (a) ribose; (b) fructose; (c) mannose; (d) glucose.

in various sugar solutions (50 w/v%) at 20°C. Also, in the SFG, TCM was hydrolyzed by apparent first-order kinetics.

The hydrolysis rate decreased in the following order: glucose < mannose < fructose < ribose. In the case of distilled water, gel formation did not occur in this study. Watanabe et al. (3) demonstrated that TCM is more stable by the addition of glycerol; that is, the amount of free water molecules contributing to the hydrolysis of TCM decreases with an increase in the amount of glycerol added. Furthermore, Suleiman et al. (13) demonstrated that the stability of diltiazem hydrochloride is affected by the variety of sugars added. Also, in this study, to obtain detailed knowledge about the relationship between the physicochemical properties of sugar molecules and the hydrolysis of TCM, the hydrolysis behavior of TCM in various sugar (e.g., ribose, fructose, mannose and glucose) solutions was investigated at 70°C. Figure 2 shows an example of the time course of the TCM hydrolysis in 0–50 w/v% fructose solution at 70°C.

The hydrolysis appeared to follow first-order kinetics. The hydrolysis rate decreased with increasing fructose content. The same hydrolysis patterns were observed in the other sugar solutions. Figure 3 shows the time courses of TCM hydrolysis in various sugar solutions (50 w/v%) at 70°C. The hydrolysis rate decreased in the following order: glucose < mannose < fructose < ribose < distilled water. This indicates that, in spite of the same concentration; the hydrolysis rates differed from each other with the variety of the sugar.

Calculation of Unfrozen Water of Various Sugar Solutions

From nuclear magnetic resonance (NMR), electron spin resonance (ESR), and dielectric relaxation spectroscopies, Yoshioka et al. (12) demonstrated that the amount of free water affects the hydrolysis rate of TCM in gelatin gel. Furthermore, Wakamatsu and Sato (14) calculated the unfrozen water in sucrose, sodium chloride, and protein solutions using DSC.

In this study, to investigate the relationship between the physicochemical properties of sugar molecules and the hydrolysis of TCM, DSC of various sugar solutions was carried out. Figure 4a shows the DSC curve of distilled water during the rewarming process after quick freezing in liquid nitrogen.

A sharp endothermic peak due to the melting of ice was seen near 0°C. Changes of the DSC curve by the addition of 10–50 w/v% glucose are shown in Figs. 4b–4f. The endothermic peak of the melt was broadened and shifted to a lower temperature. According to the procedure of Wakamatsu and Sato (14), we attempted to calculate the amount of unfrozen water of sugar solutions using the relationship

$$\Delta H = K(1 + a)x - Ka \tag{1}$$

where ΔH is the transition heat (cal/g sample [H_2O + sugar]) during rewarming, K is the melting enthalpy (cal/g H_2O) of the pure ice, a is the amount of unfrozen water (g/g sugar), and x is the weight fraction of the water (g H_2O/g sample [H_2O + sugar]) of the sample solution. Wakamatsu and Sato (14) assumed that the heat of fusion observed on the DSC curve is due only to the fusion of ice, and when a linear relationship exists between the melting enthalpy and the water content, the value of K can be extrapolated as the heat of fusion at $x = 1$, they calculated and the unfrozen water from the intercept of the x axis and the slope. Figure 5 shows the relationship between the melting enthalpy and the water content of the sugar solutions utilized in this study. The melting en-

Table 1

Transition Heat as a Function of Water Content and Unfrozen Water of Various Sugars

	$\Delta H = K(1 + a)x - Ka$		Unfrozen Water
	K (cal/g H_2O)	a (g/g sample)	(mol H_2O/mol sugar)
Ribose	$\Delta H = 99.463x - 29.660$	($r = 0.997$)	3.55
	$K = 69.783$	$a = 0.425$	
Fructose	$\Delta H = 129.90x - 40.957$	($r = 1.000$)	4.61
	$K = 89.037$	$a = 0.460$	
Mannose	$\Delta H = 124.87x - 44.474$	($r = 0.997$)	5.51
	$K = 80.396$	$a = 0.553$	
Glucose	$\Delta H = 133.77x - 51.550$	($r = 0.997$)	6.43
	$K = 82.220$	$a = 0.627$	

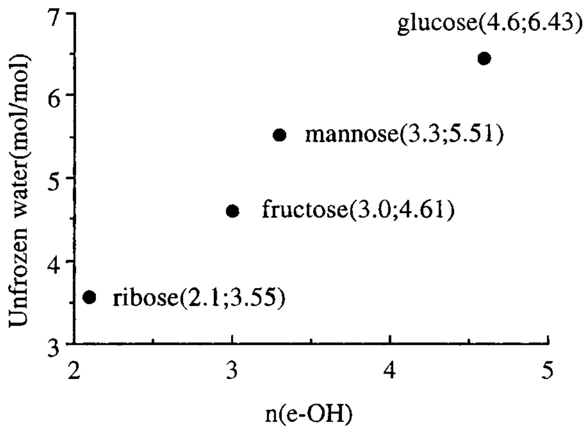

Figure 6. Amount of unfrozen water as a function of the number of equatorial–OH groups per sugar molecule.

thalpy linearly increased with the increase of the water content. The relationship between the amount of water and the transition heat in thawing and the amount of unfrozen water was calculated from Eq. 1 and is listed in Table 1.

Uedaira and Uedaira (15) demonstrated that the addition of various sugars affected the thermal denaturation of lysozyme. From DSC, they found that the denaturation temperature is closely related to the mean numbers of the equatorial OH groups *n*(*e*–OH) per molecule. That is, in aqueous solution, because the equatorial hydroxyl groups *e*–OH on the sugar molecules are able to hydrate favorably to the surrounding water molecules, lysozyme is more stable in an aqueous sugar solution having a large *n*(*e*–OH) (15). In this study, to relate the unfrozen water of sugars obtained from DSC and the hydration properties of each sugar molecule, the relationship between the amount of unfrozen water and the *n*(*e*–OH) of the sugar molecules reported by Uedaira and Uedaira (15) is shown in Fig. 6. The amount of unfrozen water increased with an increase in *n*(*e*–OH) per sugar molecule. Uedaira and Uedaira (15) demonstrated that monosaccharides have 5 or 6 hydroxyl groups per molecule; the *e*–OH groups in its molecule can be allowed to hydrate favorably with the surrounding water molecules and stabilize their molecular structure. It seems that water molecules favorably hydrate with sugar molecules that exist as unfrozen water. In this study, we attempted to investigate the relationship between the stability of the TCM in the various sugar solutions or SFGs and the *n*(*e*–OH) of sugar molecules used in this study. Figure 7a shows the apparent rate constant of the hydrolysis as a function of *n*(*e*–OH). The hydrolysis rate decreased with an increase in the *n*(*e*–OH) of the sugar molecule. The water molecules favorably hydrated with the sugar molecule seem not to take part readily in the hydrolysis reaction: The increase in the *n*(*e*–OH) increased the amount of hydrated water molecules to sugar molecules; this may result in the decrease in the hydrolysis rate. Figure 7b shows the relationship between the apparent first-order rate constant of TCM in SFGs and the *n*(*e*–OH). The hydrolysis of TCM could be depressed with an increase in the *n*(*e*–OH); in particular; glucose most effectively depressed the hydrolysis, as observed in aqueous solution. Inada et al. (16) also demonstrated that the inactivation of the myosin B Ca-ATPase activity of the myofibril was depressed to a great extent, and the amount of unfrozen water increased. Also, in this study, the amount of free water participating in the hydrolysis decreased with an increase in the amount of water hydrated to sugar molecules; glucose,

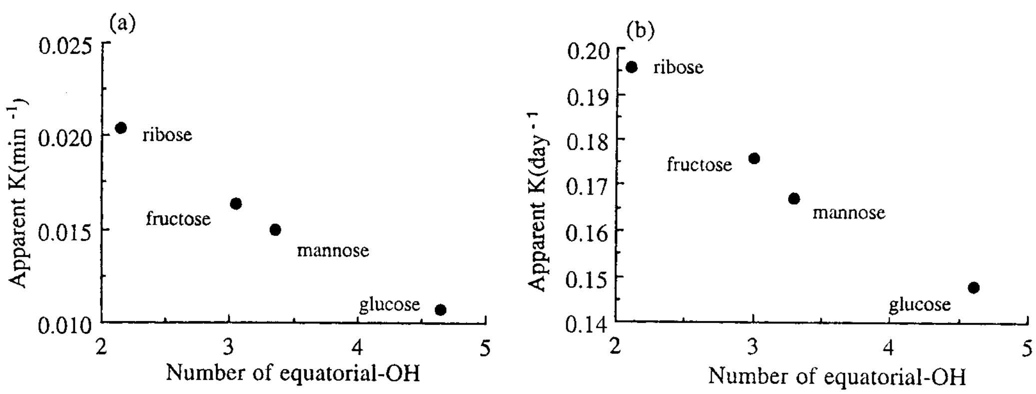

Figure 7. Apparent rate constant of hydrolysis of TCM in various sugar solutions or SFGs as a function of the number of equatorial–OH groups per sugar molecule: (a) sugar solutions; (b) SFGs.

having the largest $n(e\text{–OH})$, seems to act most effectively.

CONCLUSION

In conclusion, the hydrolysis rate of TCM seemed to depend on the amount of free water existing in sugar solutions; the increase in the sugar concentration decreased the amount of free water taking part in hydrolysis of TCM. At the same time, the alteration in the $n(e\text{–OH})$ and/or the sugar concentration might affect the hydrolysis rate; glucose, having the largest $n(e\text{–OH})$ in this study, could effectively slow the hydrolysis rate of TCM.

REFERENCES

1. M. Sugihara, Med. Digest, 51, 51–53 (1990).
2. A. Watanabe and M. Sugihara, Yakuzaigaku, 52, 69 (1992).
3. A. Watanabe, T. Hanawa, and M. Sugihara, Yakuzaigaku, 54, 77 (1994).
4. A. Ito, Y. Dobashi, K. Obata, and M. Sugihara, Jpn. J. Hosp. Pharm., 20, 41 (1994).
5. M. Iwatsuki, T. Hayashi, and H. Funaki, J. Adhesion Soc. Jpn., 27, 410 (1991).
6. N. Minoura, M. Tsukada, and M. Nagura, Biomaterials, 11, 430 (1990).
7. K. Hirabayashi and Y. Hiraiwa, New Food Ind., 35, 17 (1993).
8. C. X. Liang and K. Hirabayashi, J. Applied Polym. Sci., 45, 1937 (1992).
9. T. Hanawa, A. Watanabe, T. Tsuchiya, R. Ikoma, M. Hidaka, and M. Sugihara, Chem. Pharm. Bull., 43, 284 (1995).
10. T. Hanawa, A. Watanabe, T. Tsuchiya, R. Ikoma, M. Hidaka, and M. Sugihara, Chem. Pharm. Bull., 43, 872 (1995).
11. T. Hanawa, A. Watanabe, M. Sugihara, M. Watanabe, and T. Rikihisa, Jpn. J. Hosp. Pharm., 22, 433 (1996).
12. S. Yoshioka, Y. Aso, and T. Terao, Pharm. Res., 9, 607 (1992).
13. M. S. Suleiman, N. M. Najib, and M. E. Abdelhameed, J. Clin. Pharm. Ther., 13, 417 (1988).
14. T. Wakamatsu and Y. Sato, Nippon Nogeikagaku Kaishi, 53, 415 (1979).
15. H. Uedaira and H. Uedaira, Bull. Chem. Soc. Jpn., 53, 2451 (1980).
16. N. Inada, H. Ichikawa, Y. Nozaki, K. Hiraoka, T. Yokoyama, and Y. Tabata, Nippon Shokuhin Kogyo Gakkaishi, 39, 211 (1992).

Copyright © 2002 EBSCO Publishing