

Stabilization Mechanism of Clarithromycin Tablets under Gastric pH Conditions

Sadahiro FUJIKI, Yasunori IWAO, Mika KOBAYASHI, Atsuo MIYAGISHIMA, and Shigeru ITAI*

School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan.

Received October 25, 2010; accepted February 6, 2011; published online February 15, 2011

It has been reported that tablets of clarithromycin (CAM), a 14-membered macrolide antibiotic, are especially stable under low pH conditions such as in gastric fluid, and showed excellent antibacterial efficiency even though CAM molecules themselves are rapidly decomposed. Therefore, we aimed to clarify the stabilization mechanism of CAM tablets under low pH conditions. From the results of stability and dissolution tests, the optimal decomposition rate constant (K_{dec}) and dissolution rate constant (K_{dis}) at various pH values were calculated by curve-fitting to consecutive reactions. Consequently, $\log(K_{\text{dec}})$ increased as pH decreased. On the other hand, $\log(K_{\text{dis}})$ increased as pH decreased from 3.0 to 1.5, but decreased as pH decreased from 1.5 to 1.0. In addition, the disintegration time of commercially available tablets at pH 1.0 and 1.2 was found to be delayed, resulting in a decrease of K_{dis} . Furthermore, from powder X-ray diffraction, HPLC and elemental analysis, the delay in disintegration time might be attributable to the formation of a transparent gel, formed by the reaction between CAM molecule and hydrochloric acid under low pH conditions, on the surface of CAM tablet. On the basis of these results, this report can be considered the first case where a transparent gel prevents gastric fluid from penetrating the tablet, resulting in reduced decomposition of CAM following oral administrating.

Key words clarithromycin; dissolution rate; decomposition rate; gelation

Clarithromycin (CAM), a 14-membered semi-synthetic macrolide antibiotic, is widely used in the treatment of respiratory, skin and otolaryngology infections as well as *Helicobacter pylori* infection because it exhibits broad-spectrum antimicrobial activity. With regard to dosage form, not only tablets, but also pediatric formulations (dry syrup) are available for clinical use.^{1,2)} However, a disadvantage of CAM is its instability under low pH conditions.³⁾ Morimoto *et al.*⁴⁾ reported that decomposition of the CAM molecule occurs *via* cleavage of the neutral cladinose sugar at low pH (Fig. 1). In addition, Erah *et al.*⁵⁾ also investigated the effect of pH on the decomposition rate of CAM by calculating the decomposition rate constants of the CAM molecule in solutions and in human gastric fluid. This report demonstrated that the decomposition of CAM in solutions and gastric fluid proceeded in a pseudo-first order manner, and half-lives of CAM in pH 1.0 and 2.0 solutions were 0.1 and 1.3 h, respectively. On the

other hand, the decomposition reactions scarcely proceeded above pH 5.0. The above reports have all demonstrated rapid decomposition of CAM under low pH conditions, which simulated gastric fluid, and the resulting in decreasing CAM's antibacterial efficiency. In general, when manufacturing tablets containing an active ingredient that is unstable under low pH conditions, pharmaceutical techniques such as enteric coating and salt formation are required.^{6–8)} In fact, when manufacturing tablets of erythromycin A, another 14-membered macrolide antibiotic, an additional enteric coating of hydroxypropyl methylcellulose phthalate is necessary to maintain the drug's antibacterial efficiency. Therefore, in order to ensure the efficacy of CAM, additional pharmaceutical techniques for manufacturing CAM tablets might be necessary.

Although commercially available CAM tablets are not generally treated with such pharmaceutical techniques, they

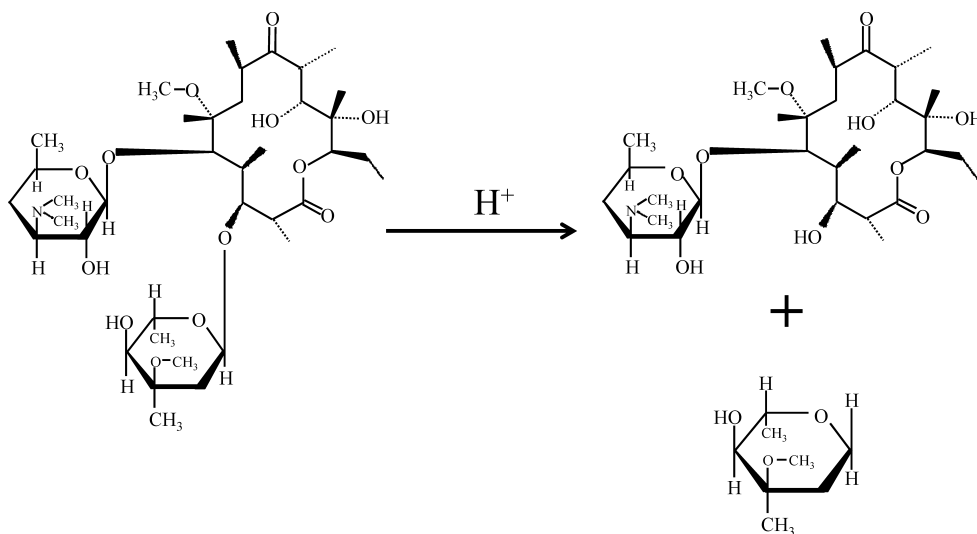


Fig. 1. Scheme of the Decomposition Mechanism of the CAM Molecule under Low pH Conditions

* To whom correspondence should be addressed. e-mail: s-itai@u-shizuoka-ken.ac.jp

still exhibit antibacterial efficiency *in vivo*. Suwa *et al.*⁹⁾ reported that when commercially available CAM tablets were administered to healthy volunteers in three groups: fasting, 30 min before and after a meal. The pH values of gastric fluid are between 1 and 2 at fasting and before a meal, and between 4 and 5 after a meal. Differences in serum CAM concentrations were scarcely observed among the three groups. This report clearly demonstrated that even when pH values were low, such as pH 1 to 2, CAM in the tablets was only barely decomposed and could still exhibit antibacterial efficiency *in vivo*. At a glance, this report may appear to conflict with the results demonstrating that the CAM molecule is rapidly decomposed under low pH conditions. However, if this interesting phenomenon is correct, the stabilization mechanism of CAM tablets in gastric fluid should be elucidated to facilitate the development of a novel drug delivery system formulation for CAM.

In the present study, using commercially available CAM tablets, the effect of pH on the release of CAM from tablets was examined. In addition, the decomposition rates of CAM in solution and tablet forms were comparatively studied. Furthermore, the stabilization mechanism of CAM tablets under low pH conditions was determined by analyzing the chemical change on the surface of 100% CAM tablets prepared by dry granulation.

Experimental

Materials Commercially available CAM tablets (Taisho Pharmaceutical Co., Ltd., Tokyo, Japan), which contain 200 mg (potency) CAM and some excipients such as lubricant, disintegrant, binder and surfactant agent, were used. Bulk CAM (purity: above 99%) was purchased from Shiono Chemical Co., Ltd. (Tokyo, Japan). All reagents used were of the highest grade available from commercial sources.

Stability Test Stability test was performed using a dissolution apparatus (Toyama Sangyo Co., Ltd., Osaka, Japan) for the paddle method. CAM (250 mg) was dissolved in 100 ml of acetonitrile. Then, 40 ml of the solution was added to 860 ml of hydrochloric acid (pH 1.0 to 3.0) at $37.0 \pm 0.5^\circ\text{C}$. The paddle rotation speed was 100 rpm. At predetermined time intervals, 5-ml aliquots of the solutions were withdrawn and neutralized with sodium hydroxide solution.

The remaining concentration of CAM was determined by high performance liquid chromatography (HPLC) system consisting of a Shimadzu LC-9A pump, a Shimadzu SPD-6A UV spectrophotometric detector, a Shimadzu CTO-6A column oven, a Shimadzu SIL-6B auto injector and a Shimadzu C-R7A plus chromatopac (Shimadzu Corp., Tokyo, Japan) under the following operating conditions: ultraviolet absorption photometer wavelength: 210 nm; column: 4.6 mm i.d. \times 15 cm stainless-steel column packed with octadecyl silica (ODS)-80TM (Tosoh Co., Tokyo, Japan); column temperature: 40°C ; mobile phase: mixture of 1/15M potassium dihydrogen phosphate and acetonitrile (13 : 7); and flow rate of 1 ml/min.

Dissolution Test The dissolution test was performed according to the paddle method listed in Japanese Pharmacopoeia (JP; 15th edition) using a dissolution apparatus. The dissolution medium was 900 ml hydrochloric acid (pH 1.0 to 3.0) at $37.0 \pm 0.5^\circ\text{C}$. The paddle rotation speed was 100 rpm. At predetermined time intervals, 5-ml aliquots of the solution were withdrawn and replaced with an equal volume of dissolution medium. The samples were filtered through a 0.20- μm membrane filter and neutralized with sodium hydroxide solution. The amount of CAM in the dissolution medium was determined by HPLC, using the procedure given in the stability test section.

Disintegration Test The disintegration test was performed according to JP 15th edition using a disintegration apparatus (Toyama Sangyo Co., Ltd.). The test medium was hydrochloric acid (pH 1.0 to 3.0) at $37.0 \pm 0.5^\circ\text{C}$.

Preparation of 100% CAM Tablets Tablets containing 100% CAM were prepared by dry granulation (slugging) because direct compaction method caused some tableting problems, such as lamination and sticking. Dry granules were prepared by compressing bulk CAM using an oil press (Japan Spectroscopic Co., Ltd., Tokyo, Japan) with a diameter of 13 mm (flat-faced punch) and then crushing the slug tablets with a mortar and pestle.

The resulting granules were sieved through a 1680 μm sieve and collected. Tablets were prepared using an oil press with a diameter of 13 mm (flat-faced punch) and tablet weight was 400 mg. The tableting force was 10 kN, and pressure was applied for 30 s.

Measurement of the Surface of 100% CAM Tablets Firstly, the disintegration test was performed by placing 100% CAM tablets in hydrochloric acid (pH 1.0, and 3.0) at $37.0 \pm 0.5^\circ\text{C}$. After 30 min, the remaining tablets were withdrawn from the disintegration apparatus and dried overnight at 37°C . Next, the tablets were crushed with a mortar and pestle and collected for analysis. To collect the unknown substance formed on the surface of tablets, the surfaces were scraped with a spatula and collected for analysis. Powder X-ray diffraction (PXRD) analysis was then performed using a Rigaku Rotaflex RU-200B powder X-ray diffractometer (Rigaku Corp., Tokyo, Japan) under the following operating conditions: target: Cu; voltage: 40 kV; current: 60 mA; scanning speed: $4^\circ/\text{min}$; 2θ range: $2\text{--}40^\circ$. To verify the changes in the intrinsic chemical structure of CAM, retention time was determined by HPLC under operating conditions similar to those of the stability test. Correspondingly, elementary analysis was performed using Yanaco CHN Corder MT-5 instrument (Yanaco Group, Tokyo, Japan).

Statistical Analysis Statistical analysis was performed with two software applications in Windows XP: Origin (OriginLab Corp., Northampton, MA, U.S.A.) for nonlinear regression analysis, and Maple (Maplesoft, a division of Waterloo Maple Inc., Waterloo, Ontario, Canada) for simulating and constructing three-dimensional graphs.

Results and Discussion

Effect of pH on Decomposition and Dissolution Behavior of CAM To clarify the stabilization mechanism of CAM tablets in the gastro-intestinal tract, the effects of pH on the decomposition reaction of CAM in solution and the dissolution behavior of CAM from tablets at low pH (1.0—3.0) were examined (Fig. 2). While CAM was barely decom-

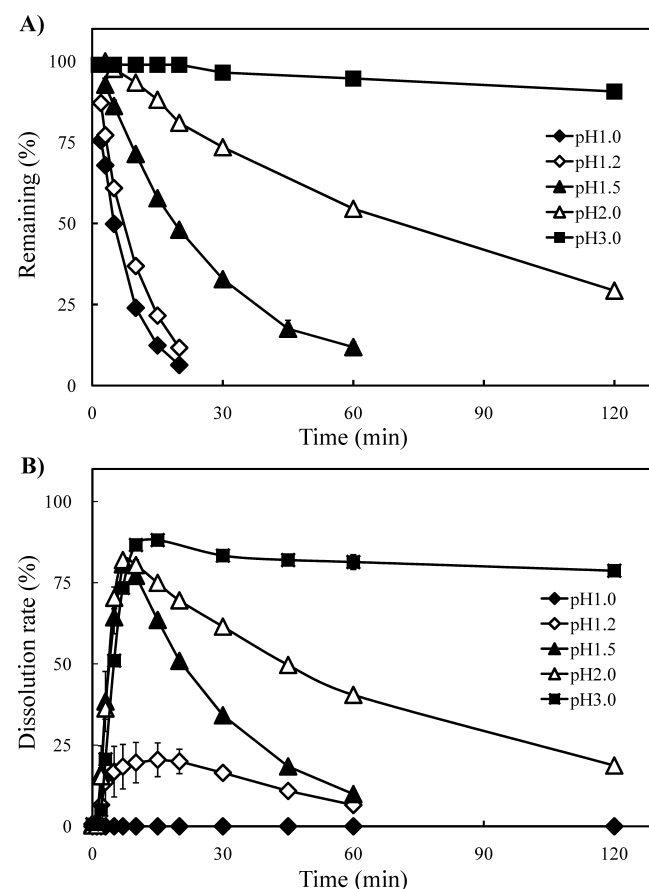


Fig. 2. Effect of pH on Decomposition and Dissolution Behavior of CAM. Each point represents a mean \pm S.D. value ($n=3$); A) decomposition behavior; B) dissolution behavior.

posed at pH 3.0, rapid decomposition was observed at pH 1.0 and 1.2 (Fig. 2A). Figure 2B shows the dissolution behavior of CAM from tablets in each pH solution. More than 80% of CAM was dissolved at 10 min after the start of incubation in pH 1.5 to 3.0 solutions, and the CAM concentration continued to decrease gradually because of CAM decomposition. On the other hand, only 20% of CAM was dissolved at pH 1.2, and no dissolution was observed at pH 1.0 throughout the dissolution test.

To calculate the decomposition rate constants (K_{dec}) and half-lives ($T_{1/2}$) of the CAM molecule under different conditions, the results of its decomposition behavior were analyzed in a pseudo-first kinetic manner (Table 1). According to the report by Nakagawa *et al.*,³⁾ an approximately linear relationship between $\log(K_{dec})$ versus pH was obtained. Similarly in this study, a linear relationship was obtained between $\log(K_{dec})$ and pH as follows (Fig. 3A).

$$\log K_{dec} = -1.13 \text{ pH} + 0.30 \quad (R^2 = 0.997) \quad (1)$$

In addition, by introducing a consecutive reaction analysis,^{10,11)} the dissolution behavior accompanied with decomposition can be estimated with the following equations.

$$Q = 100 \frac{K_{dis}}{K_{dec} - K_{dis}} \{ \exp(-K_{dis} \cdot t) - \exp(-K_{dec} \cdot t) \} \quad (2)$$

$$D = 100 \left[1 - \exp(-K_{dis} \cdot t) - \frac{1}{K_{dis} - K_{dec}} \{ K_{dis} \cdot \exp(-K_{dis} \cdot t) - K_{dec} \cdot \exp(-K_{dec} \cdot t) \} \right] \quad (3)$$

Q , D and K_{dis} denote dissolution rate at time t , decomposition rate at time t and dissolution rate constant, respectively. When each dissolution datum in Fig. 2B was curve-fitted to Eq. 2 using nonlinear regression analysis, an optimal K_{dis} could be obtained, where K_{dec} at each pH was calculated with

Table 1. Half-lives for Decomposition of CAM, Decomposition Rate Constants and the Optimal Dissolution Rate Constants

pH	$T_{1/2}$ (min)	K_{dec} (min^{-1})	K_{dis} (min^{-1})
1.0	5.0	1.39×10^{-1}	$1.35 \times 10^{-2} \text{ a)}$
1.2	6.7	1.04×10^{-1}	4.21×10^{-2}
1.5	18.9	3.66×10^{-2}	2.06×10^{-1}
2.0	68.1	1.02×10^{-2}	1.86×10^{-1}
3.0	818.4	8.47×10^{-4}	1.37×10^{-1}

K_{dec} : decomposition rate constant; K_{dis} : dissolution rate constant. a) K_{dis} at pH 1.0 could be obtained by curve-fitting the decomposition rate from the dissolution test to Eq. 3 using nonlinear regression analysis.

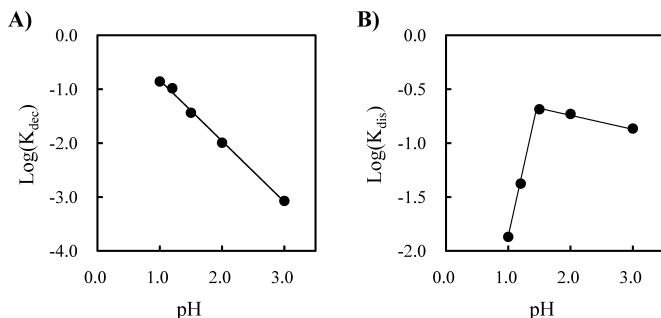


Fig. 3. Relationship between pH and A) Logarithm of Decomposition Rate Constant (K_{dec}), or B) Dissolution Rate Constants (K_{dis})

Eq. 1. Because no dissolution was observed at pH 1.0, only the optimal K_{dis} at this pH could be obtained by curve-fitting to Eq. 3 by using the data from the decomposition of CAM. The results of each parameter are shown in Table 1 and the relationship between $\log(K_{dis})$ and pH are plotted in Fig. 3B. As shown in this figure, $\log(K_{dis})$ increased as pH decreased from 3.0 to 1.5, whereas $\log(K_{dis})$ decreased as pH decreased from 1.5 to 1.0. Furthermore, $\log(K_{dis})$ was proportional to pH in over 2 pH ranges. Therefore, the following equations could be obtained.

$$\log K_{dis} = +2.36 \text{ pH} - 4.22 \quad R^2 = 0.999 \quad \text{from pH 1.0 to pH 1.5} \quad (4)$$

$$\log K_{dis} = -0.12 \text{ pH} - 0.50 \quad R^2 = 0.997 \quad \text{from pH 1.5 to pH 3.0} \quad (5)$$

Ishii *et al.*¹²⁾ also examined the dissolution behavior of CAM in commercially available tablets using the flow-through cell method. They reported an approximately linear relationship between $\log(K_{dis})$ and pH in solutions ranging from pH 3.0 to 8.0, while $\log(K_{dis})$ increased as pH decreased from 8.0 to 3.0. In this study, a similar relationship between $\log(K_{dis})$ and pH from 3.0 to 1.5 was recognized. On the other hand, the relationship below pH 1.5 in the present study did not completely fit with that reported by Ishii *et al.*, and K_{dis} decreased as pH decreased, suggesting that under low pH conditions, the dissolution of CAM from commercially available tablets might be delayed.

In Fig. 4, the decomposition rate (D) of CAM at each time and pH was simulated for both solutions and tablets by substituting Eqs. 1, 4 and 5 into Eq. 3. In the case of solutions, decomposition rate increased as pH decreased, and 94% of CAM molecules were estimated to be decomposed within 20 min at pH 1.0 (Fig. 4A). On the other hand, in the case of tablets, and below pH 1.5, the decomposition rate decreased as pH decreased, and only 16% of CAM molecules were estimated to be decomposed within 20 min at pH 1.0 (Fig. 4B). Therefore, the delay of dissolution of CAM in tablets under

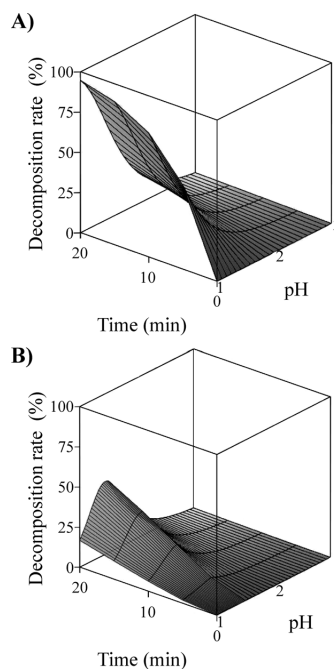


Fig. 4. Simulation of Decomposition Rate of CAM in A) Solutions and B) Tablets

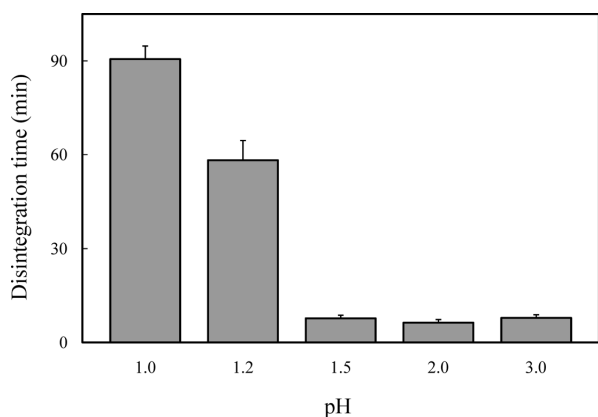


Fig. 5. Effect of pH on Disintegration Time of Commercially Available CAM Tablets

Each column represents a mean \pm S.D. value ($n=6$).

low pH conditions did not cause its decomposition.

Effect of pH on Disintegration Time of Commercially Available CAM Tablets To elucidate whether the delay of dissolution of CAM in tablets under low pH conditions was associated with the disintegration behavior of the tablets, disintegration tests using commercially available CAM tablets were performed at low pH (1.0 to 3.0) (Fig. 5). Tablets were completely disintegrated within 10 min at pH 1.5 to 3.0. On the other hand, disintegration time was drastically delayed by 90 and 58 min at pH 1.0 and 1.2, respectively. These results indicate that since disintegration of tablets themselves was delayed under low pH conditions, dissolution of CAM from the commercially available tablets did not occur, thus resulting in a decrease in the decomposition of CAM molecules.

Stabilization Mechanism of CAM Tablets under Low pH Condition To clarify whether or not the delay in disintegration under low pH condition is directly attributable to the characteristics of CAM, CAM tablets without additives were prepared, and disintegration tests were performed at pH 1.0 and 3.0. Interestingly, when the remaining tablets were removed from the disintegration apparatus just after the disintegration test, we observed the formation of an unknown transparent gel on the surface of the tablets from the pH 1.0 solution, but not from the pH 3.0 solution (Figs. 6A, B). In addition, when we performed the same experiments using commercially available CAM tablets, the formation of an unknown transparent gel on the surface of tablets, which were withdrawn from the disintegration apparatus just after 30 min at pH 1.0, was also observed (Fig. 6C), suggesting that CAM itself, not some excipients, involved in the formation of a transparent gel on the surface of tablets. Next, the unknown substance was analyzed by PXRD to determine whether CAM crystal form had changed on the surface of tablets (Fig. 7). Although CAM was found to be crystalline and differences were not observed in the PXRD patterns of CAM among the initial tablets and those of 30 min after incubation at pH 1.0 and 3.0 (Figs. 7A—C), the PXRD pattern of dried gel from the surface of tablets was different from that of the others. On the basis of these results, we hypothesized two possible mechanisms of gel formation on the surface of tablets: one due to the decomposition of CAM under low pH conditions and the other due to the interaction of CAM molecules with hydrochloric acid under low pH conditions.

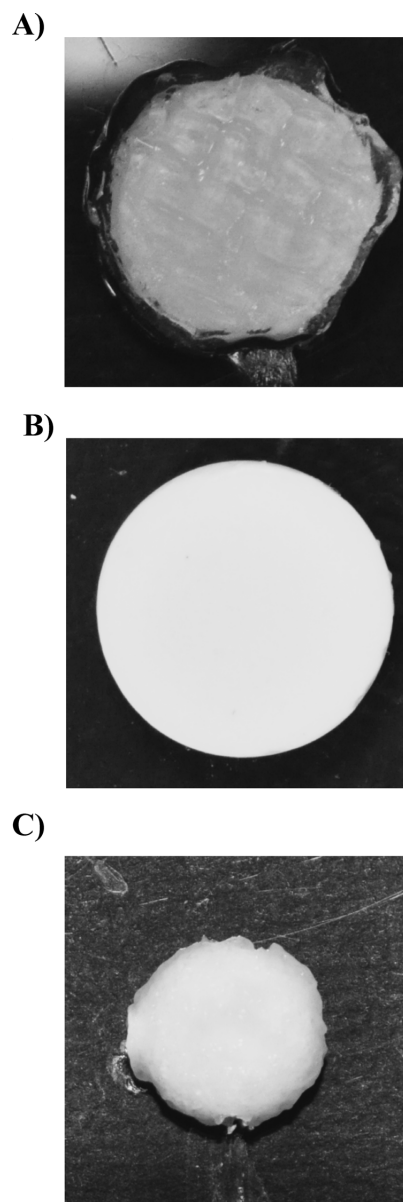


Fig. 6. Photographs of Tablets Containing 100% CAM after the Disintegration Test at A) pH 1.0 and B) pH 3.0 and That of Commercially Available CAM Tablets C) after the Disintegration Test at pH 1.0

To test these hypotheses, HPLC and elemental analysis were performed on the bulk drug of CAM and the unknown substance (Tables 2, 3). Results revealed that although the peak which was considered to be decomposition of CAM was slightly observed (3.84%), the retention time of HPLC for the bulk drug of CAM and unknown substance (96.16%) were almost equivalent (Table 2), thus suggesting that the molecular structure of CAM could be retained. In addition, from the results of elemental analysis, the value of the unknown substance was found to be almost equivalent to the theoretical value of CAM hydrochloride (Table 3). Therefore, it could be clarified that CAM molecules might react with hydrochloric acid in a 1 : 1 ratio to form a gel structure on the surface of tablets. These results indicate that CAM tablets might also form this gel structure in gastric fluid, and that such gel formation could prevent gastric fluid from penetrating the tablet, resulting in reduced decomposition of CAM in

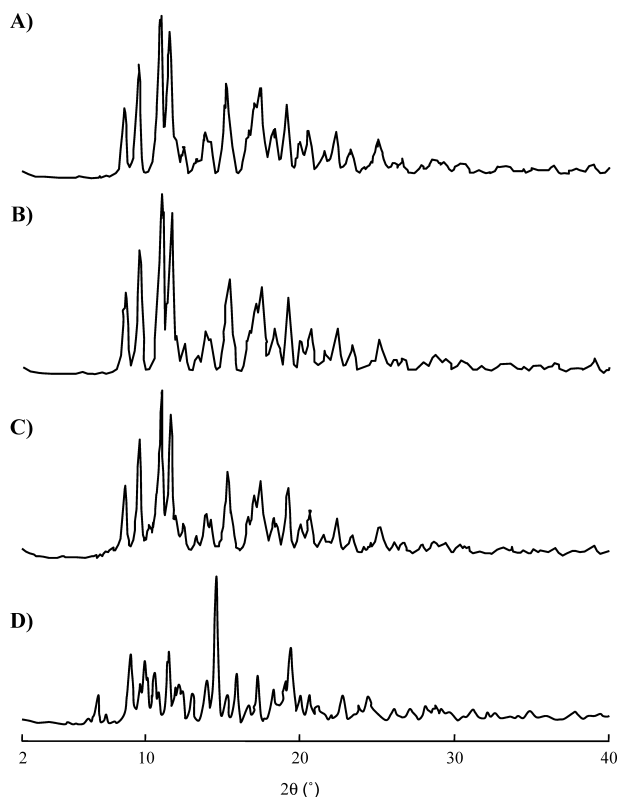


Fig. 7. Powder X-Ray Diffraction Patterns of Tablets Containing 100% CAM after the Disintegration Test

A) Initial tablet, B) tablets at pH 3.0 after 30 min, C) tablets at pH 1.0 after 30 min and D) tablet surface at pH 1.0 just after the disintegration test.

Table 2. HPLC Retention Time of Bulk Drug of CAM and the Substance on the Surface of the Tablets

	Bulk CAM	Substance on tablet surface
Retention time (min)	14.55±0.19	14.30±0.24

Table 3. Elemental Analysis of Bulk CAM and the Substance on the Surface of the Tablets

Elemental analysis	Bulk CAM	CAM T-value	Substance on tablet surface	CAM-HCl T-value
C	60.21	60.29	53.59	53.29
H	9.12	9.32	8.85	9.18
N	1.58	1.85	1.37	1.64

CAM-HCl: clarithromycin hydrochloride; T-value: theoretical value.

the same manner as an enteric-coated dosage form.^{6,7)} As reported by Suwa *et al.*,⁹⁾ when CAM is orally administered in tablet form, it is considered to be stabilized in gastric fluid by gel formation and therefore shows excellent efficacy even though the CAM molecule is unstable under low pH gastric conditions. Furthermore, in order to confirm whether CAM molecules could be released from the tablets adhered to the transparent gel in intestinal fluid, additional disintegration test was performed using commercially available CAM tablets. As the test solutions, JP first fluid (pH 1.2) and JP second fluid (pH 6.8) were chosen to use. During the first disintegration test (2 h), commercially available CAM tablets

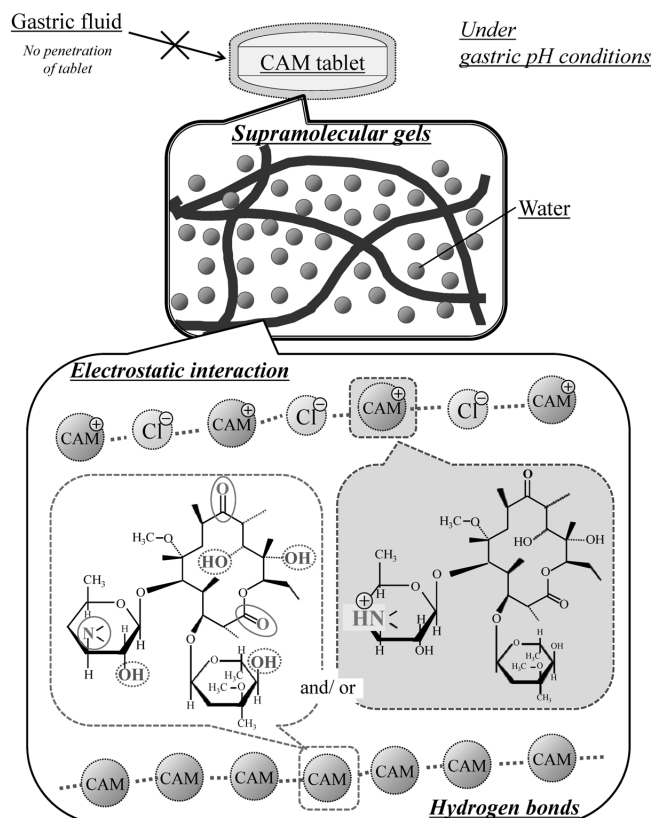


Fig. 8. Schematic Diagram of the Stabilization Mechanism of CAM Tablets in Gastric Fluid

Solid circles represent functional groups which have the possibility of proton receptors. Dashed circles represent functional groups which have the possibility of proton donors.

were not disintegrated in JP first fluid, whereas disintegration time of the remaining tablets was 3.7 min in JP second fluid. These findings indicate that even if the transparent gel was formed on the surface of tablets under low pH gastric conditions, almost CAM molecules could be released from the tablets and dissolved in intestinal fluid, suggesting that most of CAM could be absorbed in the intestine *in vivo*.

In general, gels are formed by polymers such as proteins and sugars. Recently however, some low-molecular-weight compounds have been reported to undergo gelation in water or organic solvent and are known as supramolecular gelators.^{13–16)} From this study, CAM could be classified as a supramolecular gelator under low pH conditions. Supramolecular gel is formed by entrapping solvents in a three-dimensional network structure created by entanglement of noncovalent interactions such as hydrogen bonds, van der Waals forces, π - π interaction, crystal/liquid bridges and electrostatic interaction.^{15–17)} Thus, the same mechanism could be operative in the case of CAM tablets. Specifically, in order to clarify the involvement of crystal/liquid bridges with a three-dimensional network, we mixed bulk CAM with pH 1.0 hydrochloric acid because crystal/liquid bridges are generally formed with the surface of tablets prepared by high compaction conditions. As a result, the transparent gel was easily formed by mixing (data not shown), suggesting that crystal/liquid bridges might not be involved in the formation of this transparent gel. Therefore, we speculated that electrostatic interaction between quaternary ammonium ion of CAM

cations and chloride anions and/or hydrogen bonds between tertiary amine or carbonyl groups, as proton receptors, of CAM molecules and hydroxyl groups, as proton donors, of CAM molecules on the surface of CAM tablets formed a three-dimensional network structure that entrapped water (Fig. 8). In addition, when the amounts of chloride anions and protons in pH 3.0 solutions were lower one-hundred than that in pH 1.0 solutions, the formation of gel was not observed (Fig. 6B), suggesting that abundant amounts of chloride anions and protons were necessary to form three-dimensional network structure *via* electrostatic interaction or hydrogen bonds. Supramolecular gelators have attracted special attention not only in academic fields but also in industrial fields such as cosmetics, health care, textile and foods.^{15–17)} In the pharmaceutical field particularly, novel gelators are expected to be applicable to the development of novel drug delivery system formulations such as ointments, transdermal therapeutic systems and sustained release formulations.^{13,14,18)} More detailed investigation into the mechanisms of gel formation to measure the Fourier transform infrared spectroscopy or Raman spectroscopy can be expected to facilitate the development of novel drug delivery system formulations containing CAM.

Conclusion

In the present study, we demonstrated for the first time that CAM tablets form a gel structure on their surface under low pH conditions. The gel structure is considered to prevent gastric fluid from penetrating the tablet, resulting in reduced decomposition of CAM following oral administration, with the same effect as an enteric coating. Moreover, CAM tablets may be stable under low-pH gastric conditions, even if the CAM molecule itself is susceptible to rapid decomposition,

and show excellent efficacy toward infective diseases.

Acknowledgments The authors would like to thank Dr. Takashi Ikawa at the School of Pharmaceutical Sciences, University of Shizuoka, Japan, for performing the elemental analysis.

References and Notes

- 1) Yajima T., Umeki N., Itai S., *Chem. Pharm. Bull.*, **47**, 220–225 (1999).
- 2) Yajima T., Fukushima Y., Itai S., Kawashima Y., *Chem. Pharm. Bull.*, **50**, 147–152 (2002).
- 3) Nakagawa Y., Itai S., Yoshida T., Nagai T., *Chem. Pharm. Bull.*, **40**, 725–728 (1992).
- 4) Morimoto S., Misawa Y., Asaka T., Kondoh H., Watanabe Y., *J. Antibiot.*, **43**, 570–573 (1990).
- 5) Erah O. P., Goddard F. A., Barrett A. D., Shaw N. P., Spiller C. R., *J. Antimicrob. Chemother.*, **39**, 5–12 (1997).
- 6) Watanabe Y., Sano M., Motohashi K., Yoneda R., *Yakugaku Zasshi*, **98**, 1092–1100 (1978).
- 7) Siepmann F., Siepmann J., Walther M., MacRae R., Bodmeier R., *J. Controlled Release*, **125**, 1–15 (2008).
- 8) Otto W. F., Zion I., U.S. Patent 2881163 (1953).
- 9) Suwa T., Urano H., Kodama T., Nakamura M., Watanabe T., *Chemotherapy*, **36**, 921–932 (1988).
- 10) Cai R. X., Wu X. G., Liu Z. H., Ma W. H., *Analyst*, **124**, 751–754 (1999).
- 11) Gui M., Rutan S. C., *Anal. Chem.*, **66**, 1513–1519 (1994).
- 12) Ishii K., Katayama Y., Itai S., Ito Y., Hayashi H., *Chem. Pharm. Bull.*, **43**, 1943–1948 (1992).
- 13) Sangeetha N., Maitra U., *Chem. Soc. Rev.*, **34**, 821–836 (2005).
- 14) Cao S., Fu X., Wang N., Wang H., Yang Y., *Int. J. Pharm.*, **357**, 95–99 (2008).
- 15) Suzuki M., Abe T., Hanabusa K., *J. Colloid Interface Sci.*, **341**, 69–74 (2010).
- 16) Suzuki M., Hanabusa K., *Chem. Soc. Rev.*, **39**, 455–463 (2010).
- 17) Hanabusa K., Matsumoto M., Kimura M., Kakehi A., Shirai H., *J. Colloid Interface Sci.*, **224**, 231–244 (2000).
- 18) Lim P., Liu X., Kang L., Ho P., Chan Y., *Int. J. Pharm.*, **311**, 157–164 (2006).