DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 7, pp. 715–724, 2004

The Acidic Complexation of Tetracycline with Sucralfate for its Mucoadhesive Preparation

Shoichi Higo,[1,*] Hirofumi Takeuchi,[2] Hiromitsu Yamamoto,[2] Tomoaki Hino,[2] and Yoshiaki Kawashima[2]

[1]Product Research Department, Chugai Pharmaceutical Co. Ltd., Shizuoka, Japan
[2]Department of Pharmaceutical Engineering, Gifu Pharmaceutical University, Gifu, Japan

ABSTRACT

The complex of antibiotics with sucralfate (SF) was prepared with acid. The mechanism of the complexation and some factors concerning the preparation, which influence the mucoadhering property, were studied. The complexation was confirmed by the change in color and instrumental analysis. The acidic complex appeared to be produced by reagglomeration of SF preliminary particles. It was suggested that the amide or amine groups of tetracycline (TC) and aluminum moieties of SF serve as the binding sites. The potential of multiple binding sites and a priority in them were suggested by the Scatchard plot analysis. The additional amounts of acid and the increase in the surface area increased the number of sites. The amount of the additional acid appeared to be the most important factor during the preparation of the acidic complex. The appropriate amount of acid added appeared to produce a complex rich in TC. However, an excess amount might cause the excess dissociation of aluminum moieties, which destroys the mucoadhesive paste-forming property.

Key Words: Tetracycline; Sucralfate; Complex; Binding mechanism; *Helicobacter pylori*.

INTRODUCTION

Helicobacter pylori live on the gastric mucosa locally. In actual treatment, a large amount of several kinds of antibiotics are administered concomitantly for complete eradication; however, this excessive dosage of antibiotics has caused some side effects.[1–3] A mucoadhesive formulation of antibiotics might solve these problems because the antibiotics are retained on the gastric mucosa and attack the *H. pylori* directly for

*Correspondence: Shoichi Higo, Product Research Department, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan; Fax: 81-550-87-5219; E-mail: higosui@chugai-pharm.co.jp.

DOI: 10.1081/DDC-120039361
0363-9045 (Print); 1520-5762 (Online)

an extended period of time. Moreover, a mucoadhesive formulation would make treatment possible using the least amount of antibiotic.

Sucralfate (SF) was introduced in Japan as a selective ulcer-protecting agent in 1968 and currently is accepted worldwide as a nonsystemic site protector. Nagashima and coworkers studied the behavior of SF under acidic conditions.[4–7] Its gastric mucoadhesive property comes from its adhesive paste-forming characteristic with acid consumption. In an acidic environment like the human stomach, SF might function as a good mucoadhesive material.[4–9]

Some kinds of antibiotics, such as amoxicillin, metronidazole, and clarithromycin, have been used for the treatment to eradicate *H. pylori* with a proton pump inhibitor. Tetracycline (TC) is also well known as one of the antibacterial agents effective against it. However, the problem of side effects, when these antibiotics are given in massive quantities, still remains.

The mucoadhesive preparation is a promising method to decrease the dose of antibiotics. Yokel et al. propose a simple mixture of TC and SF as a mucoadhesive preparation.[10] We have reported about the mucoadhesive property of the their acidic complex in our previous paper.[11] In the present study, we examine the mechanism of the acidic complex formulation and some factors concerning its preparation, which influence the mucoadhering property of the complex.

MATERIALS AND METHODS

Chemicals and Reagents

Two different types of SF (i.e., the original spray-dried type and the milled type) were supplied from Chugai Pharmaceutical Co. (Tokyo, Japan). The water content of SF and milled SF determined after drying was 9.1% and 10.1%, respectively. In this study, the amount of SF used was corrected for the water content. Tetracycline was purchased from Sigma Co. (St. Louis, MO). All other reagents used were of special reagent grade.

Preparation of TC–SF Complex

The TC–SF acidic complex, as shown in Table 1, was prepared by the following method. Five grams of SF, water content corrected, was mixed with water in a vessel; TC dissolved with 1 M HCl was added to the mixture and stirred for 24 hr at 400 rpm by using a propeller-type agitator with four blades. The complete mixture was entirely filtered. The product obtained on the filter was remixed in an appropriate amount of fresh water to remove free TC and then filtered again. After duplicating this process, the resulting wet powdered product was freeze-dried. After prefreezing at $-100^{\circ}C$, the freeze-dryer (Neocool, Yamato Scientific Co., Japan) was operated for 3 days or longer.

Observation of the Particles of TC, SF, and Their Complex

The appearance of the original powder of TC, SF, and the prepared complex was observed visually, and their morphologies were observed by means of a scanning electron microscope (JSM-T330A, Nihon Denshi Co. Ltd. Japan).

Instrumental Analysis of the Complex

The powder x-ray diffraction pattern of the original powder of TC, SF, their physical mixture, and their complex was determined. Data were recorded at room temperature by using a RINT x-ray diffractometer,

Table 1. The conditions for the preparation of the complexes.

Preparation no.	Type of the sucralfate	Additional amount of the acid (mL)	Volume of water (mL)	Tetracycline (g)/ Sucralfate (g)
1	SD	5	95	1/5
2	SD	5	95	1.5/5
3	SD	5	95	2/5
4	SD	10	90	1/5
5	SD	10	90	1.5/5
6	SD	10	90	2/5
7	SD	10	90	3/5
8	MD	5	95	1/5
9	MD	5	95	1.5/5
10	MD	5	95	2/5

Abbreviations: SD—spray-dried type; MD—milled type.

MJ200HS9 with a 2°/min scanning rate (Cu-*K*, 40 KV, 20 mA).

An Infrared (IR) spectrum of the above samples was measured by using a Jasco model FT/IR-230 spectrophotometer (KBr disk method). The spectrum of the complex was compared with the other samples to confirm the formation of the complex.

The Agglomeration Process of the SF Particles

The device for the size distribution measurement of the particles used a laser scattering light analysis system (LDSA-2400A, Tohnichi Computer, Japan). The measurement before dispersing in water was performed by using a dispersing-in-air method. After dispersing, the measurement was performed by using a dispersing-in-water method.

Spray-dried SF particles sieved with a 100-mesh sieve and those on the sieve were used for the experiment. The size distribution of the sieved particles was checked before dispersing in water. Five grams of these particles (corrected for water content) were suspended in 90 mL of water, and its size distribution was immediately measured. Thirty hours later, the change in the size distribution was evaluated again. After the measurement, 10 mL of 0.1 M HCl was added to the system, and the change in the particle size distribution was measured.

Measurement of Proton Consumption Amount of SF in Various TC Concentration Ratios

The influence of the presence of TC on the proton consumption capacity of SF was evaluated by use of an apparatus for the preparation of the complex using a propeller-type agitator with four blades that was used for the mixing. The varying amounts of TC (i.e., 25 mg, 50 mg, 75 mg, 100 mg, and 0 mg, respectively) were added to the spray-dried SF aqueous dispersions (1 g in 100 mL) in beakers (200 mL). Hydrochloric acid (0.1 M) was added to each beaker in 3-mL increments under mixing at 400 rpm for 5 min. After the solution became stationary, the pH of the system was measured with a precisely calibrated pH meter (TOA Auto pH STAT AUT-211 pH meter) fixed in the beaker. The pH measurements for every 3-mL addition of acid followed by mixing for 5 min were duplicated until the system did not consume the added acid. The amount of proton consumption of the system was determined by subtraction of the remaining protons (as calculated from the obtained pH values) from the added protons.

Influence of the Additional Amount of Acid on the Binding Amount of TC with SF

The saturated solution of TC was prepared, and its initial concentration was measured spectrophotometrically at 355 nm. Varying amounts of spray-dried SF, such as 70 mg, 140 mg, and 210 mg, were each dispersed in 200 mL of the saturated solution. Hydrochloric acid (1 M) (0.2 mL) was added. After mixing for 10 min, the concentration of TC in the supernatant was measured by the same above method. An additional 0.2 mL of HCl (1 M) was added to each system and mixed for 10 min. The TC concentration in the supernatant was then measured again. The binding percent of TC with SF was determined by subtraction of each measured concentration in the supernatant after acid treatment from the saturated concentration.

Determination of TC Content in the Acidic Complex

The aqueous mixture of the complex (50 mg at 50 mL) was prepared so that it was homogeneous. Three milliliters of the mixture were removed and diluted to 50 mL with 2 M acetic acid–ammonium acetate buffer to extract the TC. After gentle shaking, SF was removed by centrifugation. Tetracycline content in the complex was determined by measuring absorbance of TC in the supernatant at 355 nm. The TC content was corrected for the water content of the complex determined by the loss after drying (105°C for 3 hr).

Influence of the Additional Amount of Acid, Particle Size of SF, or Mixing Time During the Preparation on the Binding Constant and the Number of the Binding Sites

The complexes were prepared with the use of the same preparation apparatus. Tetracycline was added by dissolving it in the acid. The mixing of the system was carried out at 400 rpm for 30 hr. As shown in Table 1, the preparations were performed under two different conditions concerning the amount of the additional acid and the particle size of SF. The mean particle size of each SF was checked by using a scattering light analysis system (LDSA-2400A, Tohnichi Computer, Japan) with a dispersing-in-water method. The complexes were prepared under three or four different ratios of TC and SF per each condition to obtain the Scatchard plot.

A small amount of the dispersion was removed by centrifugation at 6-hr time intervals. The obtained

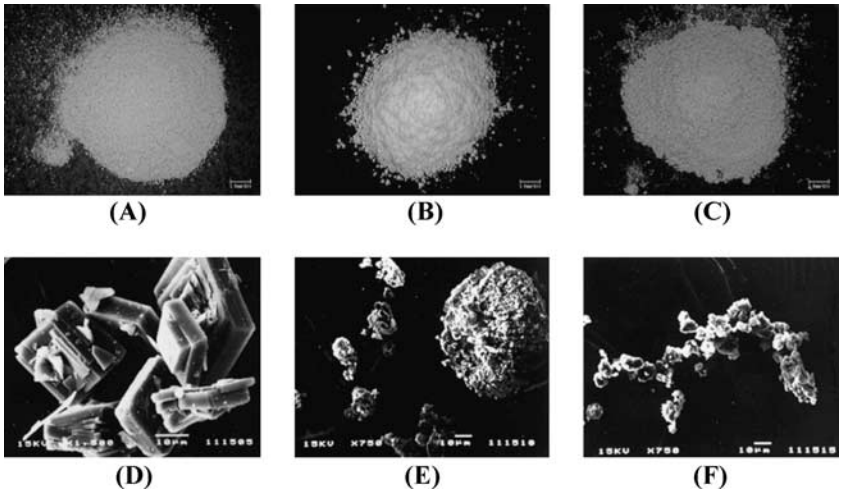

Figure 1. Optical microscopic photographs of TC original powder (A), SF (B), and the acidic complex (C). Scanning electron microphotographs of TC original powder (D), SF (E), and the acidic complex (F).

supernatant was diluted to a suitable concentration of TC and measured spectrophotometrically at 355 nm. The amount of TC bound with SF was calculated by the subtraction of the amount of free TC in the supernatant from the amount introduced into the system. Considering SF instead of the protein, the data were generated according to the Scatchard equation for a single class of binding sites:

$$r/T_f = nK - \mathrm{Kr}$$

where $r=T_b/S_t$ is the number of moles of TC bound per mole of SF, T_b and T_f are the bound and free molar concentrations of TC, respectively, S_t is the total SF molar concentration, n is the number of binding sites, and K is the binding constant for the association of TC with SF.

RESULTS AND DISCUSSION

Formation of Complex of TC and SF

All of the complexes of TC and SF, as shown in Table 1, were clearly different from the color of the original powder as shown in Fig. 1. The yellow of the TC was changed to orange by the preparation. This suggested the formation of a certain complex. It is well known that TC forms chelate compounds with metal

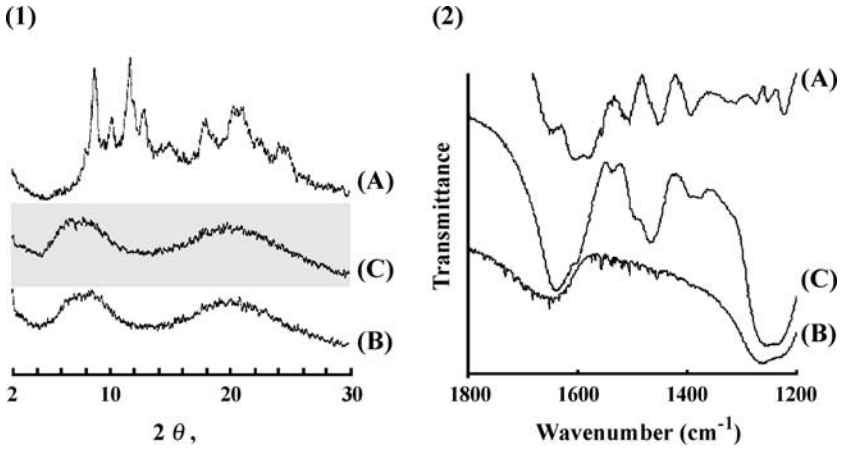

Figure 2. (1) The powder x-ray diffraction patterns of TC (A), SF (B), and complex (C). (2) FT-IR spectra of TC (A), SF (B), and complex (C).

ions such as aluminum.[12–16] Furthermore, chelating sometimes leads to the changing of its color. Considering these facts, the orange product (complex) probably was a certain kind of chelate compound.

Scanning electron microscopic photographs of the original TC and SF powders and their complex (Fig. 1) indicated that TC displayed plate crystals. They were not observed in the complex particles at all. This finding indicates that the dissolved TC was not a precipitate during the drying process but was bound to SF in a molecular form. The spray-dried SF used usually consists of a mixture of the preliminary particles and their spherical agglomerates. However, irregular agglomerates of the complex were clearly distinguishable from the original spherical agglomerates of SF. The spherical agglomerates were not observed in the complex particles at all. The agglomerates of the complex appeared to be composed of the preliminary particles of SF. These findings indicated that the complex particles were produced by the irregular agglomeration of the SF preliminary particles.

The complex formation was also clarified by the powder x-ray diffraction pattern and an IR spectrum. The amorphous SF and the crystalline structure of TC were confirmed. However, the crystalline structure of the TC disappeared by the complexation as shown in Fig. 2A. These results suggested the absence of TC crystals in the complex and offered evidence that TC binds with SF as a molecule as described above.

An IR spectrum of the complex of TC with SF was evaluated for the comparison with its compositions as shown in Fig. 2B. In the case of the original TC powder, three distinguishable peaks were observed from 1400 cm^{-1} to 1600 cm^{-1}. For example, they appeared to come from the C–N stretching vibrational bond of amide at approximately 1400 cm^{-1} and the methyl bending vibrational bond of amine at approximately 1460 cm^{-1}. Their peaks were also observed in the case of the physical mixture of TC and SF; however, the three peaks of their complex became obscure and overlapped or shifted. These changes in the peak pattern suggested the formation of a certain complex. Considering the possibility of chelate compounds, the binding mechanism by a coordinate bond was the most reasonable. Specifically, the lone electron pair of the amide or amine groups in TC appeared to be coordinated with the positively charged aluminum site of SF.

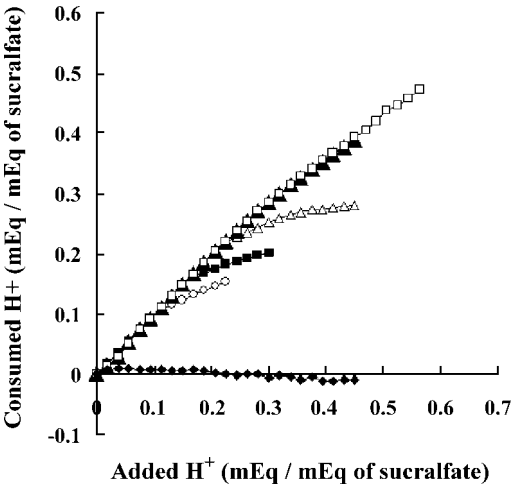

Figure 3. Proton consumption profiles of 1 g of SF in various TC concentration systems. TC alone (◆), SF alone (○), SF+25 mg TC (■), SF+50 mg TC (△), SF+75 mg TC (▲), and SF+100 mg TC (□) in the system.

The Mechanism of Forming the Complex Particles

As shown in Fig. 1, the irregular agglomerates of complex appeared to consist of the preliminary particles of SF. The particle dispersal behavior of SF in the water was studied to determine the formation mechanism of the complex agglomeration. The mean diameter of the sieved SF measured by use of a dispersing-in-air method was about 127.7 μm. The presence of the small particles despite the sieving appeared to be caused by the destruction during the measurement with high air pressure for dispersing. The mean diameter at 127.7 μm immediately decreased to about 41.6 μm by the aqueous dispersing and decreased to about 17.1 μm after a 30-hr mixing. When acid was added, the diameter of SF increased to about 21 μm. The mean diameter of the complex ranged from 20.6 to 23.3 μm. These results indicate that the spherical agglomerations of SF with a diameter over 100 μm were immediately separated into the preliminary particles during the aqueous dispersing process. The finely separated preliminary particles of SF irregularly agglomerated again by the addition of the acid.

Proton Consumption Profiles of SF in Various TC Concentration Systems

Figure 3 shows the proton consumption profiles of SF in various TC concentration systems. Each profile consisted from two distinguishable regions. On the first appearing region, named the prepaste region by Nagashima et al.,[6] all of the added acid was consumed

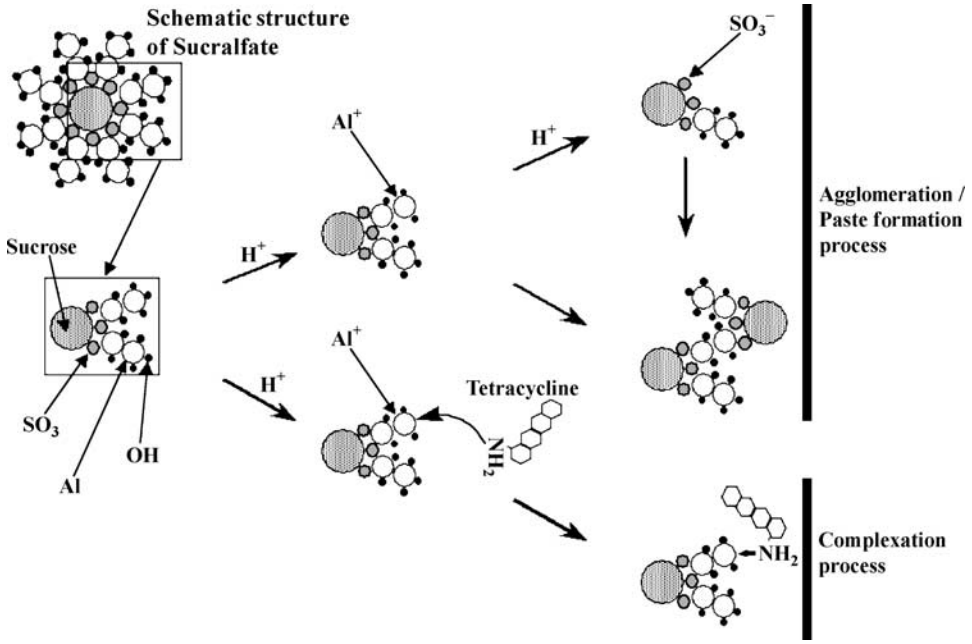

Figure 4. Binding process among the SF molecules and the complexation process with TC.

by SF. In the presence of TC, the acidic complex with SF is produced as described above. In the following region, named the paste region, SF or the resultant complex formed the paste. The mucoadhesive property of the paste formed from the complex, which was produced by a suitable ratio of TC to SF in the acidic condition, was demonstrated in our previous paper.[11] The paste-forming capacity of the complex is very important for the estimation of its mucoadhesive property.

Two such regions can be distinguished by the turning point, which shows the remarkable reduction of the acid consumption rate. It appeared to cause the rapid surface area reduction of SF for the acid consumption by the paste formation. In the presence of TC, interestingly, the turning point was shifted to a greater requirement of the acid with the increase of the amount of TC in the system. Finally, it almost disappeared with the addition of over 75 mg of TC, and SF was completely dissolved without paste formation.

The paste-forming mechanism of SF has been explained in terms of the interaction between the aluminum moieties and sucrose octa-sulfate (SOS) groups as shown in Fig. 4. The progress of their binding among the SF molecules causes the following paste formation.[4–7] If the binding was interfered with, SF does not form the paste. Considering the delay of the paste formation by the presence of TC in the system, TC appeared to interfere with the binding. In other words, it was suggested that TC bind to either the aluminum moiety or the SOS group. Considering the results of instrumental analysis, the aluminum moieties as the binding site was strongly supported as shown in Fig. 4. In addition, the excess presence of TC at the complexation appeared to cause the disappearance of the mucoadhesive paste formation capacity of SF.

Influence of the Additional Amount of Acid on the Binding Amount of TC with SF

As shown in Fig. 5, the binding efficiency between TC and SF appeared to be influenced by the additional amount of the acid during the preparation of the complex. The amount of bound TC was significantly increased with increases in the amount of additional acid. This result indicated that the number of the binding sites depends on the amount of the added acid. However, it was remarkable that the amount of bound TC was increased with the amount of SF in the system despite the same amount of acid. These findings were understandable by the aluminum moieties as the binding site with TC as follows.

Considering the process of producing the two opposite binding sites in an SF molecule, it appeared that there is a theoretical order in their production. During the acid consumption of SF, the production of

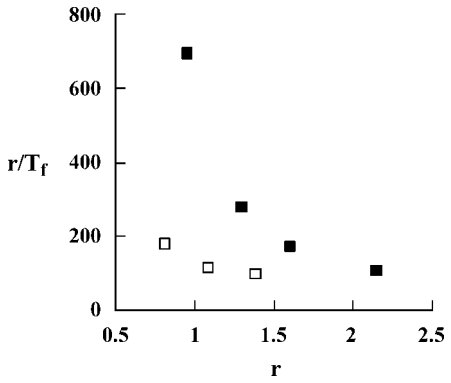

Figure 5. Influence of the amount of added acid or SF in the system on the binding amount of TC; acid was not added (▲), 0.2 mL of 1 M HCl was added (■), and 0.4 mL of it was added (●) to the system.

the positively charged aluminum site appears to precede that of the negatively charged SOS group because the SOS groups are located at the inner sites of the aluminum hydroxyl groups in the SF structure. Therefore, the negatively charged SOS group is produced after the dissociation of the positively charged aluminum sites. When a large amount of SF was supplied to the system, most of the additional acid might have been used for the production of the positively charged aluminum sites. As a result, insufficient acid remained to produce the negatively charged SOS groups. On the other hand, when a small amount of SF was supplied to the system, some aluminum moieties might be dissociated despite the same amount of acid addition. The resultant negatively charged SOS groups bound to the other positively charged aluminum moieties for the binding with TC.

Take, for example, Preparation no. 4; TC content in the acidic complex was about 11.7% (i.e., about 70% of the added TC on the preparation was bound with SF). This binding amount of TC was 23-fold compared with that for the preparation consisting of a simple mixture of these two components.[10]

The influence of the amount of additional acid on the binding amount of TC was also studied by use of the Scatchard plot. A Scatchard plot is often used for the evaluation of the interaction between the drug and protein in plasma. In this experiment, the protein in plasma was applied to SF. The plots in Fig. 6 (1) were obtained from the experiment, which was performed by the preparation of the complex with a different composition ratio of TC and SF under different amounts of added acid as shown in Table 1 (Preparation nos. 1–7). The values for the plotting were adopted after a 24-hr mixing because it appeared that the binding of TC with SF was complete. The plot for the 10 mL (1 M HCl) of acid was hyperbolic, whereas that for the 5 mL of acid appeared also to be nonlinear. The hyperbola indicates the change in the binding constant, which means the existence of multiple binding sites in an SF molecule. Furthermore, it suggested that the primary binding site(s) of SF, which had a small binding capacity, were saturated in the low concentration of TC. With increases in the TC concentration, the binding site(s) were shifted to the secondary binding sites, which had a large binding capacity. The binding sites appeared to shift to the next according to the affinity. These affinities appeared to depend on the location of aluminum moieties (i.e., the outer-located aluminum moieties reacted with the acid first). The reaction appeared to be shifted to the inner sites according to the progress of the complexation.

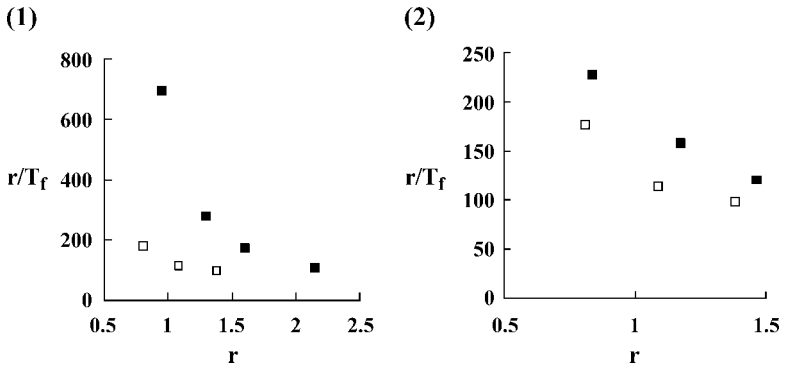

Figure 6. (1) Effect of the additional amount of the acid on the binding constant and number of binding sites; 5 mL of it was added (□) to the system and 10 mL of 1 M HCl was added (■). (2) Effect of the particle size of SF on the binding constant and number of binding sites; spray-dried SF (□) and milled of it (■).

The bend in the profile by the addition of 10 mL of acid was sharper than that by the 5-mL addition. It means a sharp change in the slope and suggests that the binding site was rapidly shifted to the next.

The intercept of the line to the ordinate when plots are linear means the number of binding sites (n). In the case of a hyperbola such as this, it seemed not to be suitable for a linear approximation. However, the plotted points for when 10 mL of acid was added were at least plotted above those for when 5 mL of acid was added. These findings tend to indicate that the number of binding sites was increased with the increased addition of acid. In other words, the greater addition of acid caused the greater dissociation of hydroxyl groups, and the resultant positive aluminum sites functioned as binding sites.

However, an excessive amount of additional acid appeared to be undesirable because it caused excessive dissociation of aluminum moieties from SF and the resultant production of negatively charged SOS groups. These sites function as the binding sites for the mucoadhesive paste formation. This paste-forming process should be performed by actual gastric juice in the stomach after oral administration. Therefore, a sufficient amount of aluminum moieties for its mucoadhesive property should be preserved in the complex.

Indeed, the complex was obtained by the agglomeration among the particles as shown in Fig. 1F. The agglomeration is produced by the binding between the aluminum moieties and SOS groups in SF as described above. Considering the amount of TC bound with SF by use of the Preparation no. 4 as shown in Table 1, the theoretical mole ratio of SF to TC to HCl was about 2.23:2.25:10, respectively. If all of the TC was bound with the SF, 1 molecule of TC was bound to 1 molecule of SF with consumption of the acid at 4.5 M. A molecule of SF includes 40 hydroxyl groups on 16 aluminum moieties. As a result, about 4.5 of the hydroxyl groups on the aluminum moieties must be dissociated from a SF molecule on the average. Theoretically, 1 molecule of TC requires an equal molecule of acid to bind with SF. Sucralfate molecules require 5 M of the acid, at least, (4 M for the negative charge of a SOS group and 1 M for the positive charge of an aluminum moiety)[6] for binding of themselves as described above. Simply, 1.5 M of the acid was lacking. These findings suggested that the binding reactions, such as complexation and binding among the SF molecules, were performed locally on the surface of SF particles, and a sufficient amount of aluminum moieties were preserved in the complex particles.

For the mucoadhesive paste formation in the actual stomach, the complex requires the fresh production of the opposite sites. In particular, SOS groups were provided by the dissociation of the aluminum moieties. A portion of the tetracycline molecules might be dissociated with aluminums when the complex contacts with fresh acid (gastric juice). As a result, the fresh aluminum moieties and the SOS groups are provided from the inner sites of the particles.

Furthermore, the binding mechanism in the nonacidic complexation should be considered. Only a small amount of TC bound with SF without the acid as shown in Fig. 5. Although the reason is not clear, this finding suggested another binding mechanism of TC with SF.

Influence of the Surface Area of SF on the Binding Amount of TC with SF

The agglomeration and complexation appeared to be performed on the surface of its particles as described above. The influence of the surface area of SF on the binding amount of TC was studied by use of the Scatchard plot. The plots in Fig. 6 (2) were obtained from the experiment, which was performed under different particle sizes of SF as shown in Table 1 (Preparation nos. 1–3 and 8–10). The values for the plotting were determined after a 24-hr mixing. The mean diameters of the spray-dried type and milled type measured by use of a dispersing-in-water method were approximately 20 μm and 8.1 μm, respectively. The smaller particle size means the larger surface area of the SF. These plots were not linear as described above. However, the curves of both of these profiles are similar. In other words, the changes in the slopes are similar. This finding indicates that the shifts of the binding sites are not different and suggested that the binding sites as a whole are the same. The points when the milled SF was used were plotted above those when the spray-dried original SF was used. This finding indicates that the larger surface area of SF increased the number of binding sites and suggested that the added acid was consumed for the complexation efficiently.

The larger surface area of SF particles appeared to provide a large number of aluminum moieties. Tetracycline appeared to be given more opportunity for the binding with the aluminum moieties. Tetracycline can bind as soon as the aluminum moiety is ionized without any further ionization because TC requires only 1 M dissociation of a hydroxyl group for the complexation. On the other hand, the added acid appeared to be partially consumed for the dissociation of aluminum moieties and the resultant agglomeration by the small surface area of SF.

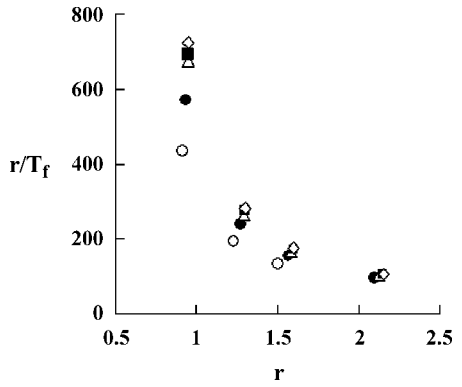

Figure 7. Effect of the mixing time after addition of the acid during the preparation of the complex. Mixing for 6 (○) hr, mixing for 12 (●) hr, mixing for 18 (△) hr, mixing for 24 (■) hr, mixing for 30 (◇) hr.

Influence of the Mixing Time During the Preparation of the Complex on the Binding Amount of TC with SF

The influence of mixing time during the preparation of the complex on the binding amount of TC with SF was studied by use of the Scatchard plot. The plots in Fig. 7 were obtained from the experiment, which was performed by use of Preparation nos. 4–7. Each symbol shows the difference of the mixing time during the preparation of the complex. The stability of TC in water under different conditions of pH was confirmed, because there was some concern that TC might decompose due to the long mixing time with acid. The remaining percentages after 24 hr at room temperature of TC in 1 N HCl (800 mg/2.5 mL; pH 0.10) and diluted solution by 100-fold (pH 2.06) were about 90% and 98%, respectively. The actual pH of the preparation system was kept above 4, because most of the acid was neutralized by the SF. Thus, the preparation of the acidic complex progressed under the stable pH for TC. Most of the TC immediately bound to the primary binding site(s) of SF, and the amount of bound TC to the primary binding site(s) of SF increased with increases in the mixing time at the low concentration of TC. This finding appears to occur because agglomerated particles were partially separated during the mixing process and produced new primary binding sites on their surface. As a result, the binding of TC to SF was almost finished after 18 hr of the mixing time. The mixing time over 18 hr appeared to be appropriate for the preparation of the complex. The binding mechanism by nonacidic complexation should be also considered. After binding by use of acid, TC might gradually bind with SF in the neutralized system.

CONCLUSIONS

The complex of TC and SF was prepared by use of the acid. The complexation was confirmed by the instrumental analysis. The amide or amine groups in TC appeared to be the binding site of TC. The aluminum moieties are suitable as the binding sites of SF by the consideration of the binding site of TC, the binding behaviors of TC and the chelate compounds. The particles of the acidic complex appeared to be produced by the reagglomeration after separating the spray-dried SF into the preliminary particles. The agglomeration was performed on the surface of the particles with competition with the complexation. Milled type sucralfate had an advantage in the formation of the complex by an increase in the surface area. It appeared desirable to have over 18-hr mixing during the preparation because of the slight gradual complexation after the main complexation at the beginning.

Tetracycline bound with SF was increased by the acidic complexation because of an increase in the number of the sites. The potential multiple binding sites and the priority in their order were suggested by the Scatchard plot analyses. This order appeared to be decided by the location of aluminum moieties. Outer aluminum moieties appeared to have higher priority than inner ones. The rate of shifting of the binding sites was accelerated by the acid addition. With an increase in the ratio of TC in the system, binding sites are shifted to the lower priority sites. In sufficient presence of the acid, an excess amount of TC appeared to cause the excess binding to the aluminum moieties for the mucoadhesive paste formation. The amount of the additional acid appeared to be the most important factor during the preparation of the acidic complex. The appropriate acid addition appeared to produce the complex rich in TC.

The acidic complexation process was kept at a stable pH for TC by the neutralizing property of SF. The preparation method shown in this paper is very useful because it is easy and ensures the complexation.

ACKNOWLEDGMENT

The authors thank Dr. Paul Langman, Chugai Pharmaceuticals, Co., Ltd. for assistance with English usage.

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